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PREFACE

The substantial research investments made by the United States through the U.S. Wheat and Barley Scab Initiative/USDA-ARS collaboration to combat Fusarium Head Blight (FHB) were justified in large measure by the argument that this scourge represents a mortal threat to the wheat and barley industries of the U.S. Earlier this year, Dr. Masa Iwanaga, Director General of CIMMYT, enabled me to see that unchecked, the detrimental effects of FHB on yield, food safety, and particularly world trade, render it a daunting ***threat to Global Food Security***. Why? Though losses in yield and quality where FHB occurs can be devastating, the real villains are deoxynivalenol and its related toxins. As indicated by Dr. Thomas Miedaner in his comments below, International regulatory bodies, prompted primarily by developed economy governments, stand poised to impose limits on the acceptable levels of DON in wheat and barley. Dr. Iwanaga showed me that the negative effects such regulatory actions will have on world trade of wheat will disproportionately affect the world's poor.

A second justification for the U.S. Wheat and Barley Scab Initiative was the self-evident fact that no single institution within the U.S. had the resources, human or otherwise, to tackle this complex problem. I argue that the *global* distribution of Institutions leading the advances in different aspects of the fight against FHB, so clearly evident in these Proceedings, now renders self-evident the need for a *global* framework which maximizes synergy and minimizes information gaps. That framework, at a minimum, should include an information hub for the collection and communication of information for scientists, policy makers, and stakeholders involved in any aspect of FHB or regulation of its toxins.

Professor Dajun Liu welcomed participants to the 2000 International Symposium in China with a talk entitled "To Defeat Scab- The Duty-Bound Task of Scientists Worldwide." I second that sentiment, and expand on it by challenging all scientists and policy makers to redouble and globally coordinate our efforts to minimize occurrence of FHB and its toxins while simultaneously ensuring that any new food safety regulations governing *Fusarium* toxins are crafted on the basis of robust toxicological and exposure data. This Symposium is the largest gathering ever of scientists and stakeholders working on this problem. Let us make the most of this opportunity to not only acquire new knowledge, but to also begin the search for strategies which weave our national and international organizations into an integrated global network with which we will ultimately defeat FHB.

These Proceedings are a compilation of over 300 poster-abstracts and papers submitted by a like number of Symposium participants representing numerous institutions in at least 27 countries. Abstracts and papers included here are organized alphabetically by first author within the relevant symposium session as described below. This compilation of unedited submissions is first and foremost a tool for participants at this Symposium.

In May of 2000, nearly 100 scientists from institutions in 13 countries gathered in Suzhou and Nanjing, China, for the *International Symposium on Wheat Improvement for Scab Resistance*. At the suggestion of Dr. S. Rajaram (CIMMYT), the International Organizing Committee (IOC) for that event agreed to remain active with the goal of convening another international symposium in four years. In 2002, the Steering Committee of the U.S. Wheat and Barley Scab Initiative (USWBSI), at the request of the IOC, agreed to host an International Symposium in 2004, in lieu of its annual Fusarium Head Blight Forum. In 2003, IOC member Dr. Akos Mesterhazy (Hungary) pointed out that the 8th International European Fusarium Seminar was also scheduled for 2004 under the leadership of Dr. Thomas Miedaner (Germany). Acting in the spirit of true international collaboration, Dr. Miedaner and his scientific board approached the

IOC with a proposal to merge their 2004 Seminar with the developing International Symposium. The IOC embraced this sensible concept and invited Dr. Miedaner to join its ranks.

The IOC, with concurrence of the hosting USWBSI, agreed upon a mixed format of invited talks organized into an opening Plenary Session followed by six Research Sessions, each comprised of Invited Talks and follow-on Poster Sessions. The Research Sessions were defined as follows:

- **Host Plant Resistance and Variety Development** - Science and affiliated technologies focused on:
  - Discovery and/or characterization of naturally occurring host plant resistance genes in wheat, barley and related species (e.g., germplasm screening, conventional genetic analyses, QTL/gene discovery and/or mapping facilitated by DNA markers or genomic techniques, studies of resistance mechanisms, etc.)
  - Breeding strategies that maximize the rate of development and adoption of resistant varieties- either conventional or GM (e.g., DNA marker assisted selection, efficacy of screening/phenotyping techniques, breeding methods in general, etc.)
- **Genetic Engineering** - Science and affiliated technologies focused on GMO approaches to host plant resistance.
- **Chemical, Cultural and Biological Control**
- **Food Safety, Toxicology, and Utilization of Mycotoxin-contaminated Grain**
- **Pathogenesis, Epidemiology, and Disease Forecasting**
- **Taxonomy, Population Genetics, and Genomics of *Fusarium* spp**.

R. Ward
President, International Organizing Committee

The European Fusarium Seminar was started in 1987 by Jerzy CheBkowski at the Warsaw Agricultural University (Poland) and since then it has been organized every two to three years in another country. The number of participants has increased steadily reaching a number of 150 to 200 scientists. The latest symposia were in Szeged (Hungary), Berlin (Germany), and Poznan (Poland) in 1997, 2000, and 2002, respectively. The permanent title of this series of conferences is still "*Fusarium* – Mycotoxins, Taxonomy, and Pathogenicity", but the spectrum of topics has been broadened by incorporating occurrence and genetics of *Fusarium* species, toxicology, resistance breeding, molecular genetics, and plant protection. The conferences used to be open to all *Fusarium* species and all crops of economic impact.

The main issue in the European Union at present is the concern for food security that is highly affected by Fusarium mycotoxins. The European Union is intensively discussing putting up thresholds for deoxynivalenol and zearalenone that will be valid for all member states.

In this perspective it is a great advantage that the ***U.S. Wheat & Barley Scab Initiative (USWBSI)*** and the ***8th European Fusarium Seminar*** are combined in the forthcoming symposium. Since problems with *Fusarium* species in our feed and food chain are of world-wide concern, it stands to reason that scientific results and conclusions should be open for discussion within the whole community of *Fusarium* researchers across national boundaries.

T. Miedaner
University of Hohenheim, Stuttgart, Germany

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CEREAL GENOME AND GENE SPACE ANALYSIS

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ABSTRACT

Cereal genome and gene-space analysis will be discussed with particular reference to wheat and barley of the Triticeae tribe. Both wheat and barley share 12 million years of co-evolutionary history and diverged from a common ancestor of rice about 40 million years ago. Diploid wheat and barley have a basic chromosome number of seven and a genome size of 5,000 Mb. Bread wheat is hexaploid and has a genome size of 16,000 Mb. Classical genome analysis in both crops was facilitated by exploiting the richness of genetic resources, such as mutants, trisomic and translocation stocks in barley and the vast number of aneuploids, such as monosomics, nullisomics and deletion lines, in wheat. Later, wheat-barley addition lines were used to demonstrate the complete conservation of gene synteny between wheat and barley chromosomes. High-resolution RFLP maps were made in the 1990's and anchored to chromosome maps by use of cytogenetic landmarks. Comparative mapping revealed rough conservation of synteny among the major cereal crops and rice, because of its small genome size (420 Mb), was the second plant to be selected for genome sequencing. In order to fully utilize the sequenced genome of rice, EST (expressed sequence tags) resources were developed in both wheat and barley, and EST-based maps were developed. The EST-based maps were compared against the sequenced genome of rice and in silico maps of wheat and barley were constructed. These maps revealed many micro-rearrangements between the Triticeae and rice chromosomes and revealed that there are limits when using the rice genome sequence for gene discovery in barley and wheat. More recently, several laboratories have initiated work on BAC-contig maps of wheat and barley chromosomes anchored to the genetic maps. The goal is to accelerate gene discovery in these two crops by map-based cloning. Both wheat and barley genomes contain more than 90% repetitive DNA and only 5% genes. The emerging concept is to focus only on the analysis of the gene space. The genic portion of the genome can be separated from the repetitive DNA by methylation filtration and Hi Cot analysis. Sequencing the gene-enriched fraction and genic BACs may be the best strategy for analysis of gene space in wheat and barley. For functional genomics, the development of other resources such as chips and mutant populations will be reviewed.

GENOMICS OF *FUSARIUM GRAMINEARUM*

H. Corby Kistler[1*], Bruce Birren[2], Sarah Calvo[2], Christina Cuomo[2], Liane R. Gale[1], U. Gueldener[3], Li-Jun Ma[2], Gary Muehlbauer[1], Kerry O'Donnell[4], Frances Trail[5], Todd Ward[4], Jin-Rong Xu[6] and members of the *Gibberella zeae* International Genomics Initiative (GIGI)

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ABSTRACT

We have generated a draft sequence assembly of the *F. graminearum* genome that is available on the web for download and query. The assembly is remarkably complete owing to near lack of repetitive sequences in the genome. After manual editing, the entire assembly currently is contained on 28 scaffolds ranging in size from 3 kb to over 8.8 Mb with an average contig length of over 71 kb. Currently, over 99.8% of the DNA sequence has been anchored to the genetic map by way of 237 genetic markers, 164 of which are sequence-tagged sites. Automated draft gene calls were conducted both at the Broad Institute and at MIPS resulting in >11,000 predicted genes. Improvements to the predicted gene sets are being made by manual annotation by members of GIGI, representing over 25 laboratories world-wide and further EST sequencing at the Broad Institute. MIPS currently is hosting web-access to the manual annotation as well as both the Broad Institute and MIPS gene models. A custom Affymetrix GeneChip microarray designed from gene models derived from the draft assembly is now available. Details of the automated annotation, efforts toward manual annotation, microarray experiments and coordination of functional analysis of the genome will be discussed. The *F. graminearum* sequencing project is funded by the National Research Initiative (NRI), through the USDA/NSF Microbial Genome Sequencing Program. The MIPS *Fusarium graminearum* Genome Database is funded by the Austrian Federal Ministry for Education, Science and Culture and the *Fusarium* microarray is funded by the USDA NRI Integrated Program: Functional Genomics of Microbes.

HOST-PATHOGEN AND PATHOGEN-PATHOGEN INTERACTIONS IN FHB

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ABSTRACT

Globally, *F. graminearum* is the predominant species associated with Fusarium head blight. However, in many situations, several fungi may be involved. These include producers of trichothecene and other mycotoxins as well as species that produce no mycotoxins. Histological studies and, more recently, the use of reporter tagged isolates have revealed how *Fusarium* species colonise host tissues but relatively little is known about the detailed molecular interactions between Fusarium species and the host plant. Within *F. graminearum* and *F. culmorum* both deoxynivalenol (DON) and nivalenol (NIV) producing isolates are present in many areas and cause disease on various cereal hosts. DON has been shown to be a virulence factor but it is not clear how host or environmental factors influence the production of DON. Furthermore, it is not known how DON production, through the loss of the ability to produce NIV, has conferred an apparent selective advantage with respect to pathogenicity towards wheat.

The genetic basis of resistance to FHB is generally complex and may involve a number of mechanisms. Studies are beginning to permit dissection of resistance into component parts and provide some insight into potential mechanisms involved. Differential expression of pathogenesis related proteins has been observed between some resistant and susceptible varieties and reduced accumulation of DON has been found among others. The imminent availability of DNA microarrays for the pathogen and a number of cereal hosts should provide the potential to reveal much about the signalling processes between host and pathogen and identify aspects relating to resistance.

In those regions where toxin-producing and non-toxin producing species form disease complexes the competitive interactions between pathogens has important consequences for disease and subsequent risks to consumers associated with the consumption of contaminated cereals or their products. Molecular diagnostic tools are beginning to shed light on some of these interactions but, as with host-pathogen interaction, much remains to be learned.

HOST PLANT RESISTANCE AND VARIETY DEVELOPMENT

Chairperson: Peter Ruckenauer

QTL ANALYSIS OF FUSARIUM HEAD BLIGHT IN BARLEY USING THE CHINESE LINE ZHEDAR 2

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ABSTRACT

Fusarium head blight (FHB) in barley and wheat caused by *Fusarium graminearum* is a continual problem worldwide. Primarily, FHB reduces yield and quality and produces the toxin deoxynivalenol (DON), which can affect food safety. Locating QTLs for FHB severity, DON level and related traits heading date (HD) and plant height (HT) with consistent effects across a set of environments would increase the efficiency of selection for resistance. A population of seventy-five double haploid lines, developed from the three way cross Zhedar 2/ND9712//Foster, was used for genome mapping and FHB evaluation. Phenotypic data were collected in replicated field trails from five environments in two growing seasons. A linkage map of 214 RFLP, SSR and AFLP markers was constructed. The data were analyzed using MQTL software to detect QTL x environment interaction. Because of the presence of QTL x E, the MQM in MAPQTL was applied to identify QTLs in single environments. MQM mapping identified nine QTLs for FHB severity and five for low DON. Only three of these QTLs were consistent across environments. Five QTLs were associated with HD and two with HT. Regions that appear to be promising candidates for MAS and further genetic analysis including the two FHB QTLs on chromosome 2H and one on 6H which also were associated with low DON and later heading date in multiple environments. This study provides a starting point for manipulating Zhedar 2-derived resistance by MAS in barley to develop varieties that will show effective resistance under disease pressure.

HIGH-RESOLUTION MAPPING OF TWO FHB RESISTANCE QTL REGIONS: *QFHS.NDSU-3BS* AND *QFHS.IFA-5A*

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ABSTRACT

Two large populations, segregating for either *Qfhs.ndsu-3BS* or *Qfhs.ifa-5A* were generated from single crosses or back-crosses of CM-82036 derived lines (resistant) by Remus (susceptible). SSD lines in the F4 or BC1F3 generation were genotyped using flanking SSR markers for *Qfhs.ndsu-3BS* (*Gwm1034, Gwm493*) or *Qfhs.ifa-5A* (*Gwm293, Gwm156*). Recombinant lines were selected and advanced for two more generations by SSD. F6 or BC1F5 plants were genotyped again using SSRs for *Qfhs.ndsu-3BS* (*Barc75, Gwm389, Gwm1034, Gwm533, Barc133, Barc147, Gwm493, Barc102*) or *Qfhs.ifa-5A* (*Barc186, Gwm1057, Barc56, Gwm304, Barc117, Gwm293, Gwm129, Barc1, Barc180, Barc40, Barc100, Gwm156, Barc141*). Marker data were used for linkage map construction and QTL mapping. Seed harvested from the same plants was used for sowing a field experiment to evaluate FHB severity in 2004. The 174 recombinant lines of the population segregating at *Qfhs.ndsu-3BS* were single-spikelet inoculated, the 180 lines of the population segregating for *Qfhs.ifa-5A* were spray inoculated.

The obtained linkage map at *Qfhs.ndsu-3BS* spanned about 25 cM. Based on the preliminary resistance data, the QTL could be placed in the interval *Xbarc133-Xbarc147-Xgwm493*, spanning about 7 cM, which is in agreement with other reports. The linkage map around *Qfhs.ifa-5A* showed suppressed recombination, and several markers were tightly linked. In order to compare genetic and physical maps for this region cytogenetic stocks were used, especially Chinese Spring deletion lines (Endo & Gill 1996). Our preliminary data indicate that the most likely position of *Qfhs.ifa-5A* is on the short arm of chromosome 5A in a region with suppressed recombination. We will repeat resistance testing in 2005 and plan to apply more molecular markers for genotyping, in order to refine the QTL maps.

ACKNOWLEDGMENTS

We thank BS Gill and the colleagues at the Wheat Genetics Resources Center at Kansas State University for supplying seed of the Chinese Spring deletion lines. This work was supported by the European Union funded research project 'Novel tools for developing *Fusarium*-resistant and toxin-free wheat for Europe', contract number QLRT-2001-02044.

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GENOTYPE-DEPENDENT ACCUMULATION OF *TRITICUM AESTIVUM* TRANSCRIPTS IN RESPONSE TO DEOXYNIVALENOL

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ABSTRACT

Deoxynivalenol (DON) is a trichothecene mycotoxin produced by cereal-pathogenic *Fusaria* and evidence suggests that it acts as a phytotoxic disease virulence factor aiding host pathogenesis. The effect of DON (20 ppm) on gene expression in roots of Fusarium head blight (FHB) disease resistant (Frontana and CM 82036) and susceptible (Remus and Riband) wheat (*Triticum aestivum*) cultivars was determined (24-h post-treatment). Despite inhibition of protein synthesis being the mode of action of this toxin, at least 70 transcripts were overexpressed in the wheat roots of different cultivars in response to DON. We assessed the effect, over time, of DON and *Fusarium avenaceum* culture filtrate on the production of specific transcripts including translation elongation factor 1a (*EF-1a*), adenosine kinase (*ADK*), retrotransposon-like homologs and genes of unknown function. We describe the genotype and treatment-specificity of transcript accumulation over time and consider the potential implications on the host cell response to trichothecenes and trichothecene-producing *Fusaria*.

INVESTIGATING FHB QTL ASSOCIATED WITH THE *VRS1* (ROW-TYPE) LOCUS ON CHROMOSOME 2(2H) OF *HORDEUM VULGARE*

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ABSTRACT

Two-rowed barley cultivars generally are more resistant to Fusarium head blight (FHB) infection than six-rowed cultivars. Several studies have found quantitative trait loci (QTLs) for FHB resistance associated with the *Vrs1* or row-type locus. However, none of these studies has conclusively determined whether this resistance is due to a QTL that is tightly linked to the *Vrs1* locus or a pleiotropic effect of the *Vrs1* locus itself. The objective of this study is to determine the relationship between *Vrs1* and FHB resistance to assess the potential for incorporating the FHB resistance QTL into a six-rowed breeding program. A population derived from a cross between Frederickson, a FHB resistant two-rowed cultivar, and Stander a FHB susceptible six-rowed cultivar, was developed by Mesfin et al. (2003), to map FHB resistance QTL. We identified a single plant at the $F_{4:6}$ stage that was heterozygous at the *Vrs1* locus and two other loci toward the telomere. After selfing this line, a homozygous two-rowed plant and a homozygous six-rowed plant were selected to be used as parents for a fine mapping population. A total of 2406 F_2 individuals from a cross between these parents were screened to identify 398 individuals showing recombination within the region of interest. A subset of 125 of these recombinant individuals was selfed to the F_3 generation, and used to create the fine map using JoinMap 3.0. The fine map consists of four molecular and the *Vrs1* morphological marker. This population was evaluated as $F_{3:5}$ lines in an unbalanced randomized design for FHB severity in two locations during the summer of 2004. We conducted simple interval mapping using PLABQTL and detected a single broad LOD peak in both environments. In St. Paul the LOD peak was centered at 0.5 cM distal to *Vrs1* with a LOD score of 8.87 and an R^2 of 30.1. In Crookston the LOD peak was centered 0.3 cM distal *Vrs1* with a LOD score of 10.7 and an R^2 of 35.2. A 1 LOD drop-off confidence interval for the position of the QTL includes *Vrs1*; therefore we cannot conclusively answer the question of pleiotropy vs. linkage. Currently, the population is awaiting analysis in China, and a second year of data with more replications in St. Paul and Crookston. New recombinant individuals with highly informative genotypes are currently under development to more accurately dissect this problem.

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PEDIGREE ANALYSIS OF JAPANESE WHEAT IMPROVED LINES FOR FHB RESISTANCE USING SSR MARKERS

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ABSTRACT

In western Japan, FHB affected 20 to 90% and 10 to 25% of wheat production areas in an average year in the 1960's and 1990's, respectively. Historically, outbreaks of FHB occurred in 1963 and 1998 in Japan. FHB struck almost all areas in Japan, covering more than 400,000 hectares (71.5% of wheat production), and estimated yield losses reached 53.7% in 1963. This outbreak of FHB devastated the production of wheat and barley in western Japan. FHB has occurred in epidemics every three to six years up to now. Farmers in western Japan, particularly in the Kyushu district, which is the main production regions of facultative wheat in Japan, experienced serious epidemics of FHB in 1998, with damage equivalent to 20 to a 100% decrease in wheat production. In the northern part of Japan, Hokkaido district where they are producing both winter and spring wheat, FHB has caused significant losses in yield and quality as a mortal threat to the wheat and barley production since 1990's. We have been missing any winter wheat materials with high level resistance to FHB for Hokkaido's production. The national breeding system of wheat and barley has been conducting active improvement and research for FHB resistance in Tokai-Kinki National Agricultural Experiment Station since 1952 to 1972, and in Kyushu National Agricultural since 1972. They screened several FHB resistant germplasms, then released cultivars and advance lines of 'Norin', 'Tokai', and 'Saikai' series. Repeated screening of genetic resources also led to the identification of several local varieties and cultivars of spring wheatl, such as Nobeokabouzu-komugi, Nyubai and Shinchunaga from the Japanese gene pool. Conversion of FHB resistance from a spring wheat Saikai 165 developed in Kyushu to winter ones through their crosses is progressing the resistance level of winter wheat for Hokkaido's production in this decade.

The genetic constitution and diversity for FHB resistant among Japanese germplasms and improved lines, however, have not yet been elucidated. It is essential to identify them along with their breeding history, so that different genes can be combined and to improve the overall resistance of wheat. We analyzed transition of chromosomal regions for FHB-resistance QTLs using SSR haplotype in Japanese Wheat Breeding Systems. We discuss genetic diversity for resistance among Japanese improved wheat and trend of QTL transition since 1960s. It shows strategic systems for future FHB breeding.

USE OF *IN VITRO* SELECTION FOR IMPROVEMENT OF FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

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ABSTRACT

Fusarium head blight (FHB) in barley is incited by *Fusarium graminearum* Schwab, which produces mycotoxins such as deoxynivalenol (DON) during pathogenesis. An *in vitro* selection study was conducted using DON, or a mixture of DON with other mycotoxins, in the anther culture system for doubled haploid production. Several barley genotypes with varying levels of resistance (3 six-row and 7 two-row) were subjected to *in vitro* selection (IVS) with the aim to select mycotoxin tolerant barley plants with enhanced FHB resistance. All IVS doubled haploid lines were evaluated for FHB resistance over 3 years (2001-2003) at Brandon, MB, in the FHB nursery inoculated with 3 isolates of *F. graminearum* using the grain spawn method, while the IVS lines produced from MNBrite were evaluated over 2 years (2002-2003). DON content was determined by the ELISA method at Ottawa, ON. Among the 10 genotypes subjected to IVS, the IVS lines produced from MNBrite and CDC Kendall showed statistically significant lower levels of DON compared with the control genotypes over 2 years and 3 years, respectively. Overall reduction in DON level was 20% for IVS lines produced from MNBrite and 27% for those from CDC Kendall. Although not statistically significant, 2 IVS lines from each of the six-row genotypes Excel and Robust (Excel 99/12-3, Excel 99/12-4, RobustDT00-6 and RobustDT00-14) showed a reduction in DON content ranging from 10 to 18%. Four IVS lines from the two-row genotype Rivers (TR256DT002-1, TR256DT0016-2, TR256DT0016-4 and TR256DT0020-3) also showed consistently lower DON content in all 3 years. When evaluated in yield trials, the agronomic performance and malting quality of the IVS lines were generally similar to the genotype for which they were developed. Thus, IVS was effective in reducing DON content and improving FHB resistance in some genotypes. The IVS lines will be evaluated further at multiple locations to confirm these findings.

EXPRESSION OF DEFENSE-RELATED WHEAT GENES DURING EARLY WHEAT-*F. GRAMINEARUM* INTERACTIONS

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ABSTRACT

Pathogen infection process may trigger the expression of an array of defense-related genes in a host plant. Differential expression of defense-related genes in different host genotypes may result in the difference in host responses to the pathogen infection. To gain more insight on the defense response of wheat to infection by *F. graminearum*, changes in gene expression in response to the fungal stress were monitored using cDNA microarrays. Microarrays containing 2306 ESTs generated from six suppression subtractive hybridization libraries were probed with cDNAs from fungal-inoculated Ning 7840 (resistant) and fungal-inoculated Clark (susceptible). The abundance of fungal-induced transcripts in the two varieties was determined by direct comparison of gene expression levels of the fungal-inoculated resistant and susceptible varieties at various time points after inoculation. Results show that up-regulation of defense-related genes can occur early at 3 hours after inoculation (hai) in Ning 7840, while mostly occur at 12 hai or later in Clark. The transcripts levels of ESTs having significant sequence similarity with cadinene synthase, proteinase inhibitors, PR-1 type pathogenesis related protein and chitinase precursor were higher in Ning 7840 than in Clark at 3 hai but lower in Ning 7840 than in Clark at later time points, indicating a slower defense response in the FHB-susceptible variety compared to the resistant variety. A selected set of differentially expressed genes from the microarray experiment was validated with real-time PCR (RT-PCR).

IDENTIFICATION OF FHB QTL COINCIDENT WITH COMPONENTS OF PARTIAL *FUSARIUM* RESISTANCE DETECTED USING A DETACHED LEAF ASSAY

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ABSTRACT

The detached leaf assay and Fusarium head blight (FHB) have been studied to see if common components of partial disease resistance (PDR) could be identified. A double haploid wheat population (91 lines from the Winter x Spring wheat cross 'Arina' x 'NK93604') was phenotyped and mapped to understand and characterise the genetic basis of the PDR components and their relationship to FHB. The map construction using SSR, AFLP and DarT markers is still in progress and identification of linkage groups is still tentative (see poster by Semagn et al.). Fusarium resistance has been assayed in detached leaves (latent and incubation period), and in the field for 3 years. Relationships have been analyzed by interval mapping (IM) and the principal component analysis derived method Partial Least Squares Regression (PLSR). In general, the latter method identified more QTL than IM and with higher (adjusted or calibrated) R^2 values. For *incubation period* IM identified 2 QTL, close to markers Dup004 (4A) and gwm 161 (3DS), explaining 19.4%. For *latent period* only one QTL was identified, gwm698 (7AL, 12.1%). Both were derived from the 'Arina' parent, contributing to longer incubation/latent periods. Using PLSR the same were identified, in addition to others from both parents, with ca. 26% R^2. Interestingly a QTL in 'Arina' was identified in both traits close to markers gwm389 and barc75, which both map to the 3BS region known from 'Sumai-3'. However, since the two traits were only correlated weakly; the QTL only explained a part of the variance. Molecular markers for FHB resistance were generally not coincident with those for incubation and latent periods, indicating that PDR components and FHB were largely under separate genetic control. However, PLSR analysis of DON also identified a QTL at barc75 (3BS), indicating an overlap with incubation period and latent period.

The present research is an important step in linking and understanding the potential utility of *in vitro* screening, including the detached leaf and seed germination assays, with major QTL for FHB resistance. This is particularly relevant to investigate the dichotomy in the relationship between incubation period and FHB resistance (Browne et al., poster presentation at this conference) that may need to be considered to effectively combine exotic and existing/adapted sources of FHB resistance.

EVIDENCE FOR MATERNAL EFFECTS IN THE INHERITANCE OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN THE WHEAT CULTIVAR HEYNE

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ABSTRACT

Fusarium head blight (FHB) is a serious disease of wheat that is best controlled by host resistance. In the eastern quarter of Kansas, farmer aversion to FHB has been a major contributing factor to the observed two-thirds decline in wheat acreage since 1980 in that part of the state. Additionally, in the large wheat-producing area in the central part of Kansas, there are increasing amounts of corn being produced and reduced tillage is gaining popularity. Both of these practices may increase the risk of FHB epidemics in this important wheat-growing area. As a result, the Kansas breeding programs began in 1998 to focus on producing cultivars with improved levels of resistance to FHB. Part of that program was to acquire the expertise to phenotype the reaction of breeding lines and common commercial wheat cultivars in Kansas to FHB. Data from field evaluation nurseries showed that several cultivars already possessed useful levels of resistance despite the fact that no effort had been directed to selecting FHB resistant cultivars. One cultivar with resistance is Heyne which has averaged about 15% blight compared with a typical susceptible cultivar like Tomahawk which has averaged about 50%. In the quest to develop additional cultivars with resistance to FHB, the initial breeding strategy has been to use resistance already present in hard winter wheat germplasm as the foundation. This strategy was chosen because it provides the least disruption to critical agronomic, quality, and disease characteristics that are necessary for a cultivar to be successful in eastern and central Kansas. The primary sources of resistance used to initiate this effort were Heyne, Hondo, KS89180B-2-1-2, Karl 92, and Lakin, along with some FHB resistant parents from Brazil. Because of the importance of Heyne as a source of resistance in the breeding program, crosses were initiated to determine the inheritance of its resistance and whether it shared genes with other resistant cultivars. An initial experiment indicated that there were differences in F1s when Heyne was used as a female verses when it was used as a male. As a result, additional experiments were conducted. In three of four greenhouse experiments, F1s with Heyne as a female and crossed with a susceptible cultivar (Tomahawk or Trego) had lower ($P<0.05$) blight scores than F1s when it was a male. Values averaged across all four experiments were: susceptible = 70.5% a; Heyne = 16.3% d; susceptible X Heyne F1 = 50.6% b; and Heyne X susceptible F1 = 29.6% c. Therefore, Heyne may have maternal determinants for resistance; however, further research needs to be done to confirm this hypothesis.

ACKNOWLEDGEMENT

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COMPONENTS OF PARTIAL DISEASE RESISTANCE DETECTED USING *IN VITRO* ASSAYS AND THEIR RELATIONSHIP TO FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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OBJECTIVES

To provide an overview on development of *in vitro* detached leaf and seed germination assays to detect components of partial disease resistance against Fusarium head blight.

INTRODUCTION

There is no complete resistance to FHB in wheat although many sources of partial resistance have been identified in a diversity of germplasm sources that frequently differed with respect to the chromosomal regions governing resistance (Liu and Anderson, 2003; Liu et al., 2003; Shen et al., 2003). Partial disease resistance is characterised by a reduced rate of epidemic development in a host population attributed to various components of partial disease resistance including lower infection frequency and longer latent period (period from inoculation to sporulation). At least 17 causal fungal species have been implicated in the Fusarium head blight (FHB) disease complex including *Microdochium nivale*, an important causal agent of FHB in the cooler maritime regions of northwest Europe; however, there is no strong evidence for species-specific resistance (Parry et al., 1995). The mechanisms of resistance to FHB are genetically complex and poorly understood, a situation that has hindered the incorporation of genetically diverse sources of resistance into commercially acceptable cultivars. As a result there has been greater interest in understanding components of partial disease resistance using *in vitro* detached leaf (Diamond and Cooke, 1999; Browne and Cooke, 2004) and seed germination assays (Browne and Cooke, in press).

MATERIALS AND METHODS

This manuscript utilises data from a number of studies which describe details of materials and methods for the detached leaf assay (Browne and Cooke, 2004) and seed germination assay (Browne and Cooke, in press). In summary the detached leaf assays were conducted using detached leaf segments inoculated with *Microdochium nivale*, which causes much more distinct leaf symptoms than other members of the FHB complex. The components of partial disease resistance (PDR) measured were incubation period (days from inoculation to first symptom development, a dull grey-green watersoaked lesion) and latent period (see above). The seed germination assay was conducted by imbibing seed in a conidial suspension of four *Fusarium* spp. and *M. nivale* var. *majus* and var. *nivale* and assessing subsequent germination to detect differences in cultivar resistance (Browne and Cooke, in press).

RESULTS AND DISCUSSION

The detached leaf assay was successful in identifying important components of FHB resistance in European wheat which were most strongly related to the PDR component latent period; longer latent periods were related to greater FHB resistance (a high UK recommended list FHB resistance rating) ($r_s = 0.70$; $P < 0.01$) (Figure 1) and to a lesser extent incubation period ($r_s = 0.53$; $P < 0.05$) (Browne and Cooke, 2004). Molecular mapping of the PDR components incubation period and latent period in a double haploid wheat population in Norway in collaborative research with University College Dublin, Ireland (Poster presentation at this conference; Bjornstad et al.) has identified QTL coincident with incubation and latent periods,

confirming the polygenic nature of both PDR components largely under separate genetic control. While map construction is still in progress results indicate QTL at 5D and 3BS are of importance for incubation period and a QTL at 2D for latent period. Identification of components of FHB resistance among wheat lines entered in the 2002 Uniform Southern Fusarium Head Blight Nursery (USFHBN), representing diverse pedigrees including known Asian sources of resistance to FHB and known US sources of moderate resistance, identifies the potential utility of the detached leaf assay for identifying components of FHB resistance (Poster presentation at this conference). However there was evidence that the effect of the PDR component incubation period on FHB resistance varies with genetic background; this may therefore need to be considered when combining exotic and existing/adapted sources of FHB resistance. Comparative evaluation of PDR components of wheat, barley and oats suggests related Graminaceae could be a potential breeding source for PDR components such as longer latent periods (Figure 2), which were longer in barley than wheat and longer in oats than either wheat or barley.

While the detached leaf assay identified an important component of moderate FHB resistance in European wheat many other resistances to FHB are not identified, including disease avoidance and escape. An example of this may be resistance detected in an *in vitro* seed germination assay significantly correlated with FHB resistance ($r_s = 0.45$; $P < 0.05$) but not correlated to PDR components detected in the detached leaf assay, suggesting a genetically distinct resistance (Browne and Cooke, in press). It is hypothesised that the resistances detected in the germination assay, common across several *Fusarium* species, primarily reflect resistances found in the grain (in the wheat head and developing seedling) (Browne and Cooke, in press).

The present work illustrates the utility of *in vitro* assays such as, but not exclusively, the detached leaf and seed germination assays in expanding our understanding of the genetics of FHB resistance and role of individual components of partial disease resistance in disease epidemic development.

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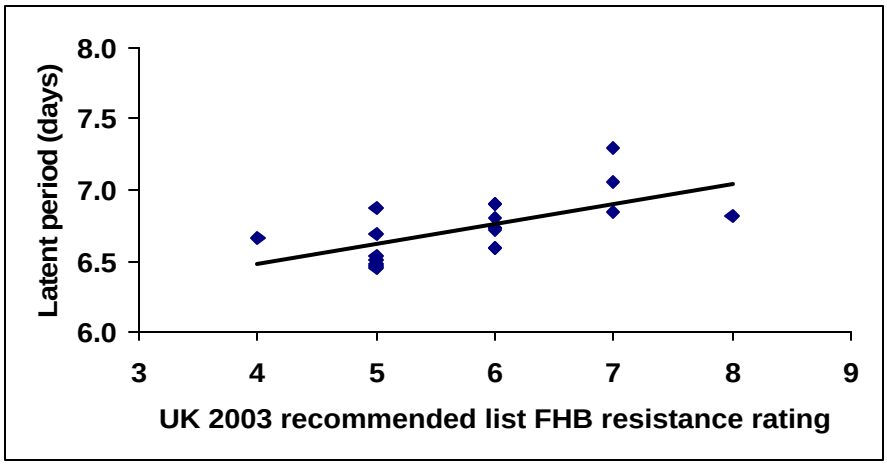

Figure 1. Latent period correlated against FHB resistance ratings for cultivars on the UK 2003 recommended list (r_s = 0.70; P < 0.01) (a higher rating = higher resistance).

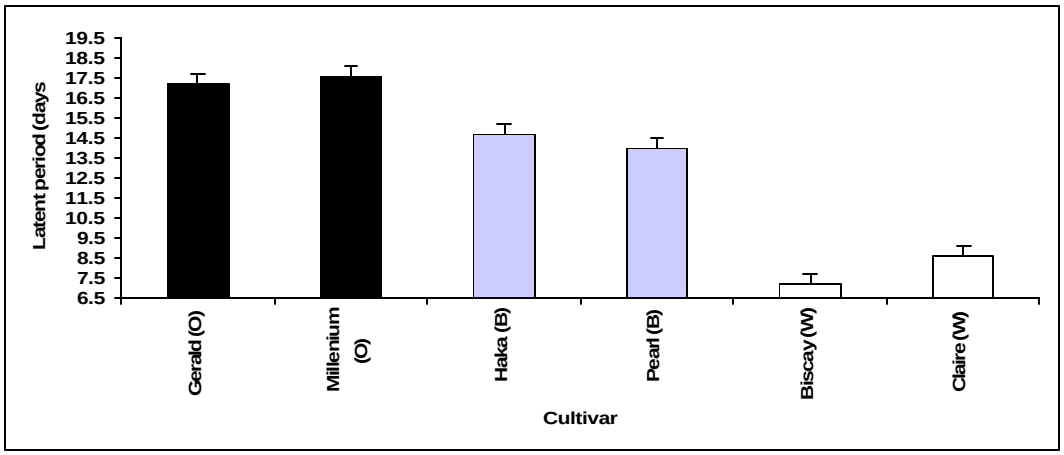

Figure 2. Latent period of oat (O), barley (B) and wheat (W) cultivars inoculated with M. nivale on detached leaves and incubated at 15ºC. Bars represent standard error of the mean.

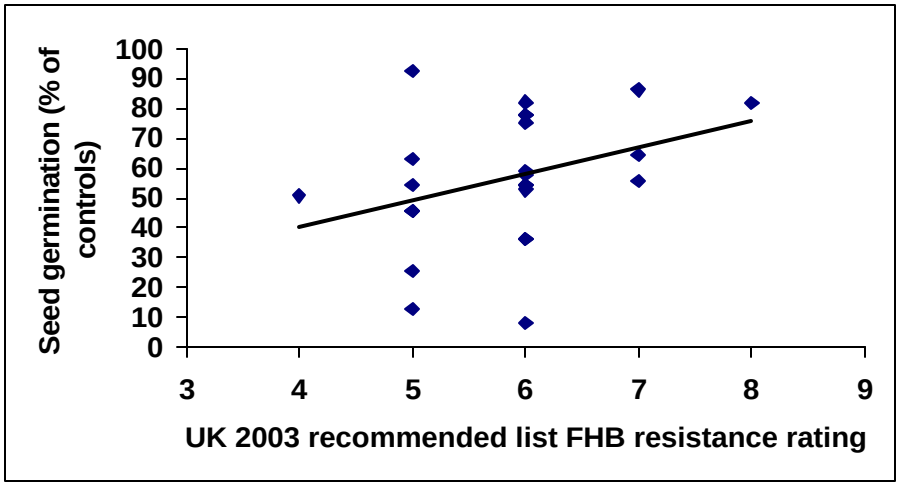

Figure 3. UK 2003 recommended list FHB resistance ratings plotted against germination of seeds expressed as a percentage of untreated controls after inoculation with isolates of M. nivale var. majus (r_s = 0.45; P < 0.05).

COMPONENTS OF PARTIAL DISEASE RESISTANCE IN WHEAT, BARLEY AND OATS DETECTED USING A DETACHED LEAF ASSAY

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ABSTRACT

The detached leaf assay was successful in identification of important components of resistance to FHB in European wheat. However, it is not known how the components of partial disease resistance (PDR) for barley and oats relate to those observed in wheat. In the present work the relative resistances of fifteen winter barley, three winter wheat and three winter oat cultivars on the UK recommended list 2003 and two spring wheat cultivars on the Irish 2003 recommended list were evaluated in detached leaf assays to further understand PDR components and Fusarium head blight (FHB) resistance across cereal species. Barley cultivars showed incubation periods comparable to, and latent periods longer than the most FHB resistant Irish and UK wheat cultivars evaluated. In addition, lesions on barley differed from those on wheat as they were not visibly chlorotic when placed over a light box until sporulation occurred, in contrast to wheat cultivars where chlorosis of the infected area occurred when lesions first developed. The pattern of delayed chlorosis of the infected leaf tissue and longer latent periods indicate that resistances are expressed in barley after incubation period is observed, and that these temporarily arrest the development of mycelium and sporulation. Incubation periods were longer for oats than for barley or wheat cultivars. However, oat cultivars differed from both wheat and barley in that mycelial growth was observed before obvious tissue damage was detected under macroscopic examination, indicating tolerance of infection rather than inhibition of pathogen development, and morphology of sporodochia differed, appearing less well developed and were less abundant. Longer latent periods have previously been related to greater FHB resistance in wheat. The present results suggest the longer latent periods of barley and oat cultivars are likely to play a role in overall FHB resistance if under the same genetic control as PDR components expressed in the head. However the limited range of incubation and latent periods observed within barley cultivars and oat cultivars are in contrast to wheat where incubation and latent periods were shorter and more variable among genotypes. The significance of the various combinations of PDR components detected in the detached leaf assay as components of FHB resistance in each crop requires further investigation, particularly with regard to the apparent tolerance of infection in oats and necrosis in barley after incubation period is observed, associated with retardation of mycelial growth and sporulation.

EVALUATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN US SOFT RED WINTER WHEAT GERMPLASM USING A DETACHED LEAF Assay

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ABSTRACT

A large environmental influence on phenotypic estimates of disease resistance and the complex polygenic nature of Fusarium head blight (FHB) resistance in wheat (*Triticum aestivum* L.) are impediments to developing resistant cultivars. The objective of this research was to investigate the utility of a detached leaf assay, inoculated using isolates of *Microdochium nivale* var. *majus*, to identify components of FHB resistance among thirty entries of United States soft red winter wheat in the 2002 Uniform Southern FHB Nursery (USFHBN). Whole plant FHB resistance of the USFHBN entries was evaluated in replicated, mist-irrigated field trials at 10 locations in eight states during the 2001-02 season. Incubation period (days from inoculation to the first appearance of a dull grey green water soaked lesion) was the only detached leaf variable significantly correlated across all FHB resistance parameters accounting for 45% of the variation in FHB incidence, 27% of FHB severity, 30% of *Fusarium* damaged kernels and 26% of the variation in grain deoxynivalenol (DON) concentration. The results for incubation period contrasted with previous studies of moderately resistant European cultivars in that longer incubation period was correlated with greater FHB susceptibility, but agreed with previous findings for the Chinese cultivar Sumai 3 and CIMMYT germplasm containing diverse sources of FHB resistance. The results support the view that the detached leaf assay method has potential for use to distinguish between specific sources of FHB resistance when combined with data on FHB reaction and pedigree information. For example, entry 28, a di-haploid line from the cross between the moderately resistant US cultivar Roane and the resistant Chinese line W14, exhibited detached leaf parameters that suggested a combination of both sources of FHB resistance. While the USFHBN represents the combination of adapted and exotic germplasm, with the exception of Ernie, the moderately resistant US commercial cultivars (Roane, McCormick, NC-Neuse and Pat) had long incubation and latent periods and short lesion lengths in the detached leaf assay as observed in moderately FHB resistant European cultivars. The dichotomy in the relationship between incubation period and FHB resistance indicates that this may need to be considered to effectively combine exotic and existing/adapted sources of FHB resistance.

RESISTANCE TO *FUSARIUM* SPP. IN A SEED GERMINATION ASSAY AND INVESTIGATIONS INTO ITS RELATIONSHIP WITH FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

Resistance of wheat cultivars from the UK 2003 recommended list were evaluated against *Fusarium culmorum*, *F. graminearum*, *Microdochium nivale* var. *majus* and var. *nivale*, *F. avenaceum* and *F. poae* using a seed germination assay and related to previously published data on detached leaf assays, using *M. nivale*, and UK recommended list *Fusarium* head blight (FHB) resistance ratings. Significant cultivar differences were found after inoculation with all fungal species with the exception of *F. poae* where the decline in the percentage of seed germinating relative to the controls was small. Correlations of the percentage seed germinating inoculated with the remaining *Fusarium* spp. and *M. nivale* were high ($r = 0.68$; $P < 0.01$ to $r = 0.94$; $P < 0.001$). Overall, isolates of *F. graminearum* caused the greatest reduction with a mean seed germination of 61.7 % relative to the controls followed by *F. avenaceum* (65.5 %), *M. nivale* var. *majus* (67.2 %), *F. culmorum* (76.6 %), *M. nivale* var. *nivale* (89.2 %) and was least for *F. poae* (92.5 %). The resistance detected in the germination assay was significantly correlated to whole plant FHB resistance ratings ($r_s = 0.45$; $P < 0.05$) but was not correlated to partial disease resistance (PDR) components detected using the detached leaf assay, namely, incubation period, latent period and lesion length. The results suggest that while resistances detected in the seed germination and detached leaf assays in part share a common genetic basis to FHB resistance, resistances detected in both *in vitro* assays are under separate genetic control. Resistances detected in the seed germination assay had a lower correlation with FHB resistance ratings against *F. culmorum*, indicating they were less effective than those detected by latent period in the detached leaf assay in European wheat cultivars.

GENETIC DIVERSITY AMONG WINTER AND SPRING WHEAT BREEDING LINES WITH VARYING LEVELS OF FHB RESISTANCE

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ABSTRACT

A set of 96 wheat genotypes was analyzed with 96 SSR markers. Markers were chosen based on several criteria: 1) we aimed to use SSRs which are evenly distributed across the genome of wheat. 2) markers which yield a simple and clear amplification pattern were preferred. SSR marker data were used to calculate genetic similarities and cluster analysis (UPGMA) was performed. To complete this analysis, we are now adding SSR markers mapping to published QTL regions: 3BS, 5AS, 6BS and 3AL in order to facilitate a haplotype comparison within the evaluated germplasm.

The 96 wheat genotypes consisted of 78 cultivars and breeding lines supplied by several European wheat breeders: Saatzucht-Donau, Austria; Sejet Plant Breeding, Denmark; Cereal Research non-profit company, Hungary; and Saaten-Union, Germany. In addition 11 winter and 7 spring wheat lines with known response to FHB were included.

With the 96 SSR markers a total number of 647 alleles were detected. Wheat genotypes generally clustered as expected by their origin. Major cluster branches included lines originating from Hungary and breeding lines derived from Hungarian winter wheat by spring wheat crosses. Other large groups were comprised of wheat germplasm originating from north-western Europe and another cluster mainly contained wheats from Germany and Austria. The remaining clusters were very heterogeneous consisting of wheat accessions from Europe and overseas. We included one *Triticum macha* accession, which was most distant from all other wheat lines.

Haplotype analysis of the same set of germplasm is ongoing but no results were ready until submission of this abstract.

ACKNOWLEDGMENTS

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MULTI-LOCATION EVALUATION OF FHB RESISTANCE OF PARENTAL LINES AND, BEST' OFFSPRING DERIVED FROM SEVERAL EUROPEAN WINTER WHEAT MAPPING POPULATIONS

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ABSTRACT

Several QTL mapping studies have either been published or are in progress in different European institutes. So far, no orthogonal comparison of the different resistance sources used is available. We therefore agreed to perform a series of experiments to directly compare the resistance level in the different populations.

In total 56 genotypes were evaluated using spray inoculation at five sites in Europe: Rennes (France), Norwich (UK), Freising (Germany), Changins (Switzerland) and Tulln (Austria).

If not stated otherwise the genotypes were comprised of both parents and the 'best' 5 offspring from the following crosses: Sumai-3/winter-wheat (4 lines), SVP72017/Capo (3 lines) Arina/Forno, Arina/Riband, Dream/Lynx, G16-92/Hussar, Renan/Recital, WEK0609/Hobbit-sib, and four lines as controls: F201-R (obtained from M. Ittu, Romania), Petrus (German cultivar) and 2 lines from a cross Sgv/Nobeokabozu// MM/Sumai-3 (obtained from A. Mesterhazy, Hungary).

At all locations spray inoculations were applied on small plots using local *Fusarium* isolates and inoculation methods. FHB severity was scored in percent infected spikelets per plot at several time points after inoculation. We present here data on FHB severity obtained at the end of the observation period (24-28 dpi, depending on location) from four locations (Rennes, Norwich, Freising, Tulln). The level of FHB severity in the different populations reflected the resistance of the parental lines. Selections from Sumai-3/Capo showed the lowest average disease severity (10% FHB) followed by SVP72017/Capo (14% FHB). The best selections from Arina /Riband had 18 % FHB, from Arina/Frono 22% FHB, and from Renan/Recital 24% FHB. The best line from each of the other crosses (Dream/Lynx, G16-92/Hussar and WEK0609/Hobbit-sib) showed around 30% average disease severity. Control lines Sgv/NB//MM/Sumai-3 showed 14% FHB, Petrus 26% FHB and F201-R 40% FHB, while highly susceptible parents like Lynx and Hobbit-sib had 71% and 82% FHB, respectively.

Results showed that winter wheat lines with a high resistance level could be obtained from crosses involving Sumai-3. However, several cycles of crossing and selection may be needed to develop agronomically adapted cultivars. Selection in crosses involving moderately resistant European winter wheat parents leads to FHB resistant lines, with a higher chance of suitable agronomic performance.

QTLS AND MARKERS FOR FHB RESISTANCE IN WHEAT

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ABSTRACT

In recent years, a number of reports on QTL mapping of Fusarium head blight resistance in wheat have been published. In the meantime on almost all wheat chromosomes putative QTL have been reported. In many earlier studies, populations were derived from highly resistant, non-adapted spring wheat lines crossed with regionally adapted susceptible lines. Resistance to fungal spread on single heads (type 2 resistance) was mostly analysed because it was considered a more heritable trait than 'field resistance'. A few reports also measured disease severity on spray inoculated field plots, which should account for most resistance factors that contribute to reduced disease severity under heavy natural infections.

The major QTL *Qfhs.ndsu-3BS* for type 2 resistance was first identified in Sumai3 and related lines. Surprisingly, other non-related Chinese lines appeared to possess a QTL in this region as well. Whether these lines carry the same or different resistance alleles could not be determined so far. Another QTL for type 2 resistance was placed on 6B by several authors, most further described putative QTL have not been thoroughly validated yet. By spray inoculation, significant QTL were mapped on 3B and 5A derived from Asian sources and on 3A, derived from Frontana. Mapping studies have also been performed using winter wheats as resistance sources, e.g. Arina (Switzerland) and Renan (France), where generally several QTL with moderate to small individual effects and a high QTL x environment interaction were found.

Recently, we were involved in the validation of three spring wheat derived QTL (3B, 5A, 3A) in independent experiments and genetic backgrounds. A large population of 1075 F_1 plants based on a four-way cross was generated: CM82036-derivative/Nandu//Frontana-derivative/Munk. From this population 15 lines in each of 8 marker classes were selected using a three-stage procedure with the SSR markers Gwm389 (3B), Gwm304 (5A) and Gwm720 (3A). All finally selected $F_{3:4}$ plants were homozygous for the above-mentioned marker combinations. They were multiplied once for seed production and the $F_{3:5}$ lines were evaluated for FHB severity using spray inoculations at four locations in Germany in 2004. Comparison of the mean FHB severity of the 8 genotype classes showed that lines with all three resistance alleles combined displayed the lowest average FHB severity (mean FHB severity: 15.8%, range: 9.4-31.6%), and the lines having all susceptible marker alleles were most diseased (mean FHB severity 34.7%, range: 26.4-51.9%). The alleles on chromosomes 3B and 3A from CM82036 and Frontana, respectively, both had a significantly lower effect than the 5A allele from CM82036. The three combinations with each of two QTLs did not differ from each other. They differed, however, significantly from those lines harbouring the 3A or 3B QTL only. We, therefore, consider all three QTL as independently validated.

VARIATION FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN SPRING BARLEY (*HORDEUM VULGARE* L.)

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ABSTRACT

Fusarium head blight (FHB) is a fungal disease of barley and other cereals, causing substantial yield and quality losses, mainly due to the contamination of the harvest with mycotoxins. We evaluated genetic variation for resistance to FHB and its association with other plant characters in diverse barley germplasm in order to identify useful lines for resistance breeding. The 143 barley lines consisted of 88 current European spring barley lines and cultivars, 33 accessions from the genebank at IPK Gatersleben, and 22 lines obtained from North American institutions. We conducted artificially inoculated field experiments with *Fusarium graminearum* Schwabe during two seasons. FHB severity was evaluated by repeated assessment of visual symptoms. On a set of 49 lines the content of the mycotoxin deoxynivalenol (DON) was analyzed.

Variation for FHB severity was quantitative. The lines with lowest FHB severity were 'CIho 4196' and 'PI 566203', both lines were obtained from North American colleagues but originate from China. Also within the European spring barley collection variation for FHB severity was highly significant. The lines with the relatively lowest FHB severity were 'Hellana', 'Pixel', 'Secura' and 'Thuringia'. From the genebank accessions 'Misato Golden' and 'Lubicki' were those with relatively low disease severity. There was a significant negative correlation between plant height and FHB severity (r=-0.55). FHB severity assessed in the field and the amount of deoxynivalenol in the harvested grains were positively correlated (r=0.87). Several lines with a useful level of FHB resistance were found or confirmed and are recommended as crossing partners. A full paper describing all relevant results in detail has been published by Buerstmayr et al. (2004).

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EXAMINING THE RELATIONSHIP OF VISUAL ASSESSMENTS OF FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN BARLEY IN A MULTI-ENVIRONMENT TRIALEXAMINING THE RELATIONSHIP OF VISUAL ASSESSMENTS OF FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN BARLEY IN A MULTI-ENVIRONMENT TRIAL

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ABSTRACT

Inoculated nurseries have been established in Canada and China to screen and evaluate barley (*Hordeum vulgare* L.) for resistance to fusarium head blight (FHB). A collaborative trial was designed to investigate deoxynivalenol (DON) content, visual assessments, and their relationships within and across five diverse locations; Brandon MB, Ottawa ON, Saint-Hyacinthe QC and Charlottetown PE in Canada, and Hangzhou in China. Twenty-five cultivars, selected to represent a range of susceptible and resistant cultivars of two-row and six-row barley, were grown in 2002 and 2003 in replicated plots and, where possible, visually rated for severity of FHB. At maturity, all plots with the exception of Hangzhou 2002, were harvested and sampled for deoxynivalenol (DON). Correlations between DON and visual ratings as well as among locations and years were examined. The 2002 and 2003 Brandon and Ottawa, as well as the 2003 Hangzhou results, indicated that significant correlations ($r>0.6$, $P<0.01$) within locations between visual assessments and DON content are possible. Correlations among visual plot severity ratings and DON were also significant among the five environments ($r>0.4$, $P<0.05$). Biplots were used as a visual summary of the data and proved a useful technique to illustrate the correlation structure. Biplots clearly showed a consensus of the DON and visual assessments from several environments. Biplots also illustrated which environments were sources of genotype by environment interaction. Environmental conditions, escapes and differences in protocol may have contributed to the lack of correlation within and among some of the locations.

ENHANCEMENT OF RESISTANCE TO FHB IN SPRING WHEAT THROUGH PYRAMIDING OF GENES FROM DIFFERENT SOURCES

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ABSTRACT

Fusarium head blight (FHB) is a world-wide problem in wheat production areas. The objective of this study was to enhance spring wheat FHB resistance through pyramiding of genes from two different sources: Sumai 3 and Frontana. Two hundred and ninety four advanced lines (F_7) were derived from three crosses: Ning 894013/Cimmyt 11// BW 264 (L651) and Frontana/Sumai 3//N 94013/Cimmyt 11 (L662) and Frontana / Sumai 3// Ning 894013 / Wuhan 2-37e (L664), using the pedigree method. In addition, one hundred and seventy four doubled haploid lines were obtained from the cross HC 467/AC Superb. After preliminary FHB screening with two replications in the field (2002), twenty-one advanced or homozygous lines were selected based on fusarium symptoms and DON level. These twenty-one lines were further evaluated in 2003 and 2004 for incidence, severity, DON content and yield with four replications in two FHB nurseries: Winnipeg (sprayed with conidial suspension) and Ottawa (spread with infected corn and barley kernels). The cultivars or lines: Quantum, Sumai 3, Roblin and HY 644 were included as checks. The results of 2003 indicated that DON level has a positive correlation with FDK, severity and incidence, with coefficients of 0.77, 0.61 and 0.53, respectively and that yield had a negative correlation with DON level and FDK, with coefficients of -0.60 and -0.76, respectively. The results of 2004 showed a similar trend. A combined analysis of 2003 and 2004 indicated that over 10 lines were significantly more resistant than the resistant check HY644. Lines L662-27-9, L662-43-8, L651-48-9, L651-24-4, HC 1090, HC 933, HC1103, HC 1123 and H12637 were close to Sumai 3 in terms of FHB incidence, severity and/or FDK. These lines also had a better yield than Sumai 3 and HY 644 in 2003. Significant year by entry interactions for incidence, severity and FDK were found in the combined analysis. This suggests that growing environment strongly affects the development of FHB. A preliminary quality test revealed that four lines: L651-15-5, L651-57-1, L651-57-3 and L651-7-2 had better dough strength and higher wet gluten content than resistant checks HY644 and Sumai 3. Some of these lines with better quality and/or higher yield, such as L651-7-2, HC 933 and HC 1123, could be used as parents for development of FHB-resistant cultivars.

ENHANCING FUSARIUM RESISTANCE IN BARLEY IN AN INTERNATIONAL PROGRAM

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ABSTRACT

FHB is an important disease in Latin America and worldwide. The ICARDA/CIMMYT barley breeding program started to research in FHB in the mid 1980's. From 5000 lines tested initially, only 23 formed the basis for resistance for the program. This research effort resulted in the widely grown FHB Chinese 2-row variety 'Gobernadora' (known as Zhenmai-1) in which resistance QTLs were mapped in collaboration with Oregon State University. All the resistance sources developed or introduced and identified in the program were made available to North American researchers, especially after the FHB outbreak in 1993. Data obtained through different locations and years shows that resistance sources identified and advanced lines bred in the program have been confirmed in Canada, China, Brazil, Uruguay, US and Mexico. Molecular-based studies in progress will look at genetic diversity among sources. In addition to the classical program, in collaboration with Busch Agricultural Resources Inc., FHB resistance is being crossed into US commercial barley varieties. Multiple disease resistance lines are now available with special low FHB levels. Preliminary results of protein analysis show that malting quality may be present in an advanced material, what makes encouraging the likelihood of identifying multiple disease resistant germplasm with enhanced malting quality. In the program's continuing studies of resistance mechanisms to FHB, a germplasm pool which combines Type I and Type II resistances is continuously being developed. Although Type II resistance may not be important or needed at the Midwest conditions, it may be crucial in other regions of the world where the period from anthesis to maturity is longer that in the Midwest. In the search for new resistance sources, germplasm from different regions of the world continues to be screened every year. This effort has identified unique sources of resistance that will probably add diversity to the resistance genes involved. New resistant lines from Palestina and Uruguay were identified in 2004. FNC-1, the first commercial variety released in 1969 that small Latin American country, showed enhanced levels of resistance at Toluca and Hangzhou. If results are confirmed, this may be the among the first malting barley commercial cultivars identified with enhanced levels of FHB resistance. A further high scale effort to screen the ICARDA barley germplasm bank looking for new resistance sources has been proposed to the USWBSI.

HAPLOTYPING OF UNIQUE NEAR ISOGENIC LINES FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN COMMON WHEAT

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ABSTRACT

QTL mapping of FHB resistance is rapidly progressing to the stage where selection of specific chromosome fragments containing resistance QTLs can be achieved via haplotype selection based on markers flanking resistance QTLs. This study was conducted to haplotype unique near-isogenic lines for resistance to *Fusarium* head blight. Near-isogenic lines of soft red winter (SRW) wheat (*Triticum aestivum* L.) conferring resistance to FHB derived from Chinese wheat lines W14 and Futai8944 at three quantitative trait loci (QTL) were developed in SRW wheat backgrounds 'Ernie' and 'Roane' via marker-assisted backcrossing. These NILs include all SSR allele combinations for the three QTLs previously identified on chromosomes 2BS, 3BS and 5AL in W14: Six SSR loci on 3BS (Barc75 - Xgwm533A/B - Barc133 - Xgwm493 – Xgwm533C), three SSR loci on 5AL (Barc100 - Barc186 - Xgwm156), and one SSR locus (Barc91) on 2BS. Roane has the same allele as W14 and Futai8944 at Xgwm493 locus, and Ernie has the same alleles as W14 and Futai8944 at Barc75 and Barc133 loci. Haplotypes of ten NILs in the Roane background include: 1) Xgwm 533A/B - Barc133 - Xgwm493 - Xgwm533C; 2) Xgwm 533A/B - Barc133 - Xgwm493; 3) Xgwm 533A - Barc133 - Xgwm493; 4) Xgwm 533B - Barc133 - Xgwm493; 5) Barc133 - Xgwm493 - Xgwm 533C; 6) Barc133 - Xgwm493; 7) Xgwm493 - Xgwm 533C; 8) Xgwm493 - Xgwm 533C and Barc91; 9) Xgwm493 and Barc91 and; 10) Xgwm493 alone. Haplotypes of four NILs in the Ernie background include: 1) Xgwm533A/B - Barc133; 2) Xgwm533A/B - Barc133 and Barc186; 3) Barc133 and Barc186 and; 4) Barc133 alone. The effect of haplotype differences on FHB resistance will be presented and discussed.

UPDATE ON QTL MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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ABSTRACT

Mapping of quantitative trait loci (QTLs) associated with Fusarium head blight (FHB) resistance and application of molecular marker assisted selection (MAS) can be used to accelerate development of FHB resistant wheat (*Triticum aestivum* L.) cultivars and provide a better understanding of mechanisms governing resistance. This study was conducted to identify QTLs in Chinese wheat line W14 and to characterize effects and interaction of these QTLs governing resistance to initial infection (Type I), spread (Type II), DON production, and kernel infection. Two doubled haploid (DH) line populations (DH1=Pioneer 2684 x W14, and DH2=Madison x W14) were evaluated for FHB resistance in two greenhouse experiments and the DH1 population also was evaluated in one field experiment. QTL analysis was done using interval, composite, and multiple interval mapping methods.

Two QTLs governing Type II resistance were consistently identified on 3BS and 5AL chromosomal regions in both DH populations evaluated twice in greenhouse experiments and in the DH1 population evaluated once in the field experiment. The QTLs located on chromosomes 3BS and 5AL also were associated with resistance to DON accumulation and kernel infection in the DH1 population on the basis of data from one greenhouse experiment. The 3BS QTL was found to have greater effect than the 5AL QTL, and these two QTLs have additive by additive epistasis towards reducing disease spread, DON accumulation and kernel infection in the greenhouse experiments. The cumulative effects and interaction of these two QTLs explained 53%, 51% and 48% of the total phenotypic variation for disease spread, DON accumulation, and kernel infection in the DH1 population, respectively. These QTLs also explained 56% of the total phenotypic variation for disease spread in the DH2 population.

In the field experiment, three QTLs having association with Type I and Type II resistance were identified in the DH1 population. Two of the QTLs were the same as those, 3BS and 5AL, identified in greenhouse experiments. The other QTL was a minor one located on chromosome 7AL. The 5AL QTL had a greater effect than the 3BS QTL in the field experiment, especially on Type I resistance. Epistasis among the three QTLs was not significant. The three QTLs cumulatively explained 37% and 36% of the total phenotypic variation for disease incidence (Type I resistance) and severity (Type II), respectively. Results from this study indicate that pyramiding 3BS and 5AL QTLs will greatly facilitate development of cultivars having more effective FHB resistance overall via reduction of initial infection, FHB spread, DON production, and kernel infection. Haplotype selection is an ideal strategy for implementing MAS of these two QTLs.

DEVELOPMENT AND UTILIZATION OF ALIEN TRANSLOCATION LINES FOR WHEAT SCAB RESISTANCE IMPROVEMENT

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OBJECTIVE

Develop, identify and utilise alien translocation lines with scab resistance.

INTRODUCTION

Scab resistance is considered to be controlled by two to three major genes and several minor genes. Recombination and convergence of different resistant components have been successfully used for scab-resistance breeding. Pedigree analysis of recent varieties indicated that genetic resources of scab-resistance were limited to only a few varieties, such as Sumai 3, Frontana and their derivatives. To broaden the genetic diversity, relatives of wheat were evaluated for FHB resistance. *Leymus racemosus* (=*Elymus gigantus* L.), *Roegneria kamoji* and *Roegneria ciliaris* were identified with high scab resistance. Three wheat-*L.racemosus* disomic addition lines, DALr.2, DALr.7 and DALr.14, one wheat-*R.kamoji* disomic addition line DA1Rk and one wheat-*R.ciliaris* disomic addition line DA2Sc#1 with good scab resistance were selected by resistance tracing and molecular cytogenetic analysis (Chen et al, 1995, Wang et al, 1999 and Wang et al, 2001). To reduce unnecessary linkage drug of alien chromatin and utilize them more effectively in conventional wheat breeding, attempt was made for the development of translocation lines between *L.racemosus*, *R.kamoji* and wheat using irradiation, genetic control system and gametocidal chromosome effect (Liu et al, 1999, 2000, Yuan et al,2003). In this paper, we will summarily report the development and characterization of wheat-*L.racemosus* and wheat-*R.kamoji* translocation lines and discuss their utilization in wheat scab resistance improvement.

MATERIALS AND METHODS

Materials *Triticum aestivum-L. racemosus* disomic addition lines DALr.2, DALr.7 and DALr.14, *T.aestivum- R.kamoji* disomic addition line DA1Rk were developed and provided by Cytogenetics Institute, Nanjing Agricultural University. *T.aestivum-Aegilops cylyndrica* disomic addition line DA2C was kindly provided by Dr. Endo, Kyoto University, Japan.

Induce translocation by irradiation treatment. Plants at meiosis stage or spikes before flowering of wheat-*L. racemosus* monosomic addition lines with scab resistance were irradiated by Co^{6} ?-ray 500R-1125R (75-100R/Min). Irradiated plants were self-fertilized or used as pollen to cross with a susceptible variety. C-banding and genomic *in situ* hybridization (GISH) were used to identify chromosome translocations among their progenies. Irradiation treatments were kindly made by Jiangsu Academy of Agricultural Sciences.

Induce translocation by gametocidal chromosome effect. Wheat-*L. racemosus* addition lines with scab resistance were crossed to wheat-*Ae. cylindrica* DA2C. Hybrid F_1 was crossed to common wheat cv. Chinese Spring (CS), and plants without chromosome 2C were identified by chromosome C-banding and self-pollinated. The progenies were used to identify translocations and deletions by C-banding and GISH.

Cytogenetic and molecular analysis. Chromosome C-banding and GISH was as described by Gill

et al (1991) and Mukai and Gill (1991). RFLP analysis was as described by Sharp (1988).

Evaluation of Fusarium Head Blight Scab resistance was primarily screened under natural or artificial severe epidemic conditions. Single-floret inoculation method was used for further identification of scab resistance using mixed conidiospore suspension of four *Fusarium graminearum* isolates with high aggressiveness.

RESULTS AND DISCUSSION

Development of translocation lines by irradiation

Irradiation of spikes

The spikes of wheat-*L.racemosus* monosomic addition lines Lr.2 and Lr.14 were irradiated at early flowering stage and the irradiated pollens were pollinated to spikes emasculated of susceptible variety. Two translocation lines were identified by chromosome C-banding and GISH. NAU601 was composed of a complete 4BS and two fifths of 4BL and a short arm of chromosome Lr.2, and designated as T4BS·4BL-Lr.2S. NAU624 was involved a half of Lr.14, part of 6BS and whole 6BL, and designated as T6BL·6BS-Lr.14L.

Wheat-*R.kamoji* addition line DA1Rk#1 with scab resistance was used to induce translocation by irradiating spikes at early flowering stage by ?-ray and the irradiated pollens were pollinated to spikes emasculated of variety Yangmai 9. One line with a pair of translocation chromosomes, which consists of 1Rk#1 short arm and a wheat chromosome segment, was identified by C-banding and GISH.

Irradiation of plants

Lines NAU611 and NAU 618 were selected from the progenies of MALr.7 irradiated at meiosis. C-banding and GISH showed that the translocation chromosome in NAU 611 was consisted of 4AL and a whole arm of Lr.7. It was designed as T4AL·Lr7S (L). The translocation chromosome in NAU 618 was consisted of five sixths (5/6) of Lr.7 (whole Lr.7S and a part of Lr.7L) and one thirds (1/3) of 1AS, the break point of translocation chromosome was located at 1/3 of the short arm. NAU618 was designated as T1AS-Lr.7.

Line NAU621 was selected from M_2 of (Ph^I/DALr.14) F_1 irradiated by -ray. Chromosome C-banding showed that a pair of chromosome consisted of 5BL and Lr.14L. GISH indicated the break point of translocation was near the centromere. This translocation line was then designated as T5BL·Lr.14L.

Irradiation is a useful method to induce chromosome breakage and develop chromosome structural aberration including translocation and deletion. In our study, the frequencies of translocation caused by irradiation of adult plants at meiosis stage or spikes before pollinating were much higher than that by irradiation of dry seeds. Monosomic addition lines were irradiated and used as male parents to cross to a susceptible variety. Theoretically, male gametes (n+1) with a whole alien chromosome without translocation were much easier to be lost during fertilizing competition. To reduce cytological works, only those plants in M_1 with 2n=42 and good scab resistance were further analysed by C-banding and GISH analysis to identify translocations.

Induce translocation by gametocidal chromosome effect

Line NAU 617, NAU631 and NAU 635 were identified from BC_1F_2 of DALr.2/DA2C//C.S. C-banding and GISH analysis showed a pair of translocation chromosomes T6AL·Lr.2S, T1DL·Lr.2S and T1BL·Lr.2S involved in NAU 617, NAU 631 and NAU 635 respectively.

Line NAU632, NAU633 and NAU634 was identified from BC_1F_2 of DALr.7/DA2C//C.S. A pair of chromosomes consisted of an arm of Lr.7 and 3BL (T3BL·Lr.7S) was observed in NAU632. Translocation of T1DS·Lr7 and T4AL·4AS-Lr.7S was identified by C-banding and GISH in NAU633 and NAU 634 respectively.

Gametocidal chromosomes can induce chromosome breakage and refusion in the gametes without this chromosome (Endo, 1988). The breakage occurs in both alien and wheat chromosomes, and resulting of many unnecessary chromosome aberrations. These materials are unstable cytologically, and need identification in successive generation.

Chromosome pairing analysis by test-cross of translocation lines with double ditelosomics of Chinese Spring

To confirm the constitution of the translocation chromosomes, translocation lines were crossed to Chinese Spring ditelosomics or double ditelosomics. Chromosome pairing involved translocation chromosome and telocentric chromosome were analysed by C-banding at MI of PMC in TC_1.

NAU 601 was primary identified as T4BS·4BL-Lr.2S. This line was crossed to Chinese Spring ditelosomic 4BS. By chromosome C-banding at MI of PMC, a heteromorphic rod bivalent consisted of 4BS and the translocation chromosome was observed in 79% PMC in TC_1. This indicated that chromosome 4B was involved in the translocation.

NAU618 (2n=44) was primary identified as T1AS·Lr.7, and was crossed to Chinese Spring double ditelosomic 1A. After chromosome C-banding at MI of PMC, a heteromorphic rod bivalent consisted of 1AS and the translocation chromosome was observed in 70% PMC in TC_1. Meanwhile, 1AL was observed to pair with a whole 1A from NAU 618. This confirmed that 1AS of wheat was involved in the translocation chromosome.

RFLP analysis

RFLP analysis indicated that chromosome Lr.2 and Lr.14 belonged to wheat homoeologous group 7 and 5, and was designated as DA7Lr#1 and DA 5Lr#1, respectively (Qi et al., 1997). According the C-banding and GISH results, 13 probes on homoeologous group 7 were used for RFLP analysis of the translocation lines NAU 601, NAU615, NAU616 and NAU617, in which 7Lr#1 was involved. The probes on corresponding homoeologous groups 1, 4 and 6 were used to determine the wheat chromosome segments involved.

NAU601 was identified as T4BS·4BL-Lr.2S. RFLP analysis using 13 probes on group 7 and 16 probes on group 4 showed that 7Lr#1S, 4BS and a part of 4BL near the centromere was involved in NAU601. The break point was located between MWG808 and ABG476.1 on 7Lr #1S and between CDO541 and PSR164 on 4BL.

NAU 615 was identified as T4BS·4BL-Lr.2S. The same probes as in the analysis of NAU601 were used in RFLP analysis of NAU615. However, the results showed that specific bands of probes BCD349 and MWG 808 for chromosome 7Lr#1 were absent in line NAU615 and present in line NAU601, indicating the fragment of 7Lr#1S in NAU 601 was shorter than that in NAU 615. The break point of 7Lr#1 in NAU615 was located between CDO595 and BCD349.

NAU 616 was identified as T1BL·Lr.2S. RFLP analysis using 13 probes on homoeologous group 7 indicated that 7Lr#1S was involved in this translocation, and the break point was located between MWG808 and ABG476.1. RFLP analysis using 8 probes on homoeologous group 1 indicated that specific bands of the probes on 1BL were present, while the specific bands of the probes on 1BS were absent. NAU 616 was confirmed as T1BL·Lr.2S.

NAU 617 was identified as T6AL·Lr.2S. RFLP analysis using 13 probes on group 7 and 7 probes on group 6 showed that the specific bands of probes on 7Lr#1S and 6AL were present, while the specific bands of probes ABG476.1, PSR690 on 7Lr#1 and the bands of probes on 6AS were absent. Therefore NAU 616 was confirmed as T6AL·7Lr#1S.

Scab resistance identification

Scab resistance was primarily screened in several progenies under natural or artificial severe epidemic conditions. Single-floret inoculation method was used for further identification of scab resistance. Most of the translocation lines showed higher resistance than susceptible check varieties Mianyang 85-45 and parent Chinese Spring, some translocation lines were similar to resistant check variety Sumai 3, but less than their resistant alien parent *L. racemosus* or *R.kamoji*.

In order to develop the lines with high scab resistance, inter-cross between different translocation lines or between alien translocaton lines and resistant wheat varieties has been used to pyramid the genes from different chromosomes or different species. To improve the agronomic traits, backcrossing with popularized varieties or elite lines with good agronomic traits was conducted. Some lines with both good agronomic traits and high scab resistance have been developed and being used in wheat breeding for scab resistance.

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MICROARRAY ANALYSIS OF SCAB RESISTANT QTL-SPECIFIC GENE EXPRESSION IN BARLEY IN RESPONSE TO *FUSARIUM GRAMINEARUM*

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ABSTRACT

Deoxynivalenol (DON), a mycotoxin produced by *Fusarium graminearum* during infection is a quality-determining factor in harvested barley grain. Our objectives were to identify genes associated with reduced DON accumulation. We used the Barley1 GeneChip to monitor differential regulation of barley genes after inoculation with *F. graminearum* in a near-isogenic line (NIL) pair containing a resistant and susceptible allele for the chromosome 3H DON accumulation QTL. The lines were developed via selfing F_7-derived lines from the Fredrickson x Stander recombinant inbred line mapping population that were heterozygous for the chromosome 3H QTL region. Stander was the donor of the resistance allele for the 3H DON accumulation QTL. DON accumulation in the line carrying the susceptible allele was 11-fold higher than the line carrying the resistant allele. Among the 22,792 transcripts tested on the chip, 70 transcripts showed qualitative differences in transcript patterns between the lines carrying the resistant and susceptible alleles after *Fusarium* inoculation. Genetic association of these 70 genes with the QTL was tested by examining transcript accumulation in another genotype. Twenty of the 70 genes showed allele- specific transcript patterns in the NIL pair. *In silico* comparative mapping of these genes against the rice genome was conducted. Among the 20 genes, five mapped to rice chromosome 1, which is synteneous to barley chromosome 3H. Further study to map all barley genes showing differential transcript patterns between the NIL pair is required to validate their genetic association with the DON reduction 3H QTL in barley.

RESULTS OF TESTING WHEAT FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN THE CZECH REPUBLIC

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OBJECTIVES

This contribution is aimed to provide wheat breeders with information about adopted methodological approaches and obtained results of testing the resistance to FHB and accumulation of mycotoxin DON.

INTRODUCTION

Fusarium head blight (FHB), predominantly caused by *Fusarium graminearum* and *Fusarium culmorum,* belongs to the most damaging diseases of wheat in many parts of the world. Because fungicides, which are now available, cannot guarantee sufficient protection (Mesterházy et al., 2003), breeding for resistance to FHB is of paramount importance. It is encouraging that genetic variation for resistance to head blight was found to be very large and valuable sources of resistance have been detected among both spring and winter wheat cultivars. However, resistance to FHB has different components (Mesterházy, 1995; Wisniewska et al., 2002) and there is a strong influence of environmental conditions on response of wheat to FHB. Evaluation of disease incidence in practice and breeding may be complicated, because multi- environment tests and many characters are needed to fully describe the state. Reliable screening methods are strongly requested by breeders.

It is also necessary in practical breeding to combine resistance to FHB with other desirable characters, such as yield ability, quality, resistance to other stress factors and adaptability. Besides exploitation of highly resistant, but genetically very distant sources, there was given evidence (Ittu et al., 2002) that substantial progress in FHB resistance may be reached through cumulating of resistance genes from different sources that are more adapted to European conditions.

MATERIAL AND METHODS

In RICP Prague-Ruzyn response of different wheat cultivars and lines to artificial infection with *Fusarium culmorum* (*F. graminearum)* is examined since 1992. Several scientific studies have been performed until now, which helped to draw conclusions in this study:

1/ The experiments in 1992-1999 period comprised registered winter wheat cultivars and available sources of FHB resistance. Type I of resistance was evaluated. Attention was mainly paid to different disease severity traits and studies of their interrelatedness (Šíp and Stuchlíková, 1997, Šíp et al., 2002a).

2/ In another experiments lasting three years (1998-2000) at two locations ten winter wheat cultivars with varying level of resistance were subjected to artificial infection (by spraying of inoculum) with two isolates of *F. culmorum* and main aim of this study was to determine effects of genotype, fungus isolate and environmental conditions on the severity of infection and accumulation of DON (Šíp et al., 2002b).

3/ In 2000-2003 period factors that influenced accumulation of DON in grain were studied in winter and spring wheat cultivars after inoculation with four isolates of *Fusarium culmorum* and *Fusarium graminearum*. Three inoculation techniques were compared in these experiments: a/ surface inoculations of selected spikes (type I), b/ surface inocula-

tions of the whole plots (type I) and c/ single floret inoculations (type II)(Šíp et al., 2003).

4/ Since 2002 national tests of resistance to Fusarium head blight are performed each year at four locations of Czech Republic and include appr. 100 winter and spring wheat materials (potential sources of resistance from international cooperation, advanced breeding lines, selected registered cultivars and three check cultivars). Each year new materials are included in tests. Prospective materials with detected resistance to FHB are tested repeatedly in national and international (reduced number of items) trials and examined for resistance to other important diseases of wheat (rusts, powdery mildew, brown leaf spot diseases).

Hill plot design became prevalent in these field tests. In the whole time period highly pathogenic isolate (B) of *Fusarium culmorum* (Šíp et al. 2002b) was used for inoculation. The spore mixture (0.8×10^7 ml^{-1}) was applied with the use of hand sprayer directly onto the plant hill in two terms (first at full flowering and second one week later). Inoculated spikes were then kept for 24 hours in polythene bags. Recently we proceeded to one term application of spore mixture on selected flowering spikes.

Head blight symptoms were evaluated usually in three terms (usually 14, 21 and 28 days after inoculation) on a 1-9 scale, where 1 = 100 %, 3 = 75%, 5 = 50%, 7 = 25%, and 9 = 0% of the spikelets with FHB symptoms. *Fusarium* damaged (scabby) kernels were calculated as percentage by total seed number (%FDK). The content of DON was determined by ELISA on RIDASCREEN^R FAST DON kits from R- Biopharm GmbH, Darmstadt, Germany. Inclusion of uninfected, control variant enabled to evaluate also tolerance to the infection. It was expressed as percent reduction from uninfected, control variant in the traits thousand grain weight and grain weight per spike.

RESULTS AND DISCUSSION

Methodological approaches adopted in national tests of resistance to FHB

Choice of fungus isolate is based on pathogenicity studies and since last year we proceeded to examine frequency and aggressiveness of different *Fusarium* species and pathotypes on the basis of detailed survey on the territory of Czech Republic. The choice of suitable fungus isolate for resistance tests should not only meet the requirement for medium to high aggressiveness (Šíp et al., 2002b), but it should also respect economical importance of fungus species and pathotypes within a species. Surveys on occurrence and economical importance of *Fusarium* pathogens are substantiated by findings that cultivar by isolate interactions cannot be neglected. It is also necessary to take into consideration that in Central Europe *F. graminearum* is becoming the prevalent *Fusarium* species. Until now predominantly highly pathogenic isolate (B) of *F. culmorum* has been used in these types of tests.

Technique of inoculation. One term spraying of inoculum (conidial suspension 0.8×10^7/ml) onto bunches of 10 flowering spikes selected within hill plots (in three replicates) is applied. Main reasons for selection of this technique were: i/ obtaining relatively higher average disease incidence and DON content, ii/ analogy to natural infections and iii/ less laborious technique (Šíp et al., 2003).

Treatment of tested material. To minimize year/location effects on results, it appeared necessary in these conditions to support disease development (when needed) by mist irrigation of plots. Precautions are also necessary to take to minimize contaminations with other diseases (protective belts, fungicide applications).

Evaluated characters. The following characters are considered as decisive in these types of experiments: visual symptom scores, percentage of *Fusarium* damaged kernels and DON (NIV) content. Symptom scoring on 1-9 scale, based on estimates of percentage of infected spikeletes (see above, ad 4), provides initial information about superficial disease spread. Useful information about pathogen colonization in grain can be obtained by determining the percentage of *Fusarium* damaged kernels. This trait was found closely related not only to DON content, but also to other disease severity parameters (symptom scores and reductions of grain yield components)(Sýkorová

et al., 2004). However, it comes from different studies that in spite of existing interrelations DON (NIV) content cannot be replaced by any other character. Data on reduction of grain yield characters (TGW, grain weight per spike) due to infection are highly important from economical point of view, but it is necessary to mention that these characters are valuable when examined in replicated, precisely conducted experiments with sufficient amount of tested material.

Results of testing the resistance and breeding for resistance to FHB

Different experiments described in Material and Methods part showed high year, location and fungus isolate effects on examined characters measuring severity of infection and content of DON. To determine the cultivar resistance to FHB we cannot avoid repeated testing and multi-replication base of trials. Examinations into resistance level of currently grown, registered wheat cultivars generally showed that high resistance to this disease is lacking and deliberate breeding programmes aimed at increasing resistance to FHB are, therefore, necessary. There is a wide choice of highly resistant sources, but many of them are genetically very distant from cultivated wheats. Due to polygenic character of resistance to FHB and necessity to reach desirable performance in many agronomically important characters, the utilization of genetically distant germplasm, has not yet been widely applied in Czech wheat breeding programmes and has not yet been a success. Major accent is laid on utilization of different more adapted wheat cultivars and breeding lines possessing medium resistance. Previous studies (Šíp and Stuchlíková, 1997) showed acceptable resistance in advanced breeding lines SG-U 513 (Table 1) or SG-U 466 (Bona), coming from the cross Brock/ Hana. There is highly advantageous that these two lines possess many other positive breeding characters. Nowadays, especially the resistant cultivars (lines) from neighboring countries (Germany), together with resistant lines of Czech origin (mentioned further) are deliberately used in our breeding programmes. Besides this, resistance to FHB is selected for also in other crosses that do not have resistant parent in their pedigree. Tests of resistance to FHB in breeding programmes of SELGEN a.s. company (Stupice, ÚhYetice) usually start in generation F6 after selection for suitable plant types and grain quality characters (F2-F4) and screening for resistance to yellow rust, brown rust and stem rust (F5). Appr. 800-900 lines are included in field infection tests of resistance to FHB. Tests in selected material are repeated in the following generation. Lines are in F6 also tested for resistance to brown leaf spot diseases (*Septoria tritici, Stagonospora nodorum, Pyrenophora tritici-repentis).*

Table 1 brings average data (2002-2004) on symptomatic reaction, % of *Fusarium* damaged kernels and DON content for three groups of cultivars (lines): 1/ examined resistance sources (1-6), 2/ resistant lines derived from crossing with highly resistant parents (Sumai3 or Nobeoka Bozu – 7-10) and 3/the most resistant lines obtained in breeding programs of SELGEN company (11-14). For making a comparison data on average performance of these characters are available for resistant Sumai 3, moderately susceptible Samanta and susceptible Corso. It is shown that the highest resistance to accumulation of DON, connected with favorable performance in other examined resistance traits, was detected in Sumai 3 and in the line SG-V NB x MM Sum 3 from Szeged, Hungary, that includes Sumai 3 and Nobeoca Bozu in its pedigree. Unfortunately this line has poor agronomic parameters. Another examined Szeged lines derived from the program that exploits resistance of Sumai 3 and Nobeoca Bozu (obtained from Dr. A. Mesterházy), which are agronomically more suitable (above all line 222), however, showed only moderate resistance to FHB. Combining FHB resistance with resistance to other diseases, yielding ability and quality traits undoubtedly represents a serious problem. Until now Czech breeders have succeeded in combining different desirable traits with moderate resistance to FHB, which was recently detected in winter wheat advanced lines of SELGEN company SG-S 1800-01 (Hana/Estica) and SG-U 7029 (Hubertus/ Dnstrjanka). In spring wheat the line SG-U143-4 (Greina / Tinos) was highly resistant and the line SG-U 947-a (NANDU/ 6182-c//NANDU/ BR-1522) moderately resistant to accumulation of DON. These materials are agronomically suitable and their obtaining is another proof that the desirable resistance level

can be reached by cumulating resistant genes from different sources possessing lower or middle resistance to FHB.

Breeders throughout Europe aim at reaching at least moderate level of resistance to FHB, but it is necessary to mention that variation over environments particularly for content of DON was in moderately resistant materials rather high (s = 10.15) compared to e.g. Sumai 3 (s= 1.57) and, therefore, risks to practical growing cannot be entirely avoided.

Present results indicate that level of resistance to FHB may be increased with the use of both above mentioned approaches. Careful selection of crossing partners is without doubt inevitable (Mesterházy, 2002), because it increases probability of finding useful gene recombinations. Programs that utilize recombination of genes from adapted germplasm evidently have time advantage and it may be more easy to reach desirable level of other important characters (especially desirable grain quality). However, to find advantageous recombination of resistance genes, high amount of material per one cross should be included in tests of resistance to FHB.

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Table 1. Average data (2002-2004) on symptomatic reaction (VSS), % of Fusarium damaged kernels (FDK) and DON content from national tests of resistance to Fusarium head blight- best performing materials.

	Cultivar	Origin	VSS (1-9)	FDK (%)	DON (mg/kg)
1	Petrus	DEU	7.0	25.2	17.5
2	Arina	CHE	6.7	22.1	14.3
3	Bizel	FRA	6.7	27.9	16.3
4	Kooperatorka	RUS	7.3	18.1	9.8
5	F 201R	ROM	7.8	19.5	14.5
6	SG-U 513	CZE	6.8	19.6	14.0
7	Szeged 219	HUN	6.3	17.6	12.2
8	Szeged 222	HUN	5.7	32.8	21.3
9	Szeged 231	HUN	6.3	19.6	11.5
10	SG-V NB x MM SUM 3	HUN	7.3	9.2	6.1
11	SG-S 1800-01	CZE	6.0	27.5	12.2
12	SG-U 7029	CZE	6.3	19.3	12.3
13	SG-U947-a (SW)	CZE	5.6	27.3	15.5
14	SG-U143-4 (SW)	CZE	5.0	10.9	5.5
15	Sumai 3 - resistant (SW)	CHN	8.0	3.8	2.5
16	Samanta - moderately susceptible	CZE	5.3	35.3	26.9
17	Corso - susceptible (SW)	DEU	5.0	79.2	66.9

SW=Spring wheat

PROGRESS IN IMPROVEMENT OF *FUSARIUM* RESISTANCE OF DURUM WHEAT

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ABSTRACT

Durum wheat is reported to be more susceptible to Fusarium head blight (FHB) than common wheat. Extensive evaluations of durum germplasm found differences in reaction to FHB but have not identified any accessions with resistance approaching that found in common wheat. Our objective is to move FHB resistance from other wheat relatives into durum wheat, and to exploit the available resistance within durum wheat. The existing variation in FHB resistance within durum may be sufficient to reduce damage under the relatively light and sporadic disease pressure experienced in the major Canadian durum production area. Higher levels of resistance would increase this protection, and perhaps facilitate durum production in the eastern prairies where FHB is a greater risk. Sources of improved resistance include the tetraploid wheats *T. dicoccoides* and *T. carthlicum*, as well as hexaploid common wheat. The extensive research to identify quantitative trait loci (QTL) for resistance in common wheat, and more limited research in *T. dicoccoides*, is being used to facilitate transfer of the resistance to durum. Current work is transferring individual QTL from chromosomes 3AS, 3BS, 4BS, and 5AS into adapted durum. Other work in progress includes mapping of Type II resistance in a *T. carthlicum* X durum population.

PRACTICAL EXPERIENCES OF BREEDING FOR LOWER FUSARIUM HEAD BLIGHT AND DON IN SPRING SIX-ROW MALT BARLEY USING DIFFERENT BREEDING METHODS

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OBJECTIVES

Identify breeding methods suitable for developing spring malting barleys with lower levels of Fusarium head blight (FHB) and Deoxynivalenol (DON) mycotoxin content.

INTRODUCTION

Fusarium head blight (FHB) has become a major limiting factor in the production of spring malt barley in the upper mid-western U.S. and Canada since an initial outbreak of the disease in 1993. Actual yield losses due to the disease are relatively minor when compared to the lost income associated with reduction from malting grade to feed grade as a function of DON levels in excess buying specifications. Anheuser-Busch has a non-detectable DON specification (defined as < 0.5 ppm DON) to qualify for malting grade.

The disease is caused by several *Fusarium* species, particularly *Fusarium graminearum*. Multiple sources of resistance to both the disease itself and the associated mycotoxin production have been reported in barley. However, the known sources of resistance are complex in inheritance (multigenic), highly influenced by environmental factors and frequently tend to be pleiotropically linked to undesirable morphological traits, such as; tall stature, late heading or late maturity and the two-row spike type. The principal source of disease resistance in six-row spring barley is from the Swiss landrace Chevron. The resistance in Chevron has been associated with elevated grain, malt and wort protein that has effectively limited the acceptance by the malting and brewing industry of resistant varieties, like MnBrite, derived from Chevron.

As early as 1995, the Anheuser-Busch barley-breeding program (BARI) started to evaluate six-row experimental spring malting breeding lines for Fusarium mycotoxin (DON) content. The methodology used to measure DON over the years has included ELISA (Neogen™ 10-10 and 5-5 kits), HPLC and GC-Mass Spec. Many of these DON evaluations have been conducted with the kind support of the USW&BSI and the greater cooperation of the barley breeding community at large, which is exemplary in it's free exchange of germplasm and information.

In addition to the relatively large environmental and genotype x environmental variances associated with DON levels, one of the most severe limitations of estimating the amount of DON that can be attributed to genetic effects is the high variance associated with sub-sample error. The difference between advancing a line and rejecting it for further testing is often well within the sub-sample error of including a single highly infected kernel in the sample or not. Coefficients of variation for DON and visual scores are typically well beyond the accepted norms (C.V. < 15) for agricultural trials. Reliable estimates of a genotype's ability to reduce DON content can only be obtained after multiple location years of testing. Even then the lines with the greatest promise often are poor under certain environmental conditions.

Until recently, our program focused mostly on reducing DON as opposed to visual scores of disease severity and incidence. The general lack of strong correlation between disease incidence and severity scores and DON content are suggestive of these being individual traits under different genetic control. Recently, methods have been developed to quantify the amount

of Fusarium mycelium in infected kernels, including real-time PCR and ELISA based procedures. We did preliminary evaluation using the ELISA based method on select samples from the 2003 crop. The method has shown itself to be highly reliable, repeatable and much less variable with consistently low coefficients of variation and more closely correlated with DON estimates than visual scores. *(Hill, et. al. see poster in this symposium).* The combined use of DON and ELISA based Fusarium quantification offers a refreshing new approach to breeding for resistance to FHB.

By 1999, (six years into the most recent outbreak of FHB) we felt we had identified several experimental breeding lines with consistently, albeit only slightly, lower levels of DON when compared to susceptible check cultivars. Examination of the direct parents of these experimental lines did not reveal much of a common background, but when the extended pedigrees were examined a preponderance of one breeding line (B7098) was noted. B7098 was itself derived from a Glenn // Hazen / Azure pedigree, which indirectly extends even farther back to Peatland and Parkland derivatives that are closely related to the resistant landrace Chevron.

The breeding methods used in our program so far have included direct selection of existing experimental breeding lines as listed above. Intercrossing the best lines in a 5 x 18 partial diallel followed by early generation testing and pedigreed selection and more recently the creation of large random mating population between the best breeding lines from our program and other spring malting barley breeding programs in North America. The relative degree of success (or lack thereof) from each of these breeding methods and their practical application in a breeding program will be discussed.

MATERIALS AND METHODS

All crosses were made by hand emasculation of the female plants with pollen from male parents applied 3-4 days after emasculation. Crosses were made either in the greenhouse or for random mating populations in the field in Ft. Collins, CO. F_1 generations were increased in the greenhouse and subsequent generations (F_2 to F_4) were grown in 1.5 m x 3 m plots sown in the field in North Dakota, Minnesota and Brandon, Manitoba, Canada.

Fusarium was established in each generation by one of several methods according to the standard protocols of the individual evaluators. F_1's were inoculated in the greenhouse with conidiospores followed by 24-48 hrs in a mist chamber. Field trials were either exposed to natural levels of inoculum (F_3, BARI Park River, ND and F_3, Harvey, Brandon, MB) or natural inoculum supplanted with Fusarium infected corn kernels (F_2 BARI Park River, ND; F_3, NDSU Osnabrock, ND and $F_3$and F_4 Brandon, MB) or spray inoculated with conidiospores (F_3, University of Minnesota St. Paul, MN). A total of six environments were evaluated over the generations.

Individual evaluators assessed field severity scores according to their own standard protocols. DON and mycelium ELISA samples were prepared by sub-sampling ~5 grams from lightly cleaned grain, except for the F_1 greenhouse test in which the F_2 seed were first passed over a $5/64^{ths}$ slotted screen to separate the thinnest kernels for the DON sample and the plumper kernels to plant the F_2 generation.

To prevent severely infected locations from totally overwhelming the results DON data were normalized relative to the location mean so that each location had an equal impact on the weighted average. Data were analyzed and graphically displayed with JMP Statistical Discovery Software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

As early as 1999 we had identified several experimental breeding lines of six-row spring malting barley that had consistently shown slightly lower levels of DON compared to check cultivars. The reduced DON content was apparently not due to early or late escape as shown in the figure below. Only the top 26 out of several hundred experimental lines and several check cultivars are shown for clarity.

We followed each of these top 26 lines thru subsequent generations of testing with selection based on

our standard criteria for agronomic and malt quality performance. As of 2004 only two of these top 26 experimental lines were still active in the breeding program. Both of these (6B97-9170 and 6B980-9022) advanced as far as AMBA pilot testing. The former line had one favorable year of pilot malt evaluation, but it did not show a significant enough decrease in DON to justify release, and was rejected in the second pilot malting for elevated wort protein. The second line did retain a slight but notable decrease in DON compared to the check cultivar Legacy, but it was rejected for slightly elevated wort protein and lower fine grind extract in its first year of pilot evaluation. Although, none of the initial 26 lines that were identified in 1999 survived to released cultivar status, most have been used extensively in cross combinations. Several of the lines identified in 1999 formed the basis of a 5 x 18 partial diallel crossing design. This material was screened as F_1 plants in the greenhouse and subsequently as F_2 and F_3 populations in the field by our own program as well as several other public breeding programs given access to the germplasm. The weighted mean DON response for the 82 possible combinations of the 5 x 18 partial diallel is listed in the combining ability table below. The mean response across a group of cross combinations provides an estimate of the general combining ability of the male and female parents with respect to DON content. The female parents are sorted from top to bottom in term of low DON across progenies and likewise the males are sorted from left to right for low DON across progenies. Highlighted cells in the table are the cross combinations with the lowest DON contents. It is apparent that most of these highlighted cells sort to the upper left corner of the combining ability table (this indicates a strong effect due to general combining ability). The lowest DON combination was a three-way cross between 6B97-2232 // C99-3012 = (Legacy / 6B97-2245). C99-3012 is not the best for DON, indicating that specific combining ability can also contribute significantly to the inheritance of lower DON.

Fifteen sub-selections were advanced from the cross combination with the lowest DON to replicated yield trials at three locations in 2003. One of the locations (Casselton, ND) had a natural infection of *Fusarium*. We measured DON of all active breeding lines in our program at this location and levels of FH infecting were measured by visual observation of severity and by mycelia quantification with ELISA as shown in figure 2.

Two of the fifteen lines had no visual levels of infection detected. One of the two lines without visual symptoms (6B03-4452 in green squares) also had the lowest level of *fc*mycelia as measured by ELISA and theoretically represented the "best line from the best cross", although several other individual lines at the same stage of testing had lower DON contents at Casselton. 6B03-4452 had acceptable malting quality and high yields across locations. 6B04-4452 was included in the NABSEN evaluation trials in 2004 where it was roughly middle of the pack for DON and ELISA *Fusarium* content, however it did not perform well in an inoculated nursery in Osnabrock, ND in 2004. Not surprisingly, pedigreed breeding methods with crosses between partially resistant parents appears to be better than simple selection, especially in terms of recovering lines with malt quality in an adapted background. The verdict is still out if this will result in a released variety, but judging from the NABSEN results the malt barley breeding community does appear to be making steady (if ponderously slow) improvements.

Most recently, we have initiated a large random mating population approach to FHB / DON. In 2002, we hand intermated 383 different cross combinations between a large set of partially resistant parents from the BARI breeding program with corresponding parents from NDSU, the University of Minnesota and the AAFC program in Brandon, MB. The F_1 plants were increased in the greenhouse and individual F_2 rows were random mated following hand emasculation to heads selected from the F_2 composite to create a BC_1 F_1 population. The BC_1 F_1 was grown in a counter-season nursery in Yuma, AZ and then as a large BC_1F_2 population in Casselton, ND during the 2004 growing season. A random sub-sample of BC_1 F_3 1,000 seeds was screened for FHB mycelia content with ELISA. The upper and lower quartiles (250 each) are currently growing in Single Seed Descent (SSD) in the greenhouse and will be available as individual BC_1 F_4 rows in 2005 to determine if we can skew segregating breeding populations toward resistant types.

The presumption that both FHB and DON content in malting barley are under multigenic controls lends itself to large random mating populations designed to accumulate individual genes for partial resistance. Having the ability to even partially skew populations toward resistance during segregating early generation stages should theoretically result in an improved probability of recovery of resistant types due not only to the skewing itself, but the fact that additional generations of selection over more environments will have occurred prior to selection on individual plants or rows at later stages. Only time will tell the relative degree of success that can be achieved with large random mating populations, and what the malting quality of such lines might be.

Probably some of the best current materials for FHB resistance at the malting barley breeding community's disposal are lines coming from the CIMMYT / ICARDA breeding program in Mexico. About 5 years ago, BARI began to support a cooperative effort with CIMMYT / ICARDA to incorporate resistance / tolerance to a wide range of barley pathogens, including FHB, into adapted malting backgrounds. The ability to achieve intense levels of diverse diseases on large numbers of lines in the Mexican highlands near El Baton permit development of multi pathogen resistant lines on a scale that could never be achieved in the U.S. but difficult to do without also having access to adapted malting lines from the U.S. as a starting point for malting quality.

In conclusion, many different approaches to developing six-row malt barleys that are more resistant to FHB and / or that have lower DON content have been tried. Progress seems to be slow but positive. The best weapon we have in fighting this devastating disease is a barley community that works well together, shares information and germplasm as it becomes available. BARI is proud to be one member of that community and we look forward to continued cooperation with all of you in the future.

ACKNOWLEDGEMENTS

The authors would like to thank, Drs. Richard Horsley, Kevin Smith, Brian Steffenson, Mario Therrien, Bryan Harvey and Eric Lefol for access to their best germplasm and their respective staffs and graduates students for extensive field evaluations of segregating populations. We also thank Drs. Paul Schwartz and Nick Hill respectively for DON and FHB contents.

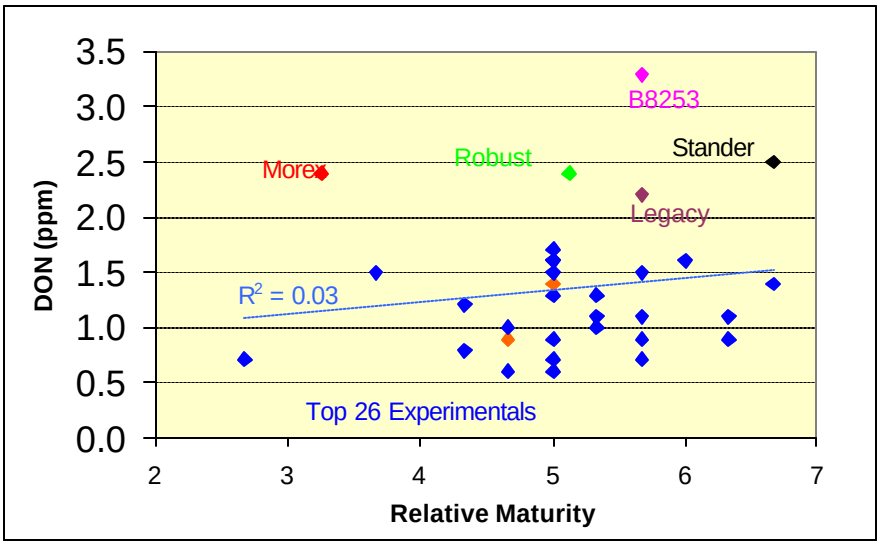

Figure 1. DON vs. Relative Maturity 1999.

Table 1. Combining Ability for DON.

Ave. DON	Male		lowest DON					=selfs											
Female	**6B97-2232**	**C99-3032**	**C99-3013**	**6B97-2037**	**6B98-9032**	**6B97-2195**	**6B97-2245**	**C99-3033**	**6B98-9015**	**6B98-9025**	**6B98-9022**	**C99-3034**	**6B97-2601**	**6B97-2063**	**C99-3012**	**6B98-9438**	**6B97-2311**	**C99-3011**	**Grand Ave.**
6B97-2232	18.3	14.8	18.4	16.6	15.8	16.6	22.0	20.0	19.0	18.1	17.5	17.6	19.9	16.7	12.0	24.0	17.8	34.6	**18.9**
6B98-9438	15.9	19.2	21.4	19.1	14.9	19.1	18.7	23.5	15.4	24.4	18.8	20.7	16.4	22.2	19.9	20.7	17.7	22.0	**19.4**
6B97-2063	15.5	18.0	19.8	20.8	22.2	19.4	15.7	18.8	19.3	18.7	16.1	18.3	25.8	21.0	23.7	22.5	29.4	15.8	**20.0**
6B98-9015	21.8	23.9	14.1	19.8	16.7	19.7	25.7	21.8	20.8	20.9	26.1	25.0	16.0	16.6	27.7	18.5	23.4	24.8	**21.3**
6B98-9025	17.6	17.0	21.2	19.1	27.1	23.3	18.6	16.6	27.7	22.6	28.7	26.4	30.0	32.3	26.5	23.2	30.7	34.4	**24.6**
Grand Ave.	**17.8**	**18.6**	**19.0**	**19.1**	**19.3**	**19.6**	**20.1**	**20.1**	**20.4**	**20.9**	**21.4**	**21.6**	**21.6**	**21.8**	**22.0**	**21.8**	**23.8**	**26.3**	**20.9**

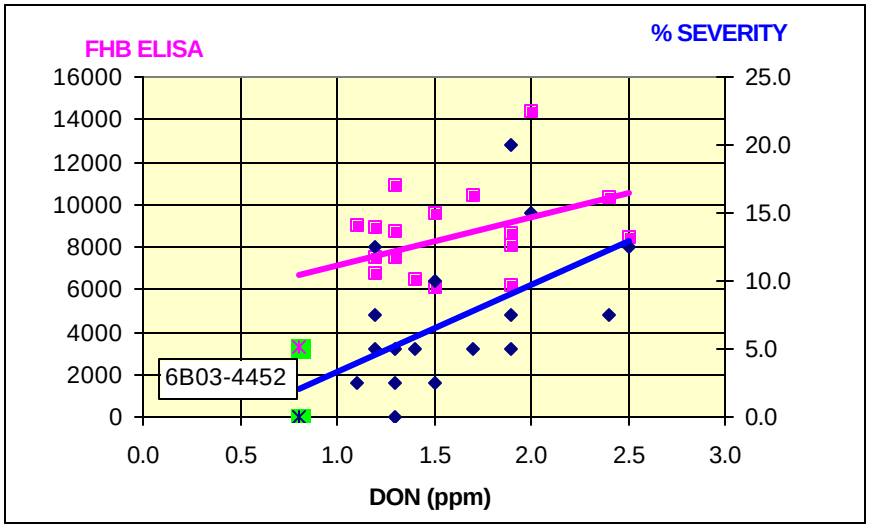

Fig.2. *Fusarium* ELISA and % Severity vs. DON.

SCAB SCREENING OF SOFT RED WINTER WHEAT GENOTYPES IN MARYLAND

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ABSTRACT

The 2003/2004 wheat growing season presented favorable environmental conditions for the development of a scab (*Fusarium graminearum*) epidemic in Western Maryland. Scab damage, however, was not as severe or widespread as it was during the 2002/2003 season although it significantly reduced test weight and in some cases, farmers ploughed under their wheat. A cooperative test of winter wheat advanced lines from three state programs (VA, KY, and MD) was grown under field conditions in Clarksville (MD) and the level of scab severity, percentage of tombstones, and Deoxynivalenol (DON) were assessed as well as heading date, height, test weight, and kernel weight. Fifty-two genotypes were tested and the incidence of the disease was fairly uniform across this nursery. Average test weight (52.1 lbs/Bu) was much lower than that observed (58.1 lbs/Bu) for the same set of genotypes at Queenstown (MD) indicating that test weight was affected by the presence of scab. There were significant genotypic differences for scab incidence and severity. A small group of advanced lines that included the moderately resistant genotype 25R37 showed moderate levels of resistance to scab with low scab severity values. On the other hand, there was a large number of genotypes that were very susceptible although variation in scab severity was large (C.V. = 45%). These lines do not have any of the chinese or other exotic sources of resistance to scab in their pedigree. It is important, however, to continue to screen adapted advanced lines of soft red winter wheat for even moderate scab resistance. This can be useful for future breeding in combination with other major sources of resistance to scab to reach the goal of developing disease-resistant varieties in the near future that are adapted to the mid-Atlantic region of the USA.

IDENTIFICATION OF QTLS ASSOCIATED WITH FHB RESISTANCE IN A ACCA/CHEVRON BARLEY POPULATION OF DOUBLED HAPLOID LINES

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ABSTRACT

The development of barley (*Hordeum vulgare*) cultivars with resistance to *Fusarium* head blight is difficult due to the complex inheritance of FHB resistance. Identification of QTLs for FHB severity and DON level would provide the basis for marker-assisted selection and facilitate the selection of resistant lines. A segregating population of 293 doubled haploid lines developed from a cross between ACCA, a relatively susceptible elite cultivar, and Chevron, classified as "moderately resistant", was tested for FHB severity and DON levels. Doubled haploid lines and their parents were inoculated under field conditions at Laval University (Québec, Canada). Inoculations were done by spraying heads with a conidial suspension of *Fusarium graminearum* strain *Fg*9903. Over five years (2000-2004), a randomized complete block design with three replications was employed. In the field, spikes were visually assessed for FHB infection 15 days after inoculation. In 2002, 2003 and 2004, seed harvested were tested for DON levels using an ELISA test. The same population was genotyped using microsatellite markers developed by the SCRI (Scotland) and chosen to be well distributed over the length of barley genome. A preliminary analysis allowed us to identify one QTL for FHB severity on chromosome 1 (near EBmac0603) that accounts for 29% of the phenotypic variation for disease resistance observed in the segregating population.

ESTIMATION OF FHB RESISTANCE AND DON ACCUMULATION IN FIELD GROWN WHEAT USING GC-MS ANALYSIS

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ABSTRACT

Fusarium fungal diseases of wheat, maize and other cereal crops are on the increase in Europe, North America and China and they pose a serious threat to cereal yield, grain quality and consumer safety (1, 2). Fungal targets for intervention are sought in an attempt to control ear blight disease either within growing cereal crops or on infected crop residues. Both *Fusarium graminearum* and *F. culmorum* are flower and stem base attacking specialists that rarely invade leaf tissue. During infection both fungal species produce water soluble sesquiterpenoid, group 'B' trichothecene mycotoxins. In the USA and NW Europe, the mycotoxins of greatest concern are deoxynivalenol (DON), nivalenol (NIV) and 15-acetyl DON. The target site for DON in plant, microbial and animal eukaryotic cells is the peptidyl transferase catalytic unit in the ribosome. Binding results in the inhibition of protein synthesis (3). Our two main research objectives are (a) to determine which *Fusarium* genes are required to cause disease on cereal ears and (b) to identify the plant and fungal determinants that regulate mycotoxin production during invasive plant growth. Most *TRI* genes encoding for trichothecene mycotoxin biosynthesis are located in a single cluster within the *Fusarium* genome (4). We are carrying out GC-MS analysis to measure DON content in the grain of wheat genotypes selected on the basis of their disease resistance derived from UK field trial data collected over four years. We are also planning to construct a *TRI5:GFP Fusarium* reporter strain to track the onset and spread of mycotoxin contamination during fungal hyphal colonisation of wheat tissue.

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THE FUSARIUM MYCOTOXIN DEOXYNIVALENOL INHIBITS PROGRAMMED CELL DEATH IN *ARABIDOPSIS THALIANA*

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ABSTRACT

Some of the most commercially devastating diseases of crop plants are caused by fungi of the genus *Fusarium*. They not only severely reduce yield but also contaminate grain with a variety of mycotoxins that are poisonous to humans and livestock alike. Two such mycotoxins are; deoxynivalenol (DON – *F. graminearum* and *F. culmorum*) and fumonisin B1 (FB1 – *F. moniliforme*). Both are known to trigger PCD in animal cells. Only FB1 has been shown to cause PCD in plants. Here we investigate the effects of DON treatment on *Arabidopsis* cells. Although DON induces PCD in animal cells, we found that it does not in plants. In fact our studies show that DON actually blocks PCD in *Arabidopsis* cells.

INVESTIGATING THE GENETICS AND MECHANISMS OF FUSARIUM HEAD BLIGHT RESISTANCE IN THE WINTER WHEAT VARIETY ARINA

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ABSTRACT

Resistance to Fusarium head blight (FHB) caused by *Fusarium culmorum*, was evaluated in a doubled haploid population of a cross between the winter wheat varieties Arina and Riband. A molecular marker map was constructed with AFLP and published SSR markers, and was used to identify resistance QTL associated with measured phenotypic traits.

There was high correlation between AUDPC and DON content of grain and more moderately with RSW within years. DON accumulation was highly correlated between years and the measure of AUDPC was found to be a good predictor of the level of FDNA, DON and RSW within each year. The genetic map constructed identified a total of 24 linkage groups covering 976 cM, with all except one linkage group anchored to a known genomic location with SSR markers. QTL for resistance traits were found on 5 different chromosomes of Arina (1B, 2B, 2D, 4D and 6B) and 2 chromosomes of Riband (5BL/7BL and 7D). The FHB resistance controlled by QTL on chromosomes 4D and 6B were stable over all years. However, there were no QTL for FHB resistance that were coincident with those previously reported in Arina. Both phenotypic and genotypic analysis indicated that in Arina there was a strong association between AUDPC, DON and RSW.

Fungal growth, toxin accumulation and expression of a trichothecene biosynthetic gene were analysed in Arina and Riband to elucidate the underlying mechanisms of resistance to FHB. Analysis of DON accumulation and FDNA content indicated that Arina has resistance to initial fungal infection of the grain. DON accumulation was also shown to be lower in the chaff of Arina compared to Riband during early infection. Fungal activity (^{2}-tubulin) and expression of *Tri*5 in the grain and chaff of Arina and Riband was shown to be tissue specific, rather than variety specific, with no evidence for host dependent differential regulation of trichothecene biosynthesis. In Arina and Riband *Tri*5 activity was reduced in the grain compared to the chaff. This is the most comprehensive analysis of spatial and temporal disease progress undertaken to date. It is hoped that the identification of novel resistance to FHB in Arina could be used to improve resistance of winter wheat to this disease.

CHARACTERIZING BARLEY NEAR-ISOGENIC LINES FOR A QTL CONDITIONING DEOXYNIVALENOL ACCUMULATION FOR FUSARIUM HEAD BLIGHT SEVERITY AND DEOXYNIVALENOL ACCUMULATION USING FIVE ISOLATES OF *FUSARUIM GRAMINEARUM* UNDER FIELD CONDITIONS

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ABSTRACT

Two near-isogenic lines (NILs), for a QTL demonstrated to influence deoxynivalenol (DON) accumulation on greenhouse inoculated plants and derived from a cross between the barley cultivars Fredrickson (moderately resistant) and Stander (susceptible), were evaluated under field conditions for accumulation of DON in inoculated spikes. The experiment was designed as a randomized complete block split-plot with three replicates; main plots being near-isogenic line and sub-plots being inoculation treatments. Inoculation treatments included five isolates of *Fusarium graminearum*, inoculated separately, and a water-inoculated control. Inoculum was applied at heading, with two applications of inoculum (1 x10^5 macrocondia ml^{-1}) applied one hour ~~hour~~ apart, using a CO_2 powered backpack sprayer. Inoculated plots were assessed for Fusarium head blight (FHB) incidence and FHB severity at 14 days post-inoculation. Spikes, four per plot, were arbitrarily collected from each plot immediately following the second application of inoculum and at 36, 48, 72, 96, 120 and 240 h post-inoculation. A sub-sample of the grain harvested from plots at maturity was also collected. Harvested spikes were placed in plastic bags and stored at -80 °C until analyzed for trichothecenes. Trichothecenes were extracted from sub-samples taken from the bulked spikes, ground under liquid nitrogen using a mortar and pestle, and also from the harvested grain, ground for 2 min with a Stein Laboratories Mill. Deoxynivalenol and other trichothecenes were extracted using acetonitrile, derivatized as their trimethylsilyl ether derivatives and analyzed using gas chromatography-mass spectrophotometry and selected ion monitoring. The mean FHB severity in inoculated plots 14 days post-inoculation ranged from 1.4% to 32% while non-inoculated controls were all below 1.9%. The average mean FHB severity for the two near-isogenic lines differed slightly across all inoculation treatments. The NIL carrying the Stander allele at the QTL (conditioning lower DON accumulation), had a mean FHB severity of 10.8% while the NIL carrying the Frederickson allele (conditioning higher DON accumulation), averaged 12%. Of the five *F. graminearum* isolates tested, four generated FHB severities averaging between 12% and 22%, and one appeared to be significantly less aggressive with an average FHB severity of 3%. Differences in FHB severities were significant between the NILs for the four more aggressive *F. graminearum* isolates. Deoxynivalenol and other *Fusarium*-produced trichothecenes were not detected in any spike tissues sampled prior to 240 h post-inoculation. At 240 h post-inoculation DON and 15-acetylDON were detected, however levels were low (<~~>~~1 ppm) and no significant differences among treatments were detected. In grain harvested from inoculated plots at maturity, DON ranged from 2.5 ppm to 31 ppm with significant differences evident for DON accumulation among the five isolates tested although not among the NILs examined. In St. Paul in 2004 it was an unusually cool summer, with July temperatures well below average. The cooler temperatures slowed disease development and likely also my-

cotoxin production following inoculation, resulting in the observed lack of trichothecenes in spikes sampled prior to 240 h post-inoculation. Sampling after 240 h but before grain maturity was not conducted, but may have detected differences in DON accumulation between the NILs, as observed in greenhouse studies. The cool weather extended the growing season by almost two weeks and it is likely that the DON detected in mature grain accumulated in late July and early August. We plan to repeat the study examining these NILs in 2005.

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GERMPLASM ENHANCEMENT FOR FHB RESISTANCE IN SPRING WHEAT THROUGH ALIEN INTROGRESSION

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OBJECTIVES

To introgress FHB resistance from wild wheat relatives into bread wheat.

INTRODUCTION

In our epiphytotic nurseries we routinely observe about 20% floret infection on resistant accessions such as Sumai3, Frontana, Nyu Bai etc. The progeny obtained from three and four was crosses involving some of the latter accessions, were slightly superior to the accessions themselves in terms of symptoms and DON content (Cao *et.al.*, 2003) but still showed measurable amounts of infection. In an attempt to enhance the FHB resistant of bread wheat we embarked on a program of screening several wheat-related wild species, to find sources of resistance, then introgress these into wheat germplasm (Fedak *et.al*., 2003 a,b). This report will describe the lines obtained from three such combinations.

MATERIALS AND METHODS

The *Tritium timopheevi* accessions that were screened for FHB resistance were obtained from Dr. Gina Brown Guidera of USDE, ARS Manhattan Kansas while accessions of *T. monococcum* were obtained from Dr. Maxime Trottet of INRA Le Rhen Cedex, France and the *Aegilops speltoides* accessions were obtained from Dr. Maria Zaharieva of INRA Centre de Montpellier in France.

The screening methods involved growing the plant materials in growth rooms, inoculating spikes at 50% anthesis (point and spray) with a 50 000 spore suspension of *F. graminearum*. Plants with inoculated spikes were "misted" for 48 hours and symptoms scored at 21 days. Inoculation was repeated on accessions showing minimal symptoms. Resistant accessions of *T. monococcum* and *Aegilops speltoides* were crossed onto the cultivar Superb and hybrid embryos were cultured on B5 medium. The *T. monococcum* hybrid was backcrossed to the cultivar Fukuhokomugi whereas three backcrosses to Superb were required to restore fertility of the hybrid involving *Ae. speltoides*.

The *T. timopheevi* accession was crossed to the experimental line Crocus, backcrossed once and then 1300 BC1 seeds were advanced to F9 by SSD.

The derived lines were seeded as one meter rows in the FHB nursery, inoculated with corn spawn and irrigated twice a day. Symptoms were scored at 21 days after 50% anthesis. Incidence and severity scores were assigned visually and FDK was determined on threshed samples. Samples of seed were ground and submitted for DON analysis.

RESULTS AND DISCUSSION

The incidence, severity, FHB index and FDK values for the interspecific derivatives and check cultivars are shown in Table 1. The fact that the check varieties performed as expected, from previous experience, indicated that a high level of FHB infection occurred in the 2004 epiphytotic nursery. A total of nine interspecific-derived lines were evaluated and compared to three resistant and two susceptible checks. Sumai3 gave lowest values for all four of the parameters measured. Fukuhokomugi gave a low FHB index but level

of FDK. Nyu Bay had a higher FHB index than Fukuho but a lower FDK value. The *T. monococcum* derivative had a similar FHB index to Sumai3 but a higher level of FDK. The *T. timopheevi* derivative had a FDK level similar to Nyu Bay but a lower FHB index.

Of the seven *Ae. speltoides* derivatives evaluated, all except line 1 had low FHB index values. The FDK levels among the seven lines ranged from 6.3 to 14.7%. Four of the lines had FDK levels numerically lower than Sumai3 and most were lower than Nyu Bay. If the levels of resistance of the interspecific derivatives persist through further testing they will be evaluated for their ability to enhance the levels of resistance in accessions such as Sumai³. Studies are underway to determine if the QTL carried by the interspecific derivatives are different from those derived from resistant wheat accessions.

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Table 1. FHB symptoms in progenies of interspecific crosses with bread wheat

Source of resistance	Generation	Incidence(%)	Severity(%)	FHB index	FDK(%)
Ae. Speltoides					
Line 1	BC3F4	30	25	7.5	11.7
2		15	10	1.5	7.0
3		10	10	1.0	6.3
4		15	10	1.5	14.7
5		15	10	1.5	6.7
6		15	10	1.5	10.0
7		10	10	1.0	7.0
T. monococcum					
Line 1	BC2F4	10	5	0.5	15.3
T. timopheevi					
TC 67	F9-SSD	19.0	11.3	2.1	13.9
Checks					
Sumai3		10	5	0.5	9.0
Nyu Bay		33	16	5.3	13.2
Fukuhokomugi		15	5	0.8	50.0
Roblin		80	80	16.0	90.0
AC Barrie		45	10	4.5	20.3

EVALUATION OF BARLEY IN CHINA FOR FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

Accessions and breeding lines of spring barley (*Hordeum vulgare* L.) must be evaluated multiple times to adequately determine their reactions to Fusarium head blight (FHB), incited commonly by *Fusarium graminearum* Schwabe, and their relative ability to accumulate deoxynivalenol (DON). Inoculated field nurseries were established in Minnesota and North Dakota to assess FHB resistance in locally adapted cultivars and breeding material. Since these studies are designed to evaluate type 1 reactions, material can be tested only once during the year. Winter and wild barley accessions can not be evaluated in the Upper Midwest. Thus, off-season FHB screening nurseries were established in eastern China at Zhejiang University, Hangzhou and at the Shanghai Academy of Agriculture Sciences, Shanghai in 1995. FHB is endemic to this region and no other confounding head diseases were observed. Barley is grown under short-day conditions in eastern China and most accessions flower within a two-week window of time. The FHB screening nurseries near Hangzhou and Shanghai are used for 1) assessment of Chinese and Japanese cultivars, 2) testing of spring, winter, and wild (*H. v.* subsp. *spontaneum*) barley accessions from world collections, 3) collection of data for QTL mapping studies, 4) identifying morphological traits associated with FHB resistance, and 5) evaluation of elite breeding materials. Only the nursery at Hangzhou has been used since 1999. Disadvantages of the Chinese nurseries include transportation costs, plants need to be staked to prevent lodging, and moderate to low correlations between FHB and DON data from China and that from the Upper Midwest. Yet, the data correlations are little better among test sites in the Upper Midwest. The FHB nurseries in China have aided in confirming the FHB resistance of accessions previously reported to have resistance; identifying additional accessions with resistance among winter; wild, and East Asian spring barleys; mapping of major QTLs for FHB resistance to chromosome 2H; verifying strong associations in chromosome 2H between QTL for FHB resistance and genes for spike type, spike length, maturity, and plant height; and selecting breeding lines with low FHB severity scores and DON accumulation. This cooperative research has also provided information about genes controlling photoperiod responses and plant height. The *Eam5* and *eam9* genes for short-day response, the *Eam1* gene for long-day response, and a new semi-dwarfing gene were identified in Chinese cultivars. Continuation of the cooperative nurseries at Hangzhou is anticipated because it speedups FHB evaluations and may lead to sooner release in both countries of malting barley cultivars with FHB resistance.

DEVELOPMENT OF WHEAT LINES NEAR-ISOGENIC FOR DIVERSE SCAB RESISTANCE QTLS FOR COMPARATIVE GENETIC AND GENOMIC ANALYSIS

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ABSTRACT

Extensive efforts have been directed at identifying novel sources of Fusarium head blight (FHB) resistance that may be used to develop new wheat cultivars with enhanced scab resistance. In many instances reportedly unique FHB resistance quantitative trait loci (QTLs) have been mapped to different chromosome locations both in a diverse range of common wheat genotypes and in related species, but have not yet been introgressed into elite U.S. hard red spring wheat (HRSW). To continue improving FHB resistance in HRSW, it is imperative that new FHB resistance QTL from wheat and its relatives be validated or their effectiveness in HRSW backgrounds, that the efficacy of reported markers for unique QTLs be tested, and that validated QTLs be incorporated by breeding programs. Otherwise the fruits of FHB resistance mapping will not be fully realized and future improvements in FHB resistance will slow. In 2001, we initiated a program to use marker-assisted backcrossing to individually introgress five FHB resistance QTL from diverse germplasm sources into three different FHB-susceptible HRSW backgrounds (Norm, Wheaton, Apogee). The initial QTLs selected include two from Sumai 3 (on chromosome arms 3BS and 5AS) to serve as reference QTLs, one from the soft red winter wheat Freedom (chromosome arm 2AS), one from the Brazilian wheat Frontana (chromosome arm 3AL), and one from wild emmer, (chromosome arm 3AS). Our goal is to develop BC_4-derived near-isogenic lines (NILs) that are principally HRSW in genome composition but possess one of the five different QTLs (QTL-NILs). To date we have completed QTL-NIL development for three QTLs in Apogee, and will have equivalent QTL-NILs completed in the Norm and Wheaton in 2005. These lines will be subjected to comparative FHB resistance evaluations to determine which of the new introgressed QTL confer resistance in HRS backgrounds, and what level of resistance each QTL confers. This program is intended to be an ongoing endeavor to incorporate diverse new FHB resistance QTL into HRSW over time as they are identified. The QTL-NILs that harbor new validated FHB resistance QTLs will be available to HRSW breeding programs. Further, there are many additional scientific benefits to be gleaned from these lines. For instance, the QTL-NILs will be used to quantify the effects of gene pyramiding, to examine the molecular basis of host-pathogen interactions, and to explore the biological basis of differences between type I and type II resistance.

MOLECULAR GENETICS OF FUSARIUM HEAD BLIGHT SUSCEPTIBILITY ASSOCIATED WITH CHROMOSOME 2A FROM THE WILD EMMER LINE "ISRAEL A"

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ABSTRACT

The LDN(DIC) set of disomic substitution lines, in which chromosomes of durum wheat cv. Langdon (LDN) are replaced by corresponding ones from the wild emmer line 'Israel A' (DIC), have been useful in locating genes for resistance to Fusarium head blight (FHB). The line LDN(DIC-2A) shows increased FHB susceptibility relative to Langdon, suggesting the presence of a susceptibility factor on chromosome 2A from Israel A. We are interested in understanding the underlying biology associated with this increased FHB susceptibility. In previous studies we obtained evidence that this chromosome not only increases susceptibility, but also appears to suppress the action of a major FHB resistance QTL located on chromosome 3A from Israel A. The gene or genes involved appear to act in an additive fashion. We are now seeking to further dissect the genetics of the FHB susceptibility through molecular mapping. A population of 95 recombinant inbred lines (RICL) from the cross LDN (DIC-2A) x LDN was screened for FHB resistance. DNA from the parents was isolated and screened with microsatellite markers for chromosome 2A to identify polymorphisms that could be used for construction of a molecular map of this chromosome. The FHB disease index of the RICL ranged from 18 to 95, with a mean of 61. The disease index for LDN and LDN (DIC-2A) were approximately 45 and 80, respectively. Molecular markers spanning over 100 cM of chromosome 2A were scored in the RICLs. QTL analysis revealed at least one region of chromosome 2A that was associated with increased susceptibility to FHB. Disease index means for individuals with alternate alleles at the microsatellite marker most strongly associated with FHB susceptibility differed by nearly 17% (71% vs 54%). We are adding additional molecular markers to the chromosome 2A map and are obtaining more FHB resistance data on the RICL population to be able to describe in more detail the molecular genetics of FHB susceptibility associated with this chromosome.

BACKCROSS RECIPROCAL MONOSOMIC ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANCE IN 'FRONTANA' WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

The Brazilian bread wheat cultivar 'Frontana' has resistance to Fusarium Head Blight (FHB), and it is thought to primarily inhibit fungal penetration (Type I resistance) and perhaps degrade the mycotoxin, deoxynivalenol (DON). Its resistance mode of action might be different than that of the commonly used FHB resistant genotype, 'Sumai-3'. We used a set of 'Chris' monosomic (2n-1) chromosome lines in crosses to Frontana (2n) and followed a backcross reciprocal monosomic hybridization scheme to produce disomic lines. Each disomic line is expected to have on average a similar genetic background but critical chromosomes derived from either Frontana or Chris. Our objective was to produce lines which might help to identify the chromosomes involved in the Type I FHB resistance attributable to Frontana. We were successful in producing disomic chromosome lines for all Frontana critical chromosomes except chromosomes 3B, 4A, 5A, and 7D. In 2004, production of the reciprocally derived disomic lines was completed, and lines were evaluated in one greenhouse and two field experiments. Plants in greenhouse and field experiments were spray-inoculated at anthesis with a 25,000 spore per milliliter suspension produced from a single field isolate of *Fusarium graminearum* (Schwabe). There were two replications for each field experiment at Fargo and Prosper, ND, and experiments were planted according to a RCB design with a split-plot arrangement. Whole plots consisted of the critical chromosome and sub-plots were either the chromosome of interest from Frontana or Chris. A RCB design with three replications was employed for the greenhouse experiment. Data were obtained for severity of FHB, incidence of FHB, grain DON-content, and number of tombstone kernels. We will summarize results and data comparisons made based on the greenhouse and field experiments.

DNA MARKERS LINKED TO FLOWER OPENING AND LOW FHB INCIDENCE IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, is an important fungal disease in many wheat growing areas of the world. The objectives of this study were to determine the relationship between width and duration of flower opening and incidence of FHB in wheat; and to identify DNA markers associated with narrow flower opening and low FHB incidence. It was hypothesized that wheat lines whose flowers open briefly and narrowly have a reduced risk of infection. To test the hypothesis, wheat cultivars Patterson and Goldfield were crossed to generate a recombinant inbred (RI) population consisting of 100 F_2-derived lines. Florets of Patterson open wide; florets of Goldfield tend to stay closed. The population of RI lines was characterized for FHB incidence and flower opening width and duration in the $F_{7:9}$ and $F_{7:10}$ generations. The RI population was genotyped with 79 SSR markers. Three markers were found to have significant marker-trait association with low FHB incidence and narrow flower opening. The major QTL effect associated with narrow flower opening and low FHB incidence was found in the map interval Xbarc200–Xgwm210, explaining 29% of the phenotypic variation for FHB incidence over three locations. This adds credence to the hypothesis that narrow flower opening is responsible for low FHB incidence in this population. Breeding wheat lines for both morphological avoidance, such as narrow flower opening, and physiological resistance to FHB may be valuable in future breeding research to reduce crop production and grain quality losses in wheat due to FHB.

QTL MAPPING OF NOVEL RESISTANCE SOURCES IN TETRAPLOID WHEAT

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ABSTRACT

A lot of efforts worldwide have been undertaken to improve Fusarium head blight resistance in durum wheat. However, the resistance level occurring naturally in certain hexaploid wheat lines (e.g. Sumai3) is not reached in durum wheat. In addition, transfer of resistance from bread wheat into durum wheat did not yield highly resistant tetraploid lines yet. Recently tetraploid wheat lines were identified, which could be used for durum improvement. Hence a mapping of tetraploid resistance sources was initiated. Therefore, two moderately FHB resistant *Triticum dicoccoides* lines and one *T. dicoccum* line were crossed with three adapted but susceptible *T. durum* cultivars. To obviate agronomic difficulties and to use the advances of backcross mapping populations (Tanksley et al.,1996) the F_1 was backcrossed once with the recurrent durum parent. Using single seed descent for four to five generations recombinant inbred lines were developed. For genotyping SSRs and AFLPs are used. The polymorphism rate with SSR markers was about 65% in the *T. durum*-by-*T. dicoccoides* cross and about 50% in the *T. durum*-by-*T. dicoccum* cross. To phenotype the lines we use two different artificial inoculation systems: the first is single spikelet inoculation and the second is spray inoculation. Because of the high logging tendency and the partial vernalisation requirement in certain *T. durum*-by-*T. dicoccoides* lines, a field experiment is not feasible. Therefore, we apply the single spikelet method in this population in the greenhouse. For spray inoculation we use a macrospore suspension of 25,000 conidia ml^{-1} for the single spikelet method 500,000 conidia ml^{-1}. The field trials are under irrigation to keep humidity high and optimise the infection. Preliminary results of the first season of resistance testing showed that both evaluated populations segregated significantly for FHB severity. The best lines were almost disease free.

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TRANSFER OF TWO MAJOR QTL FOR FHB RESISTANCE INTO DURUM WHEAT

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ABSTRACT

Improving Fusarium head blight (FHB) resistance in wheat is a major challenge worldwide. Particularly durum wheat is known to be highly susceptible against FHB. As durum wheat is mainly used in direct human consumption, the contamination with *Fusarium* mycotoxins needs strict control.

We tried to transfer the two FHB resistance QTL *Qfhs.ndsu-3BS* (Anderson et al., 2002) and *Qfhs.ifa-5A* (Buerstmayr et al., 2003) derived from the hexaploid cultivar Sumai3 into three regionally adapted durum cultivars. To restore the specific durum traits, the F_1 was continuously backcrossed (up to 6 times) with the recurrent durum parent. In 2003 and 2004 inoculation experiments in the field were carried out. More than 100 lines (BC_4F_3, BC_4F_4, BC_5F_3 and BC_6F_3) were sown in two-row plots in spring 2003. During flowering they were spray inoculated with a *F. graminearum* suspension containing 25,000 macroconidia ml^{-1}. FHB severity was scored 5 times - 10, 14, 18, 22 and 26 days after infection (dai). To evaluate disease severity an area under disease progress curve (AUDPC) was calculated. The best 30 lines showing reduced FHB severity in 2003 were tested again in 2004 using the same procedures as before. Some of the backcross lines showed a reduced disease progress compared to their susceptible durum parents. However, 26 days after infection most of the heads were bleached also on the backcross lines, while the resistant parents did only develop slight FHB symptoms. The results of the introgression of the two QTL on 3B and 5A into durum wheat did not meet our expectations. (I) Only 5 lines out of more then 100 showed an improved resistance level although they contained the Sumai3 QTL. Just one line reached a moderate and proper level of resistance. (II) In later back-cross generations lines tended to loose FHB resistance completely. This fits with the hypothesis that epistatic factors could possibly influence FHB resistance in durum wheat (Stack et al., 2002). The fact that the best lines were derived from the same BC_3 plant also confirms this hypothesis.

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GENETICS OF THE RESISTANCE TO FUSARIUM HEAD BLIGHT IN THE HEXAPLOID WHEAT WANGSHUIBAI: THE ROLE OF EPISTATIC INTERACTIONS

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INTRODUCTION

Fusarium Head Blight (FHB) or scab has become one of the most serious diseases of wheat in North America, and around the world. In North Dakota alone, it is estimated that the impact of FHB on the state economy exceeded 6 billion US$ in the last ten years.

Scab is caused by different species of *Fusarium*. In North America, the predominant species is *Fusarium graminearum*. Infection takes place during anthesis; ideal conditions are warm temperatures (25-30 °C) and high humidity. The disease reduces yield through floret sterility and poor seed filling. Quality losses are also important due to reductions in storage proteins, cellulose, and amylose (Boyacioglu and Hettiachchi, 1995). Additional economical impact is due to the accumulation of a vomitoxin (deoxynivalenol, or DON) in the seed, which makes it unsuitable for human and animal consumption.

Several types of resistance have been described. Three of these types are commonly accepted as Type I, resistance to the initial infection, Type II resistance to the spread of the pathogen through the spike, and Type III, resistance to the accumulation of DON. Type II resistance is most frequently measured and used in breeding programs.

The most predominant source of type II resistance is the Chinese cultivar 'Sumai 3'. Several QTLs for type II resistance have been identified on the genome of this cultivar. A major QTL has been identified on 3BS (Waldron et al, 1999, Anderson et al, 2001, and Del Blanco et al 2001). Additional QTLs have been identified on chromosome 5A (Buerstmayer, et al, 2002), 6B and 6A (Anderson et al, 2001) and 7D (Sneller et al, 2001).

Other sources of type II resistance to FHB have been studied and several QTLs have been identified on chromosome 3A of *Triticum dicoccoides* (Otto et al, 2001), chromosome 2D of 'Wugham', 3BS of 'Maninga' (Somers et al, 2003), and chromosomes 1B, 3A, 3D and 5A of 'Fundulea 201R' (Shen et al, 2003). Other potential sources of resistance to FHB are Wangshuibai (the subject of this paper) from China, citr9445 from China, PI157593 from S. Korea, PI362463 from Yugoslavia, and citr9429 from China.

Previous genetic diversity studies suggested that Wangshuibai (a landrace from the Chinese province of Jiangsu) may have different resistance genes than that in Sumai 3. Bai et al (2003) showed that Wangshuibai is not closely related to Sumai 3. In a more recent study looking at the genetic diversity of the short arm of chromosome 3B, Liu and Anderson (2003) found that Wangshuibai has no alleles in common with Sumai 3 for any of the molecular markers around the QTL found on chromosome 3BS. Given these data and the high level of resistance in this source, it is logical to contemplate the possibility of Wangshuibai carrying different resistance genes than Sumai 3.

In this study our objectives were to 1) identify the chromosomal location of genes responsible for the resistance to FHB of Wangshuibai, 2) estimate the effect of these genes, both in single locus and epistatic models, and 3) identify PCR markers closely linked to these genes, so they can be used in a marker assisted selec-

tion (M.A.S.) breeding scheme to develop resistant cultivars.

MATERIALS AND METHODS

An F_6 derived population consisting of 388 recombinant inbreed lines (RIL) was developed by SSD from the cross between Wangshuibai and ND671 (an elite HRSW line from the NDSU-HRSW breeding program). A random subset of 88 lines was used for a QTL analysis. The remaining lines were used to validate results from the QTL analysis. This validation was done by genotyping the lines with the significant molecular markers found in the QTL analysis.

Phenotyping of these lines was done in 3 greenhouse replicated trials during the years 1999, 2000, and 2001. Lines were grown in 36X21 cm buckets with two rows of five plants per bucket. At the time of anthesis, heads were single point inoculated in a floret in the middle of the spike with 10 µl of a suspension with 50000 spores/ml. After inoculation, the spikes were covered with plastic bags and misted for 3 days to ensure high humidity conditions. Temperature in the greenhouse was kept between 25 and 30°C. Disease scores were taken 14 and 21 days after inoculations. The NDSU variety 'Alsen' (derived from Sumai 3) was used as a resistant check.

Genotyping of these lines was done using 2 sets of SSR PCR primers, GWMs (Röder et al, 1998), and BARCs (http://www.scabusa.org/pdfs/BARC_SSRs_12011101.html). Amplification was done in accordance to those described by the developers of both sets. Amplified products were separated using either 6% acrylamide non denaturing gel, or 6% acrylamide denaturing gel visualized with silver staining. Several STS markers developed by Liu and Anderson (2003) were also included in the linkage map. In addition, several Target Region Amplified Polymorphisms (TRAPs) were developed from wheat EST sequences. In total, the linkage map contains 185 loci across the genome.

The linkage map was constructed using MAPMAKER v 2.0 (Lander et al 1987). The genome was scanned for QTLs using NQTL (Tinker and Mather 1995). Important regions were analyzed for epistatic interactions. Analysis of variance to estimate the effect of epistatic interactions was done with SAS system for Windows v. 8.01 (SAS Institute). Analysis of variance was also used in the validation step.

RESULTS AND DISCUSSION

The population segregated for the spread of FHB both at14 and 21 days after infection (DAI). The range of the scores 14 DAI were between 0% and 15%. Wangshuibai showed an average infection of 3.8%, and the average infection level of ND671 was 7%. Alsen had the same level as Wangshuibai. The range of score at 21 DAI (Fig 1) was between 2.5% and 50%. The average infection in Wangshuibai was 3.8%, no change from the scores at 14 DAI, for ND671, the average was 22 %, a three fold increase from 14 DAI. Alsen had an average infection of 10%, almost a 3-fold increase from 14 DAI.

After scanning the genome for significant QTL peaks, we found a major peak on chromosome 3B, directly over *Xsts138* (Fig 2). This peak explains 17% of the total

phenotypic variance for 14 DAI, and 31% 21 DAI. Other smaller peaks were also found. The importance of these peaks by themselves was very limited with the explained variance never exceeding 3% of the total phenotypic variance. After estimating the effects on single locus and additive models, we scanned the genome for loci interacting with locus *Xsts138(3B)*. The results from this scanning are shown in table 1. However couldn't be validated with the addition of the 300 RIL remaining in the population. This suggests that the epistatic interactions found in the initial subset of lines were probably due to statistical artifacts resulting from the relatively small number of lines in each genotypic class.

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Dr. Franckowiak told me that these are ci (short for cereal introduction) and tr (short for triticum) or ho (short for hordeum). So please make the correction to citr everywhere.

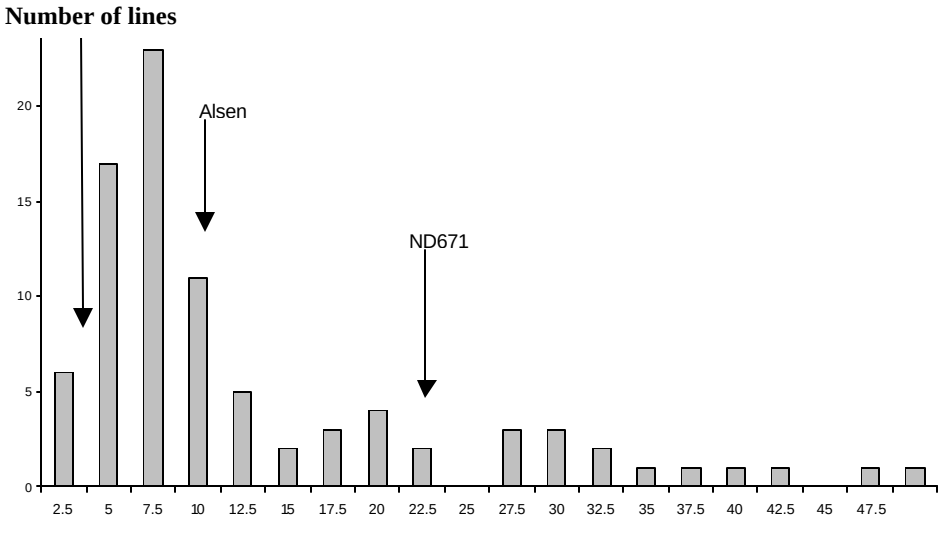

Figure 1. Frequencies histogram for FHB 21 DAI

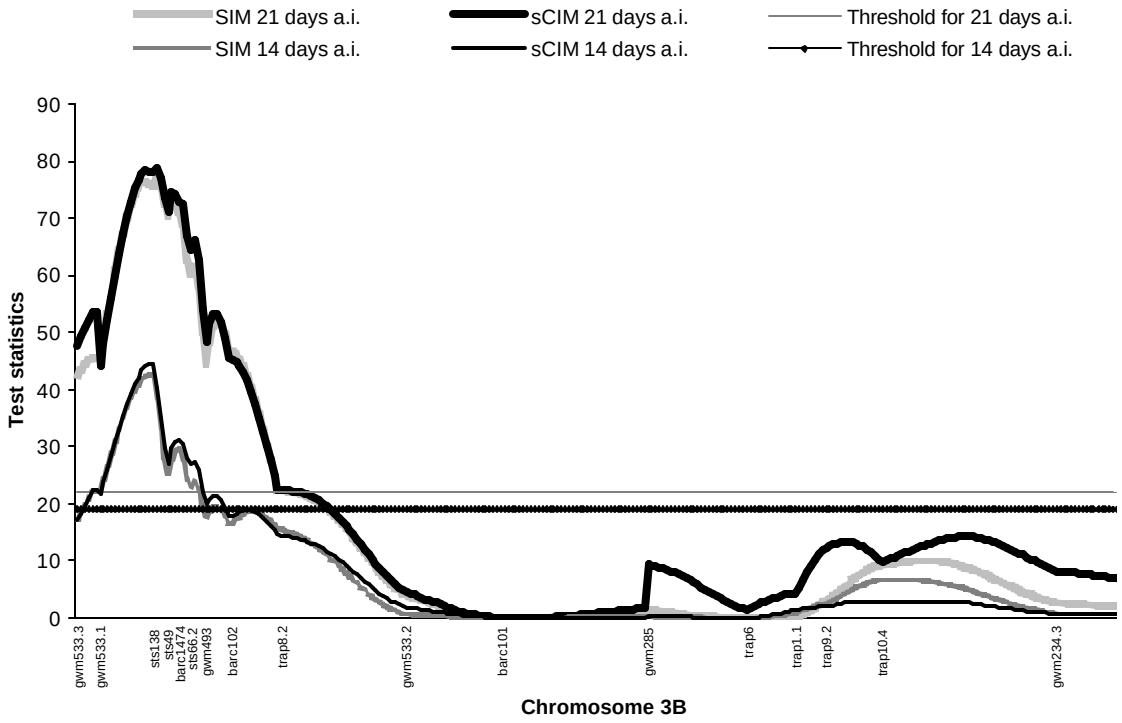

Figure 2. Simple interval mapping (SIM) and simplified composite interval mapping (sCIM) graphs on chromosome 3B for readings 14 and 21 DAI.

Table 1. Loci found to have epistatic interactions with locus *Xsts138*.

Locus	Phenotypic Variance explained (%)	Time of observation (DAI)
Xgwm2 (3A)	67	21
Xgwm333 (7B)	47	21
Xgwm156 (5A)	55	21
Xbarc101 (3B)	51	21
Xtrap9.2 (3B)	59	21
Xbarc1033 (6B)	55	21
Xgwm304 (5A)	53	14
Xbarc117 (5A)	42	14
Xgwm192 (6B)	40	14

MAPPING OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO FUSARIUM HEAD BLIGHT AND *IN VITRO* TOLERANCE TO DEOXYNIVALENOL IN THE WINTER WHEAT CULTIVAR WEK0609®

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ABSTRACT

Fusarium head blight in wheat (FHB) can cause devastating reductions in grain yield and quality and contaminate grain with mycotoxins such as deoxynivalenol (DON). The identification and characterisation of sources of resistance is vital to prevent the emergence of resistant pathogen strains. The objectives of this study were to characterise and map QTL for potentially novel resistance in the FHB resistant American winter wheat cultivar WEK0609 and establish whether germination on medium containing DON (*in vitro* DON tolerance (IVDT)) is associated with QTL for resistance. Symptom development, fungal DNA (FDNA) and deoxynivalenol (DON) content of grain and IVDT was assessed among doubled haploid lines (DHLs) developed from a cross between WEK0609 and the FHB susceptible cultivar Hobbit 'sib'. Major QTL for resistance were detected on chromosomes 3BS, 5A and 1B plus a minor effect QTL on 2D. On 3BS, reduced symptom development was coincident with minor QTL for reduced DON and FDNA levels in grain and increased *in vitro* tolerance to DON. On 5A, reduced symptom development was coincident with a minor QTL for increased RSW, a major QTL for increased IVDT and the awning suppression gene (*1B*). On 1B, reduced symptom development was coincident with a major QTL for increased RSW and minor QTL for reduced DON and FDNA and associated with the 1RS / 1BL rye translocation and the high molecular weight glutenin gene (*Glu-1B*). The contribution to FHB resistance made by QTL on 3B and 5A was confirmed by analysis of inter-varietal chromosome substitution lines (IVCSLs) in which WEK0609 chromosomes were substituted into the Hobbit 'sib' genetic background. Microsatellite allele size analysis indicated that the QTL on 3BS may be allelic with that in the same interval in the Chinese variety Sumai-3 (*Qfhs.nsdu-3BS*) and the QTL on 5A and 1B may be allelic with those that lie in the same intervals in the Romanian cultivar Fundulea F201R (*Qfhs.ifa-5A* and *Qfhs.ics-1B* respectively).

FUSARIUM HEAD BLIGHT RESISTANCE INCORPORATED INTO SOFT RED WINTER WHEAT

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ABSRACT

Fusarium head blight (FHB) epidemics in 1998 and 2003 devastated much of the soft red winter (SRW) wheat (*Triticum aestivum* L.) crop in the mid-Atlantic region. To develop high yielding, scab resistant SRW wheat lines, we have deployed a combination of top-cross, doubled haploid, backcross, and molecular-marker assisted breeding methods. We first verified Type II resistance levels in resistant sources currently used in breeding programs. Additionally, we characterized currently cultivated and adapted SRW wheat genotypes for scab resistance or susceptibility. We found and confirmed high levels of Type II resistance in six wheat lines from China, three from Canada, one from France, and two from Japan. We also identified or confirmed the presence of tolerance to kernel infection, yield loss, and DON production in SRW wheat cultivars or lines. Initially, we developed a doubled haploid line, VA01W-476, which expressed a high level of resistance in both greenhouse and field trials. This line has been used as parent in many breeding programs in the eastern United States. We have also made great progress in the development of FHB resistance lines using top-crossing and backcrossing methods. VA02W-713, a top-cross (Ning7840/Pioneer2691//Roane) derived elite FHB resistant SRW wheat line, ranked 1st in grain yield (77 Bu/Ac) among 54 entries in Virginia's Advance Wheat Test over three locations, and will be evaluated in Virginia's Official Variety Trials in 2005. Type II FHB resistance has been successfully transferred from diverse sources, such as Chinese wheat lines W14, Shaan85, Futai8944, Futai8945, Futai8946, Ning9016, Ning7840, Yumai 7, Er-Mai 9, Wuhan 1, and French line VR95B717, into adapted SRW wheat backgrounds Roane, Ernie, Pioneer 2684, Renwood 3260, Madison, Jackson, and a Sisson sib via backcrossing. Twenty-six SRW wheat lines possessing both high yield potential and scab resistance were selected among 268 lines evaluated in Virginia's 2004 Scab Observation tests. These lines are included in a regional FHB haplotyping project and will be evaluated in 15 states as part of a collaborative research initiative. In addition, a set of near isogenic lines incorporating FHB resistance QTLs from W14 and Futai 8944 into Roane and Ernie backgrounds have been developed using molecular-marker assisted backcross breeding.

STRESS-DIRECTED SELECTION: INDUCING VARIATION IN SPRING WHEAT TO ENHANCE RESISTANCE TO FUSARIUM HEAD BLIGHT AND OTHER DISEASES

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ABSTRACT

We showed earlier that the stresses from repeated cycles of wheat streak mosaic (WSM)or barley yellow dwarf (BYD)-plus-WSM disease pressure appeared to reveal unexpected variation in heritable traits in populations of elite wheat germplasm. This was exploited by co-selecting virus and FHB resistance in early generation progeny of a cross between an FHB-resistant (HY644) and a WSMV-resistant (CO960293-4) parent. Following a protocol of 'Stress-Directed Selection' (SDS), progeny of the cross HY644/CO960293-4 were selected in F_1 and F_2 generations under combined BYD-plus-WSM pressure and 11 WSMV-resistant F_3 lines evaluated in a Fusarium head blight (FHB) field nursery in summer 2002. The F_4 progeny lines of a single F_3 line that combined resistance to FHB and leaf rust were selected indoors under BYD-plus-WSM pressure, followed by severe FHB from spray inoculation of heads at anthesis. The best progeny F_5 plants were again selected indoors under severe BYD-plus-WSM pressure. Following selection of F_6 lines in the summer 2003 FHB nursery and winter increase of the best resulting 49 F_7 head-row lines, F_8 lines were tested in a 2004 FHB field nursery; 8 of these performed better than the resistant HY644 parent. The iterative application of SDS to selfing populations of HY644 also generated progeny lines with heritable, altered FHB resistance. Plants grown from seed of a single head of HY644 collected from the 2002 FHB field nursery were subjected indoors to BYD-plus-WSM pressure and selected for FHB resistance, followed by a second indoor round of selection under BYD-plus-WSM pressure. Seed from the best heads were grown as 10 head-row lines in the 2003 FHB field nursery, and the best lines selected. A subsequent indoor test of one of these lines (#8785) compared with a line of original, 'pre-SDS' HY644 breeder's seed showed that the selected SDS-generated line sustained significantly less FHB damage than HY644 grown from breeder's seed. Genomic DNA fingerprints of the two lines were indistinguishable except for #8785's lack of a doublet band at ca. 215 bp, indicating the altered FHB resistance could not be attributed to seed admixture or cross-pollination.

CAN METABOLIC PROFILING DISCRIMINATE QUANTITATIVE RESISTANCE AND METABOLIC PATHWAYS IN WHEAT-FHB SYSTEMS?

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ABSTRACT

Plants of wheat cultivars Sumai3 and Roblin, highly resistant and susceptible to fusarium head blight (FHB), respectively, were grown in greenhouse, and the four central spikelets in spikes were inoculated at anthesis with a spore suspension of *Fusarium graminearum*. Spikelets inoculated with sterile distilled water served as control. Inoculated plants were incubated for 24 h, spikelets were harvested and crushed in liquid nitrogen. Metabolites were extracted using methanol and chloroform, derivatized using MSTFA and analyzed using a gas chromatograph and mass spectrometer system. A total of 86 consistent compounds were detected, of which 30 were specific to a treatment: Sumai3-pathogen (SP), Sumai3-water (SW), Roblin-pathogen (RP) or Roblin-water inoculated (RW). The compounds specific to SP and RP can be used qualitatively to discriminate quantitative resistance to FHB. Some compounds, however, were common to 2, 3 or to all 4 treatments. Compounds such as cinnamic acid, acetoacetic acid, (E, E, E)-(5-phenylsulfonylgeranyl) geraniol, butanoic acid and 2, 3, 4, 5-tetrakis-OTMS-D-Ribose were detected only in SP. 4-hydroxycinnamic acid was detected in all treatments but the abundance in SP was 6 and 8 times more than in SW and RP, respectively. Benzeneacetic acid, 3-methoxy-alpha., 4-bis [trimethylsilyl)oxyl]-, TMS ester was detected in both SP and SW spikes, but the abundance was about 4 times higher in SP. 1, 2, 3, 4, 5, 6-hexakis-OTMS-myo-inositol abundance increased following pathogen inoculation in both the cultivars and the increase was higher in SP. Compounds such as 2, 3, 5, 6-OTMS-ethyl ²-D-galactofuranoside, bis-TMS phenylpyruvic acid and glutamine tris-(TMS) were detected only in SP and RP, but the abundances were higher in SP. Malonic acid (TMS), ²-DL-lyxopyranose, 1,2,3,4-tetrakis-OTMS, 1,2,3,4-tetrakis-OTMS-²-D-xylopyranose, 1,2,3,5-tetrakis-OTMS-arabinofuranose and 5-methoxy-2-[(TMS)oxy] benzoic acid TMS ester were unique to RP. For compounds that were common to all treatments, but that varied in their abundance, data were subjected to factor analysis to derive relative loadings of compounds to each treatment. Thus, metabolic profiling can be used not only to discriminate cultivars but also to discriminate resistance levels based on pathogenesis related metabolites (metabolites induced following pathogen inoculation). The two cultivars appear to follow different metabolic pathways, Sumai 3 follows PAL and Roblin follows melonate pathway, to defend against the pathogen attack. The possible role of various compounds detected in this study in plant defense against pathogen stress, especially the antimicrobial properties and signal transduction, and their potential application for screening cultivars of wheat for quantitative FHB resistance is discussed.

MAPPING OF THE FHB QTL ON THE SHORT ARM OF WHEAT CHROMOSOME 2D USING THE CO-LINEARITY BETWEEN WHEAT 2D AND RICE CHROMOSOMES 4 AND 7

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ABSTRACT

A "finished" rice genome sequence with 99.99% accuracy will come to us by the end of 2004 through the International Rice Genome Sequencing Project (IRGSP). In addition to the sequence, the Japanese Rice Genome Project (RGP) has provided much information, full-length cDNAs, a high-density EST map, rice insertion mutants, micro-arrays, several databases including proteome, etc. All of this information is very useful and important for promoting wheat genomic research. Recently we started working on improving Fusarium head blight (FHB) resistance in wheat using a comparative genomics approach in collaboration with three research institutes (NIAS, JIRCAS, and the STAFF Institute). To date we have identified three genomic regions on wheat chromosomes 5AL, 5BS, and 2DS associated with Type I resistance to FHB in a population of doubled haploid lines (DHLs) from the F_1 cross of Sumai #3 and Gamenya. The QTL on chromosome 2DS revealed a risk factor for both Type I and Type II resistance as a negative effect contributed by Sumai #3. In this study, we focused on this QTL region of wheat chromosome 2DS, which has synteny with two rice chromosomes, 4 and 7. As a first step we have done *in sillico* mapping of wheat markers and ESTs on pseudo-molecules of rice chromosomes 4 and 7. About two-thirds of the markers or ESTs on wheat 2D could be assigned to the rice chromosomes, which shows the co-linearity between wheat 2D and rice chromosomes 4 or 7. Our *in sillico* mapping of wheat markers and ESTs restricted our target FHB QTL to the 6Mb region of rice chromosome 4. Several disease-related genes, not only classical disease resistance proteins but also genes for cytochrome P450s and ABC transporter-like multi-drug resistant proteins, were included in this chromosome region. We report on our progress in developing new markers based on information on the rice genome sequence in this region.

FHB RESISTANCE BREEDING IN WHEAT PROGRAMME AT SELGEN

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ABSTRACT

The principal pathogens associated with FHB in Czech republic are *F. culmorum, F. graminearum* (Schwabe) and its teleomorph, *Gibberella zeae* (Schw.) Petch.. While other *Fusarium* spp. including and *F. poae* (Peck) Wolenw., *F. avenaceum* (Corda ex Fr.) and *Microdochium nivale* (Ces ex Berl. & Vogl.) Sammuels & Hallett and its teleomorph *Monographella nivalis* (Schaffnit) Muller are also present in FHB complex. Fusarium Head Blight epidemy doesn´t appear every year in the Czech Republic, therefore a breeding program based on natural infection is not possible. The occurence of FHB mostly depends on climatic conditions, crop rotation and removing or ploughing in previous crop residues. After all developing varieties with high levels of FHB resistance has become an essential component to the wheat breeding program.For effective selection in our breeding program we use inoculation techniques. The mostly used technique at our breeding program is inoculation by spraying heads with the conidial suspension (Mesterházy). Because of screening about 1000 lines every year we prefer to inoculate about 10-20 heads/a line in a hilll plots (about 40 seeds is sowed for one plot), so the size of nursery can be kept manageable. The inoculations are done once at the stage of flowering (we are carefully looking for flowering heads and label them). After inoculation the screening nursery is under mist irrigation for about one month. Although *F. graminearum* and *F. culmorum* do not have vertical races, isolates differ significantly in their aggressivness. Therefore, over the entire inoculation period and all locations we use the same inoculum. The first symptoms are observed 7-10 days after inoculation. Breeding for resistance to FHB was started at Plant breeding Station Stupice in 1988 by crossing resistant variety Nobeoca Bozu with winter wheat Sparta and spring wheat Sandra. Nobeoca Bozu had good resistance to FHB but was not very good in other traits. The variety as well as the new lines were very high with low yield and suspectibility to other diseases. That is why we used back-crossing in F1 generation with our high yield varieties.¨. From this program we did not registred any variety but sources with better tolerance to FHB were obtained.Screening in breeding program for resistence to FHB is divided into two inoculated field nurseries - for winter wheat and spring wheat and a control without infection.Basic yield components have been mesured and contents of mycotoxins have been anylsed at Research Institute of Crop Production in Prague.The testing for resistance to FHB is done with the Czech varieties, worldwide known sources and breeding lines from the F4 generation. In cooperation (programme QD1311) with Research Institute of Crop Production in Prague and other breeding stations in the Czech Republic and in Europe we have participated on testing sources of resistance to FHB as well as advanced breeding lines at more locations since the year 2001. In the last years we have used the most resistant proben cultivars (SUMAI3, FHB21, NOBEOCA BOZU, F201R, CM82036, line ST144-01, SG-S1875) for crossing. Most of the resistant sources are spring wheat. So there are many crosses between spring and winter wheats, with other selection to acceptable frost resistance for winter type.

BREEDING SCAB TOLERANT HARD WINTER WHEAT IN SOUTH DAKOTA

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ABSTRACT

Development of resistant hard winter wheat varieties is the main component of our integrated strategy to combat the increasing FHB threat in eastern South Dakota. Our main objective is to develop FHB resistant hard winter wheat varieties with superior agronomic performance and resistance to biotic and abiotic stresses. Our short term objectives are to: 1) Screen and identify elite FHB resistant lines and develop segregating populations, and 2) enter promising resistant lines into regional nurseries to facilitate development of varieties with broad adaptation. In 2003 – 2004 we screened 1498 genotypes in the mist-irrigated field nursery. These included South Dakota experimental lines, the Northern Regional Performance Nursery (NRPN), the Southern Regional Performance Nursery (SRPN), and the Regional Germplasm Performance Nursery (RGON). The percentage of the South Dakota experimental lines that were superior to the FHB resistant check 'Expedition' (Disease index = 16.8%) was 14.6%. Two experimental lines, SD97380-2 and SD98102, with good FHB, leaf and stem rust resistance, and superior agronomic performance, are being increased with intention to release. Lines included in the South Dakota Crop Performance (CPT) Variety Trial and the Advanced Yield Trial were also evaluated in the greenhouse using needle inoculation and were also screened for the 3BS QTL associated with the Sumai3 type resistance. In the 2004 – 2005, we planted 36 F_3 and 57 F_2 bulks containing Sumai3, Ning7840, or their derivatives in three-way crosses (unadapted/adapted//adapted) with local varieties and elite lines. This will enable us to combine the major 3BS and 5AS QTLs with our local indigenous resistance in an adapted background. Seed from these populations will be available for interested programs in the region. Scab-resistant advanced lines from these populations will be entered into regional nurseries to facilitate development of varieties with broad adaptation to the northern Great Plains.

ADAPTIVE EVOLUTION AND PATHOGEN RESPONSIVE EXPRESSION OF NEW TAXI-TYPE XYLANASE INHIBITOR GENES IN WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

TAXI-I (*Triticum aestivum* xylanase inhibitor I) is a wheat grain protein that inhibits arabinoxylan fragmentation by microbial endo-ß-1,4-xylanases. We have now demonstrated the presence of TAXI-I family members with isolation of two mRNA species, Taxi-III and Taxi-IV. At the nucleotide sequence level, Taxi-III and Taxi-IV were 91.7% and 92.0% identical, respectively, to Taxi-I, and Taxi-III and Taxi-IV were 96.8% identical. Notably, analysis of nucleotide substitutions between Taxi-I and Taxi-III revealed trace of adaptive evolution. In an attempt to understand the physiological significance of the TAXI family members, the level of Taxi-I and Taxi-III/IV transcripts was measured. Accumulation of Taxi-III/IV transcripts was most evident in roots (at all stages investigated) and older leaves (partially etiolated) where transcripts of Taxi-I were negligible. When challenged by a fungal pathogen Fusarium graminearum, the concentration of Taxi-III/IV transcripts was induced by approximately 20-fold at 24h post-inoculation. In contrast, the increase of Taxi-I transcripts in response to the fungal pathogen was rather limited. Furthermore, the Taxi-III/IV genes were also strongly expressed in wounded leaves. Recombinant TAXI-III protein inhibited Aspergillus niger and Trichoderma sp. xylanases and some spelt xylan-induced xylanases of F. graminearum. Thus, some, but not all, TAXI-type xylanase inhibitor genes may have a role in plant defense.

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MULTIDISCIPLINARY APPROACHES TO BREEDING FUSARIUM HEAD BLIGHT RESISTANCE INTO COMMERCIAL WHEAT CULTIVARS: CHALLENGES AHEAD

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OBJECTIVES

An important goal of wheat scientists is to incorporate Fusarium head blight (FHB) resistance into commercial cultivars of bread wheat and durum wheat using all available tools**:** conventional breeding, cytogenetics, molecular genetics, and biotechnology. This article summarizes the progress made in this direction and outlines some of the problems involved in resistance breeding.

INTRODUCTION

Fusarium head blight (FHB) or scab, caused primarily by the fungal pathogen *Fusarium graminearum* Schwabe, is a serious disease of bread wheat (*Triticum aestivum* L., 2n = 6x = 42; AABBDD) and durum wheat (*Triticum turgidum* L. var. *durum* Desf., 2n = 4x = 28; AABB). The disease greatly reduces grain yield and quality, although its severity varies from year to year depending on environmental conditions favoring the spread of pathogen. The combined direct and secondary losses incurred to wheat and barley producers in scab-affected regions of the United States during the period from 1998 to 2000 were estimated at $2.7 billion (Nganje et al., 2002); the states of North Dakota and Minnesota suffered about 55 percent of the total losses. Due to accumulation of the mycotoxin deoxynivalenol (DON), the grain is rendered unfit for human or even animal consumption. Incorporating FHB resistance into commercial wheat cultivars using all available methods of traditional breeding and tools of cytogenetics and biotechnology would be necessary for controlling this ravaging disease. This article summarizes the progress made in this direction and outlines the problems encountered and challenges that lie ahead.

SOURCES OF RESISTANCE AND THEIR EXPLOITATION

Bread Wheat Cultivars - Current cultivars of cultivated wheats, particularly of durum wheat, have limited variability for FHB resistance. The most commonly used source of resistance is the Chinese wheat cultivar 'Sumai 3' that has been used by wheat breeders worldwide. A major QTL (quantitative trait loci) for type II resistance was identified on short arm of chromosome 3B of this cultivar (Anderson et al., 2001; del Blanco et al., 2003). Cytologically based physical mapping showed that this QTL is located in the distal region of 3BS (Zhou et al., 2002). Additional QTLs have been identified on 5A (Buerstmayr et al., 2002), 6B and 6A (Anderson et al., 2001), and 2AS and 2BL (Zhou et al., 2002). The FHB resistance of Sumai 3 has been successfully used in wheat breeding in the US. Alsen, a hard red spring wheat variety released by North Dakota State University, carries FHB resistance from Sumai 3, and has accounted for about 30% of the planted acres in North Dakota for three consecutive years since 2002 (North Dakota Wheat Commission, 2004).

A Romanian winter wheat with FHB resistance has been developed at the Agricultural Research-Development Institute, Fundulea; it has QTLs located on 1B, 3D, and 5A (Alexander and Ittu, 2003). Other potential sources of FHB resistance are 'Wangshuibai', a landrace from the Jiangsu province of China, PI157593 from South Korea, and PI362463 from Yugoslavia. Genetic diversity studies have shown that resistance genes in Wangshuibai are different from those in Sumai 3. Liu and Anderson (2003) found that the former has no alleles in common with Sumai 3 for any of the molecular markers around the QTL on 3BS.

Primary Gene Pool of Durum Wheat - Wild emmer wheat (*Triticum turgidum* L. var. *dicoccoides* Körn, 2n = 4x = 28; AABB) shares the A and B genomes with durum wheat. It is in the primary gene pool of durum wheat and readily crosses with it. Several QTLs have been identified on chromosome 3A of wild emmer (Otto et al., 2002). Other tetraploid wheats with the genomic constitution AABB include Persian wheat [*T. turgidum* subsp. *carthlicum* (Nevski in Kom.) Á. Löve & D. Löve], Polish wheat [*T. turgidum* subsp. *polonicum* (L.) Thell.], oriental wheat [*T. turgidum* subsp. *turanicum* (Jakubz.) Á. Löve & D. Löve], Georgian emmer wheat [*T. turgidum* subsp. *paleocolchicum* (Menabde) Á. Löve & D. Löve], and poulard wheat (*T. turgidum* subsp. *turgidum*). All these subspecies belong to the primary gene pool of durum wheat and desirable traits in these tetraploid wheats can be easily transferred into durum wheat by conventional breeding approaches. However, these resources have not been adequately utilized so far.

Among the tetraploid wheats, reaction to FHB has been systematically evaluated only in *T. turgidum* L. var. *dicoccoides*. Of the 282 accessions evaluated for type II resistance, Miller et al. (1998) found 10 accessions to be more resistant than the best durum cultivar. Buerstmayr et al. (2003) evaluated 151 accessions from different geographical areas in Israel and Turkey and identified eight accessions to be resistant to FHB. However, the levels of resistance shown by these accessions were not as high as in Sumai 3. *T. turgidum* var. *dicoccoides* is being exploited as a source of FHB resistance for durum wheat. Durum cultivar Langdon – *dicoccoides* disomic substitution lines [LDN (Dic)] were evaluated for type II resistance. Langdon with a pair of chromosome 3A from *dicoccoides*, i.e., LDN (Dic-3A) had the least FHB infection (19.8%) compared to other LDN (Dic) disomic substitution lines (Stack et al., 2002). A single major QTL for resistance and a microsatellite marker linked with resistance have been identified (Otto et al., 2002). The FHB resistance of LDN (DIC-3A) is currently used in durum wheat breeding programs in North Dakota, although material showing stable FHB resistance has not been obtained so far.

In an effort to transfer FHB resistance from *T. turgidum* var. *dicoccoides* to durum wheat, two new sets of Langdon – *T. turgidum* var. *dicoccoides* (LDN-Dic) disomic substitution lines were developed using two *dicoccoides* accessions (PI481521 and PI478742) with FHB resistance as chromosome donors. These substitution lines are currently being evaluated for resistance to FHB (J. D. Faris, pers commun). They were molecularly characterized using the high-throughput TRAP (target region amplification polymorphism) marker technique and 642 chromosome-specific TRAP markers have been developed (Xu et al., 2003). These studies could facilitate the transfer of FHB resistance from *T. turgidum* L. var. *dicoccoides* to durum wheat using marker-assisted selection (Bai and Shaner, 2004).

Other tetraploid wheat subspecies with AABB genomes have also been evaluated for resistance to FHB, although on a limited scale. Gagkaeva (2003) identified FHB resistance in some accessions of *T. turgidum* subsp. *dicoccum (T. dicoccum)* and *T. turgidum* subsp. *carthlicum (= T. persicum).* In a preliminary study, type II resistance was identified in several accessions of *T. turgidum* subsp. *dicoccum* and *T. turgidum* subsp. *carthlicum* (Xu and Cai, unpublished). Some accessions in these two subspecies showed 5.0 – 8.0% infection in a greenhouse evaluation. In contrast to *T. turgidum* var. *dicoccoides*, both *T. turgidum* subsp. *dicoccum* (*T. dicoccum*) and *T. turgidum* subsp. *carthlicum* are cultivated and it should be easy to transfer their FHB resistance into durum wheat.

Wild Relatives of Wheat - Wheatgrasses and other wild relatives in the secondary gene pool have a good potential as donors of FHB resistance to wheat. Tetraploid wheatgrass (*Thinopyrum junceiforme* (Löve & Löve) Löve, 2n = 4x = 28; $J_1J_1J_2J_2$ genomes) and diploid wheatgrass (*Lophopyrum elongatum* (Host) Á. Löve, 2n = 2x = 14; EE) show excellent resistance to FHB. Thus, *L. elongatum* shows a mean infection of 3.8%, compared to 60% to 90% infection in susceptible durum cultivars (Jauhar, 2001). Other wheatgrasses, e.g., *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve (2n = 2x = 14; JJ), may also be potential donors of FHB resistance to wheat. Because

of their low chromosome number, it would be desirable to use diploid wheatgrasses in resistance breeding work. These grasses are in the secondary gene pool of wheat. Although they can be easily crossed to both bread wheat and durum wheat using embryo rescue techniques, lack of pairing between wheat and alien chromosomes can be an obstacle to intergeneric gene transfers. Problems encountered in transferring alien FHB resistance into durum wheat were outlined by Jauhar (2001).

Transfer of Alien Chromosome Segments and Reconstruction of the Wheat Genome - Manipulation of the *Ph1* system (located in chromosome 5B) that suppresses pairing among less related chromosomes is the key to alien gene transfers into durum wheat. Thus, the use of the 5D(5B) disomic substitution line (that lacks chromosome 5B and hence the *Ph1* gene) could help promote wheat-alien chromosome pairing. In triploid hybrids (2n = 3x = 21; ABJ) involving diploid *Th. bessarabicum* and 5D (5B) substitution line, Jauhar and Almouslem (1998) observed more than 4-fold pairing compared to their counterparts with normal *Ph1*. Another method of at least partially solving this problem is to use appropriate genotypes of wild grasses that suppress the activity of *Ph1*, resulting in elevation of wheat-alien chromosome pairing (see Jauhar, 2001). Using these techniques of cytogenetic manipulation, we have incorporated alien chromatin in the durum genome and produced scab-resistant germplasm (Jauhar and Peterson, 2000, 2001; Jauhar, 2003). Thirty lines from the advanced hybrid derivatives were evaluated in 2004 in the Field Scab Nursery in Prosper, North Dakota. Stability of these alien integrations remains somewhat of a problem. However, we have isolated several stable durum disomic addition lines with a pair of chromosomes from *L. elongatum*. Some disomic additions have only 11-21% infection compared to 60-80% in the Langdon checks under both greenhouse and field conditions. We plan to use X-radiation to break this chromosome and integrate its segments into the durum genome. Unfortunately, whole alien arm or large segments are not well accepted by durum wheat that has far less genetic buffering than bread wheat. Introduction of the shortest possible alien segment in the wheat genome should be a cytogeneticist's goal. This is particularly true of alien integrations into durum wheat where only alien segments of minimal size would have stability and consequent practical usefulness. Introduction of small chromosome segments by manipulation of the *Ph1* gene is an effective strategy.

Genetic Transformation and FHB Resistance - As discussed above, traditional methods involving chromosome-mediated gene transfers have led to some improvement of FHB resistance in bread wheat; and FHB-resistant germplasm has been produced in durum wheat. These techniques involving sexual hybridization can be tedious and time-consuming (Jauhar and Chibbar, 1999; Repellin et al., 2001). Modern biotechnological approaches facilitate direct and asexual incorporation of desirable genes into otherwise superior wheat cultivars (see, for example, Jauhar and Khush, 2002). Such transgenic approaches to combat FHB in wheat and barley were described by Dahleen et al. (2001). FHB resistance in wheat was achieved by expressing antifungal genes, including TRI101, PDR5, and thaumatin-like protein (TLP) genes that degrade structural components of the fungus and/or interfere with biochemical and metabolic processes in the pathogen (Okubara et al., 2002; Anand et al., 2003). However, a major obstacle to durum wheat transformation has been the lack of an efficient *in vitro* regeneration protocol. We standardized the *in vitro* regeneration method as well as transgenic technology for durum wheat (Bommineni and Jauhar, 1996; Bommineni et al., 1997; Satyavathi and Jauhar, 2003; Satyavathi et al., 2004). Using these technologies, we are incorporating antifungal genes into durum cultivars and testing them for FHB resistance (see also paper by Satyavathi et al. in these proceedings).

CONCLUSION

Although the stability of alien chromatin integrations into the durum genome remains a problem, we have produced several stable durum disomic addition lines with a pair of chromosomes from *L. elongatum*. Some of these disomic additions have only 11% infection compared to 60-80% infection in the Langdon checks, which is encouraging. However, moving genes from the added chromosome to the durum genome remains

a challenge that we are currently undertaking. We feel that all available tools of traditional plant breeding, cytogenetics, molecular genetics, and biotechnology should be used to combat this devastating disease of wheat. We hope that with concerted, multidisciplinary approaches, plant scientists will be able to win the race against this fungal pathogen.

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ENHANCEMENT OF SOFT RED WINTER WHEAT CULTIVARS WITH FUSARIUM RESISTANCE

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ABSTRACT

An overall goal of the breeding program is to enhance the development of Fusarium head blight (FHB) resistant cultivars for the Southeast. During the 2003 wheat season, a severe epidemic of scab occurred in Georgia resulting in economic losses of millions of dollars in the region. Numerous producers had difficulty in marketing their grain due to high DON concentration that was above the 2% level established by the millers in the state. Currently, the leading cultivars available to southeastern producers are moderately susceptible to scab. Wheat cultivars with higher level of scab resistance are needed to reduce the economic losses from this disease. The level of resistance within the elite lines of Georgia's program has significantly increased. Three elite breeding lines, GA941523E21, GA941320E24, and GA941318E22, evaluated in both greenhouse and field screening were identified with high level of Fusarium resistance similar to Ernie. Several elite breeding lines with good scab resistance from both native and exotic sources are also being evaluated in multi-locations for yield and agronomic performance. Recently several new cultivars (Coker 980582, Truman (MO 980525) and IN 97395B1-4-3-8 are available as parents with good scab resistance and better resistance to other diseases that should make excellent parents. Double haploid and backcross techniques are being used to facilitate the transfer of Fusarium resistance. Marker assisted selection (SSR) is being employed on backcross and double haploid lines to identify FHB resistance derived from exotic and native sources. DNA samples were extracted from 147 DH plants generated from 8 crosses. Fifty-six plants showed positive for the QTL on 3BS with markers, Xgwm 533 and BARC 133. The plants will also be screened for the other QTLs on chromosome 5AL and 2BS.

PROGRESS FROM FIVE YEARS OF SELECTING FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN SPRING WHEAT

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ABSTRACT

Some effort aimed at the improvement of resistance to Fusarium Head Blight (FHB) has been practiced within spring wheat breeding programs for many years. With the advent of the US Wheat & Barley Scab Initiative, however, such efforts have become major resource expenditures. As such, it seems worthwhile to periodically monitor progress from such endeavors. In our first attempt to gauge progress, the objective of this project was to determine whether resistance to FHB in spring wheat germplasm selected in the upper Midwest has increased from 1998 to 2003. To facilitate such measurement, a test was composed which included 10 varieties that were released to growers between 1998 and 2003, as well as 21 breeding lines that were selected within the same time frame to continue their advancement through their respective breeding program (i.e., advanced to statewide preliminary yield trials). Five additional lines were included in the test as checks. These artificially inoculated tests were grown under mist-irrigation at Brookings, SD and Prosper, ND during the 2004 growing season. FHB severity data were collected from each four-replication test. Data were subjected to analysis of variance over locations. Year of release or advancement and entry means were used as independent and dependant variables, respectively, to fit a simple regression model. It was found that entries were significantly different with respect to FHB severity, however, the slope of the simple regression line was not statistically separable from zero. These results suggest that much phenotypic variation for FHB severity is present within this germplasm. At the same time, it appears that the entries sampled from the 5-year time span are still too variable, with respect to FHB severity, to begin monitoring progress in the advancement of FHB resistance. This becomes more intuitive when one considers that within the South Dakota State University spring wheat breeding program, as an example, populations formed after 2000 in the hopes of increasing FHB resistance, have yet to reach the preliminary yield trial stage of the program where lines used in this study were selected. Additional attempts to examine progress in FHB resistance breeding will likely be initiated in the future.

GENETIC AND PHYSICAL MAPPING OF THE BARLEY CHROMOSOME 2H FUSARIUM HEAD BLIGHT RESISTANCE QTL

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ABSTRACT

Our long-term goal is to clone the genes responsible for Fusarium Head Blight (FHB) tolerance conferred by the barley chromosome 2(2H) QTL. Towards this end, we are saturating the region with molecular markers, identifying BAC clones for each molecular marker, and developing isolines with minimal segments of the resistant parent (CI4196) genome in the susceptible parent (Morex) genome background. We have used three sources of molecular markers targeted to the QTL region. These are: 1) synteny with the rice chromosome 4 region including the BAC/PAC clones OSJNBb0091E11 to OSJNBa0029H02 (~365kb) and OSJNBa0014K14 to OSJNBa0010H02 (~2.2Mbp). There is a gap of unknown distance between OSJNBa0029H02 and OSJNBa0014K14. Ninety-seven unique barley ESTs were identified from the 28 rice BACs; 2) wheat ESTs mapped to group 2 deletion lines; 3) barley ESTs mapped by Gary Muehlbauer's laboratory to the long arm of chromosome 2(2H) using the Barley1 microarray (Muehlbauer et al., in preparation). Additional map saturation will be achieved using the Diversity Arrays Technology (Wenzl et al., 2004. Proc. Natl. Acad. Sci. USA 101:9915-9920). Each molecular marker identified to map to the target region will be used to isolate barley BAC clones. Fingerprinting of these BAC clones is in progress in collaboration with Tim Close and results to date can be viewed at "http://phymap.ucdavis.edu:8080/barley". Lines with recombination in the Bin8-11 region are being used to develop isolines using back crossing method.

EVALUATION OF FHB RESISTANCE IN DURUM WHEAT BREEDING

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ABSTRACT

Resistance against FHB is becoming increasingly important for new durum wheat varieties. No-tillage sowing is increasing and with it the infection pressure for FHB among other diseases. Therefore it is important for a breeder to have knowledge about the resistance of his material. This concerns breeding lines and also varieties that might be used as parents.

Saatzucht Donau is a private breeding company with programs for both winter and spring durum wheat. The conventional assessment of FHB was to score it when it occurred naturally. Three years ago we started to use artificial inoculation, a method which had been already established for screening winter bread wheat. To avoid the problem of too early yellowing of the ears hindering the scoring of FHB the trials are grown at a location which is not a durum wheat region but has generally later maturity.

For spring durum wheat we sow triple rows which are inoculated by spraying with a spore solution that has half the concentration of that used in winter wheat to prevent overkill. Inoculation is done twice to account for differences in flowering. Heading (days after June 1st) and the percentage of bleached ears per plot (on a scale from 1 to 9) are scored. Usually two to three FHB scorings are possible. Parallel natural occurrence of FHB is scored in the yield trials at the breeding station where the durum wheat program is located.

The first year didn't give any results due to bad plant establishment. To have more of a micro plot we went from double rows to triple rows and try to sow with a high density. Particular attention is paid to the pH of the soil as durum wheat turned out to be a lot more sensitive to acidity than bread wheat. The two following years we could compare a set of 21 varieties/breeding lines over years and locations. Although the heading dates showed a very good correlation over sites and years the FHB scores did not. The only significant correlation was between the scores under natural infection over two years. Surprisingly in both years the scores from artificial inoculation showed a much higher (negative) correlation with heading date than those from natural infection. Partly this might be due to the generally lower scores under natural infection. Our results also did not confirm the ratings of some of the varieties in the French official variety list. Overall we are not past the judgement that "the bad are probably susceptible and the good ones might be more resistant".

In winter durum wheat, 1m2 plots were sown adjacent to the winter bread wheat inoculated trial but were not sprayed, again to prevent overkill. Even so the infection pressure was sufficient for producing up to 90% infected heads. 2004 was the first year of winter durum wheat testing and those results correlated quite well with a very heavy natural infection occurring at the yield trial site. Breeding lines that performed well under natural infection in 2004 will be tested together with the winter bread wheat in 2005 to verify the results.

A QUICK AND CHEAP METHOD FOR MASS PRODUCTION OF CONIDIAL *FUSARIUM* SUSPENSIONS

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ABSTRACT

Artificial inoculations with Fusarium spp. may require large amounts of fungal biomass. Production of inoculum should be cheap and easy. Ideally, the inoculum should be produced before the inoculation period, it should be storable under stable conditions and available quickly when required. The method described here fulfills these requirements and is suitable for the production of conidial suspensions in very large quantities. We use mung bean (Vigna radiata (L.) Wilczek) broth and produce fungal biomass with the bubble breeding method (Mesterhazy, 1978). Mung bean broth is produced by adding 20 g beans in one liter of boiling water. The beans are cooked for about 21 minutes until the pericarp starts to burst. The liquid is immediately filtrated to remove the beans and autoclaved. After seeding, the flasks (10 L) are continuously aerated with sterile air for 5 days at room temperature. Thereafter the bottles are stored overnight at 5°C (to collect microconidia 36 hours are recommended). The conidia will settle at the bottom. Above the conidial layer the supernatant is present consisting of a mycelial layer and the remaining clear liquid medium. By aspirating the supernatant, the mycelium and the surplus medium can be removed. The conidia from several bottles can be collected and the procedure can be repeated if further concentrating of conidia is required. Conidial concentration is determined with a hemacytometer. The suspension can be frozen (-80°C or at –20 to –30°C) in small aliquots. The aliquots must be quickly thawed for example in lukewarm water (25-30°C) before use. We successfully produced inoculum with all Fusarium spp. tested including F. graminearum, F. culmorum, F. avenaceum, F. cerealis, F. equiseti, F. acuminatum, F. poae, F. sporotrichioides, F. tricinctum, F. subglutinans, F. verticillioides, F. proliferatum, F. oxysporum and F. solani. Additional remarks: 1) conidial concentrations after bubble breeding typically varied from $5*10^5$ to $2*10^6$ conidia/ml, 2) after removal of the supernatant the conidial concentrations routinely increased with a factor 10, 3) aggressiveness of the frozen strains was tested with a seedling test and remained stable for at least 4 months, 4) freeze-thawing increased aggressiveness (compared with the non-frozen inoculum), 5) different strains of a single Fusarium spp. may produce different amounts of conidia, 6) the ratio of macro- to microconidia can vary extremely from strain to strain within the same Fusarium spp.

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NON-SPECIES SPECIFICITY OF *QFHS.NDSU-3BS* AND *QFHS.IFA-5A* IN WHEAT

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ABSTRACT

A wheat population of 96 doubled haploid (DH) lines from a cross between 'CM82036' and 'Remus' was investigated in detail for Fusarium head blight (FHB) resistance. 'CM82036' carries two QTLs for FHB resistance (*Qfhs.ndsu-3BS* and *Qfhs.ifa-5A*) and all 4 QTL classes resulting from all possible combinations of these QTLs were present in the nursery (24 lines in each class, including the parents). Resistance was assessed after spray inoculation with 8 different fungal strains on separate plots. The strains used were: *F. graminearum* (2 strains), *F. culmorum* (2), *F. avenaceum* (2), *F. sporotrichioides* (1) and *Gerlachia nivale* (1). Disease incidence (a measure for Type I resistance) and disease severity (Type I plus II) was assessed 4 to 5 times at different time points after inoculation. Experiments were done over two years on two locations (Tulln, Austria and Szeged, Hungary). In addition the lines were tested for resistance to fungal spread (Type II) with point inoculation (2 seasons in Tulln, Austria). Two Fusarium strains (*F. graminearum* and *F. culmorum*) came to use. Spread of the symptoms (number of diseased spikelets) was assessed 4 times at different time points after inoculation. AUDPC was calculated and used for further analyses. The results showed that: 1) highly significant differences in FHB resistance were present between genotypes and QTL classes both after spray and point inoculation, 2) strains varied highly significant in aggressiveness, 3) *Qfhs.ndsu-3BS* did not reduce disease incidence, 4) both *Qfhs.ndsu-3BS* and *Qfhs.ifa-5A* improved resistance to spread, but the effect of *Qfhs.ndsu-3BS* was significantly higher than *Qfhs.ifa-5A*, 5) in general additive effects of both QTLs were observed on disease severity and resistance to spread, 6) after spray inoculation *Qfhs.ifa-5A* was effective against all strains and this QTL decreased disease incidence and disease severity, 7) after spray inoculation *Qfhs.ndsu-3BS* significantly reduced disease severity of all strains except *F. sporotrichioides* and one strain of *F. avenaceum*. It is concluded that *Qfhs.ndsu-3BS* improved only Type II resistance and *Qfhs.ifa-5A* mainly Type I resistance. *Qfhs.ifa-5A* was effective against all tested fungal species whereas the results for *Qfhs.ndsu-3BS* were inconsistent in this respect.

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THE ROLE OF RESISTANCE TO DEOXYNIVALENOL IN THE COMPLEX FUSARIUM HEAD BLIGHT RESISTANCE COMPLEX IN WHEAT

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ABSTRACT

Two wheat populations were tested for resistance to deoxynivalenol (DON) and Fusarium head blight (FHB). In the first population 96 doubled haploid (DH) lines originating from a cross between 'CM82036' and 'Remus' were investigated. All 4 QTL classes, depending on the presence/absence of *Qfhs.ndsu-3BS* and/or *Qfhs.ifa-5A*, were represented (24 lines each, including the parents). In the second screening nursery (SN population) 127 genotypes were tested. Included were well-known highly resistant lines, breeding material and negative checks. Both populations were tested for DON resistance (DONR) with 3 methods: electrolyte leakage of flag leaf disks, germination of seeds and application of DON in the ear. They were investigated for FHB resistance (FHBR) after spray inoculation (2 locations, 2 seasons). The DH and the SN population were tested with 8 and 3 fungal strains, respectively. Disease incidence (Type I resistance) and disease severity (Type I+II) were assessed. Resistance to spread of the fungus (Type II) was investigated using single spikelet inoculation with 2 strains (2 seasons). In selected wheat lines concentrations of DON, DON-3-glucoside and other glucoside conjugates were determined with HPLC - Tandem Mass Spectrometry. QTL analyses were done with the DONR data for the DH population. DONR and FHBR data were compared (correlation analyses). The most important results were: 1) in both populations ANOVA analyses showed highly significant differences in DONR between the wheat lines for each DONR evaluation method, 2) DONR in the ear was not related with DONR in other plant tissues, 3) application of DON in the ear resulted in typical FHB symptoms, 4) 'Sumai3', 'Nobeokabozu' and their derivatives expressed high DONR in the ear, 5) *Qfhs.ndsu-3BS* improved DONR (AUDPC was reduced from 12.47 to 2.37 units), 6) *Qfhs.ndsu-3BS* was the only QTL detected for DONR in the DH population (LOD = 47, R^2 = 0.90), 7) DONR in the ear was correlated with Type II resistance only (r = 0.78), 8) DON-3-glucoside was detected after DON application in the ears, and 9) there was a highly significant positive correlation between the DON-3-glucoside/DON ratio and DONR in the toxin treated ears (r = 0.81). It is concluded that DONR plays an important role in the FHBR complex. We hypothesize that *Qfhs.ndsu-3BS* either encodes a glucosyl-transferase or regulates expression of such an enzyme.

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IDENTIFICATION AND MAPPING OF A QTL FOR TYPE II RESISTANCE TO FUSARIUM HEAD BLIGHT ON CHROMOSOME ARM 2DL OF WHEAT

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OBJECTIVES

To identify and map quantitative trait loci (QTL) for Type II resistance (resistance to spread) from a potentially new source of resistance.

INTRODUCTION

Fusarium head blight caused by *Fusarium* spp. and *Michrodochium nivale* has caused major economic losses in wheat and barley industries worldwide. Other than 'Sumai 3' and Sumai 3-derived lines, few sources of strong resistance have been identified and verified. The identification and mapping of QTL from new sources of resistance is needed to enable breeders to prevent against genetic uniformity and to most efficiently develop cultivars with effective combinations of resistance.

MATERIALS AND METHODS

A population of 130 dihaploid lines was developed from a cross between 'Flycatcher', an FHB susceptible wheat, and CASS94Y00009S-51PR-4B-0M-1Y-0M (designated 'CASS94' from this point onward), an FHB resistant F6 line derived from a cross between a synthetic hexaploid (TK SN1081 / *Ae. tauschii* (222)) and 'Mayoor'. (Mujeeb-Kazi, 1995; Mujeeb-Kazi et al., 1999).

One hundred and eight of the 130 dihaploid (DH) lines in addition to CASS94, Flycatcher, 'W14' (an FHB resistant check, Jiang, 1997) and 'Norm' (an FHB susceptible check) were planted sixteen times over a period of seven and a half weeks in the greenhouse. At the seedling stage, plants were vernalized for 2 weeks. Single floret inoculation was conducted just prior or post dehiscence by pipetting 10¼l of *F. graminearum* macroconidial suspension at 50,000 spores/ml [a 1:1 mixture of two isolates: PH-1 (NRRL 31084) and a second isolate from a MI wheat field] into a single floret cavity. Immediately following inoculation, plants were misted for 72 hours. All spikes inoculated on a single date were considered a single replication. Replications included plants from different planting dates, the number of spikes inoculated per genotype per replication varied, and not every replication included all genotypes. In total, 12 replications of inoculation were conducted over a 10-week period. Over the entire experiment, 21 to 42 spikes were inoculated per DH line (i.e. approximately 1.75-3.5 spikes were inoculated per DH line per replication).

The number and percent of visually scabby spikelets was evaluated at 7, 10, 14, 17(or in one replication at 18) and 21 days post inoculation (dpi). Area Under the Disease Progress Curve (AUDPC) was calculated at 10, 14, 17 and 21dpi for the number and percentage of scabby spikelets according to Shaner and Finney (1977). Genotypic means and experiment-wise LSDs were estimated in SAS® 8.2 using Proc Mixed LSmeans.

One hundred and thirty DH lines, in addition to CASS94 and Flycatcher, were genotyped using microsatellite (SSR) primer pairs. Two hundred and twenty two microsatellite primer pairs (169 BARC,

18 GDM, 32 GWM and 3 WMC) were polymorphic between CASS94 and Flycatcher and were used to genotype the population. Linkage map construction was performed in JoinMap® 3.0 (Van Oijen and Voorrips, 2001). QTL analysis was performed in Windows QTL Cartographer V. 2.0 (Wang et al., 2001-2004) using Single Marker Analysis (SMA, $p<0.05$) and Composite Interval Mapping ($p<0.01$). The LOD threshold value of each trait was empirically derived for a 0.01 experiment-wise error rate using 1000 permutations. Tests for epistasis were conducted using Multiple Interval Mapping function of QTL Cartographer V 2.0.

RESULTS

There were significant differences among progeny for all disease scoring methods at all dpi. Neither CASS94 nor Flycatcher exhibited a high level of resistance, revealing that the population exhibits a high level of transgressive segregation.

The population showed the expected 1:1 segregation ratio for the majority of SSR loci. One hundred and twenty –nine loci, amplified by 123 primer pairs were mapped to 26 linkage groups on 16 chromosomes, covering a total of 700.8 cM. Overall, the map was consistent with other previously published wheat maps (map not shown).

Single Marker Analysis (SMA) and Composite Interval Mapping (CIM) both identified a major QTL for FHB resistance on chromosome arm 2DL, having R^2 values ranging between 0.29-0.60 and LOD scores as high as 25.1 (Fig. 1, Table 1). This QTL was identified in every disease measure at every dpi. This QTL is most closely linked to *Xgwm539* and resistance is from CASS94. The average phenotypic mean for 99 of the 108 DH lines (data for this locus was unavailable for 9 lines) with the CASS94 allele versus the Flycatcher allele at *Xgwm539* is shown in Table 2.

Other QTL and putative QTL with smaller R^2 values (between 0.04 and 0.15) were significant in some measures of disease at some dpi according to CIM and/or SMA on chromosomes 3B (in the region of *Qfhs.ndsu-3BS*), 1B, 5A, 5D and 4A. All of these QTL, except two QTL identified on 4A, coincide with QTL for FHB resistance identified by other researchers. No epistatic interactions were identified between any of these QTL and/or between these QTL and the QTL identified on chromosome arm 2DL.

DISCUSSION

These results strongly confirm the presence of a QTL on chromosome arm 2DL for Type II resistance to FHB, as first reported by Somers et al., 2003. This QTL has been named *Qfhs.crc-2D*. Somers et al., 2003, investigated FHB resistance in a dihaploid population in which resistance was associated with the parent 'Wuhan-1' (a Chinese accession of unknown pedigree, D. Somers *pers comm.*). Wuhan-1 and CASS94 (the resistant parent in the population reported here) do not have any known common parentage. Although the four different measurements of disease and five different dpi used in this study were not independent of one another (i.e., different experiments were not conducted for each measurement method and dpi), *Qfhs.crc-2D* was identified as a major QTL using all disease measurements/dpi combinations, reflecting the importance of this QTL in resistance.

Xgwm539, the marker most closely associated with *Qfhs.crc-2D*, is highly polymorphic across diverse collections of germplasm (McCartney et al., 2004; Quarrie et al., 2003) and amplifies only a single locus (Quarrie et al., 2003). These characteristics of Xgwm539 make it ideal for marker-assisted selection. Although *Qfhs.crc-2D* appears to be closely linked to Xgwm539, the density of the genetic map in the region of *Qfhs.crc-2D* should be increased. Either the *Qfhs.crc-2D* QTL has an even greater effect than indicated here, or *Xgwm539* is extremely closely linked to the QTL.

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Table 1. R2 values from Composite Interval Mapping analysis for the QTL near Xgwm539 on chromosome arm 2DL at different days post inoculation (DPI) using different measures of disease. #SS = Number of Scabby Spikelets. %SS = Percent of Scabby Spikelets. AUDPC #SS and AUDPC %SS = AUDPC of the number and percent of scabby spikelets, respectively.

DPI	#SS	%SS	AUDPC #SS	AUDPC %SS
7	0.54	0.49	n/a	n/a
10	0.60	0.56	0.58	0.54
14	0.54	0.59	0.53	0.53
17	0.42	0.55	0.48	0.49
21	0.29	0.39	0.34	0.45

Table 2: Effects of the Xgwm539 locus on four measures of FHB in a subset of 99 DH lines of the CASS94 x Flycatcher population. Data are averages for all DH lines carrying the indicated allele. #SS and %SS = Number and Percent of Scabby Spikelets, respectively. AUDPC #SS and AUDPC %SS = AUDPC of the Number and Percent of Scabby Spikelets, respectively. DPI = days post inoculation.

	Allele at Xgwm539	
Measure of Disease	**CASS94**	**Flycatcher**
#SS 7 dpi	1.6	3.6
#SS 10 dpi	3.4	8.5
#SS 14 dpi	6.0	12.3
#SS 17 dpi	8.5	13.7
#SS 21 dpi	11.0	14.5
%SS 7 dpi	9.9	22.3
%SS 10 dpi	20.7	51.9
%SS 14 dpi	36.3	74.4
%SS 17 dpi	50.2	82.3
%SS 21 dpi	64.7	87.5
AUDPC #SS 10 dpi	8.3	20.1
AUDPC #SS 14 dpi	13.6	27.6
AUDPC #SS 17 dpi	17.0	29.5
AUDPC #SS 21 dpi	22.0	31.1
AUDPC %SS 10 dpi	51.3	122.9
AUDPC %SS 14 dpi	83.5	168.5
AUDPC %SS 17 dpi	103.1	179.4
AUDPC %SS 21 dpi	132.0	189.0

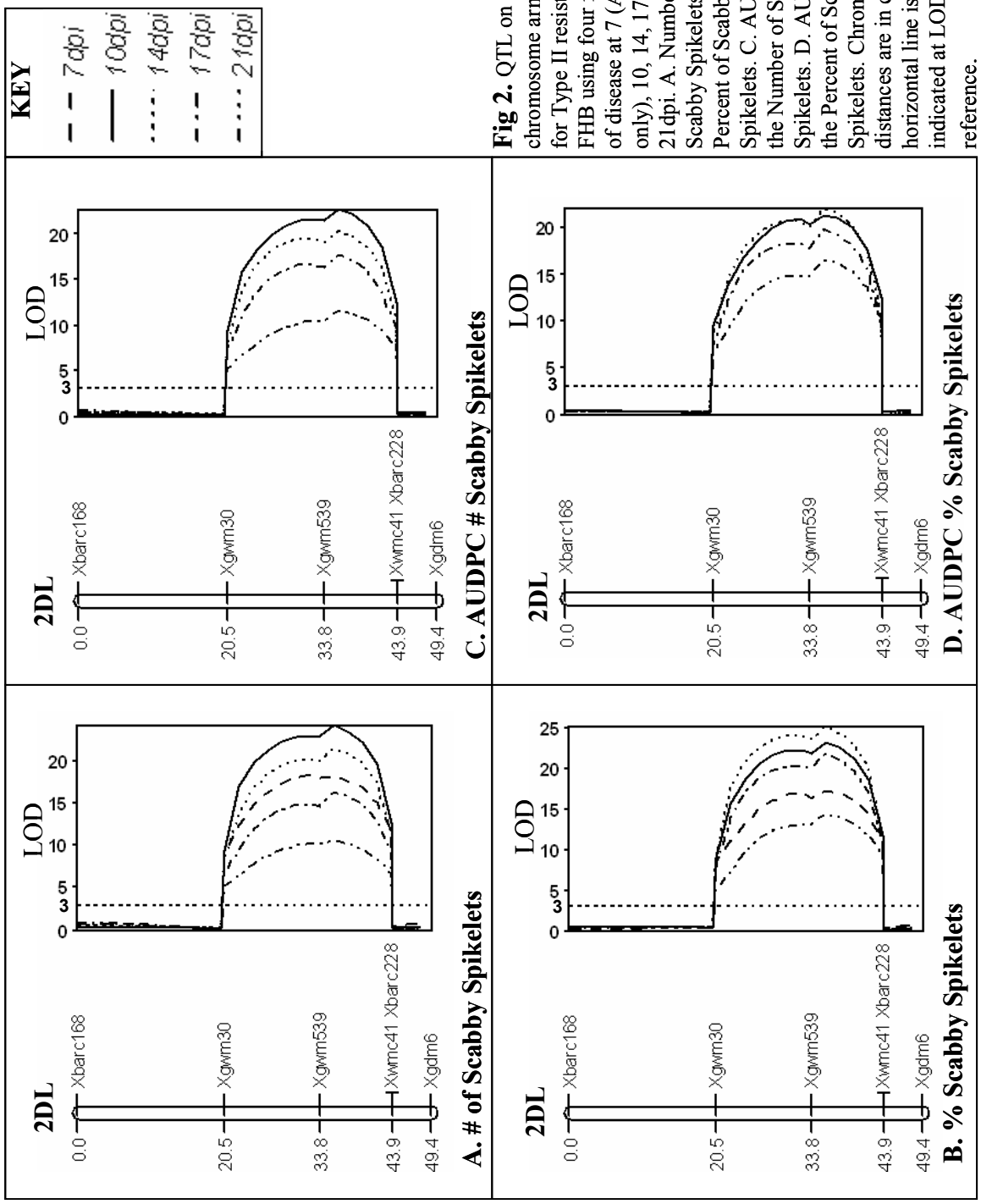

Fig 2. QTL on chromosome arm 2DL for Type II resistance to FHB using four measures of disease at 7 (A and B only), 10, 14, 17 and 21dpi. A. Number of Scabby Spikelets B. Percent of Scabby Spikelets. C. AUDPC of the Number of Scabby Spikelets. D. AUDPC of the Percent of Scabby Spikelets. Chromosome distances are in cM. A horizontal line is indicated at LOD 3 for reference.

RESISTANCE REACTION OF BRAZILIAN WHEAT CULTIVARS TO SCAB

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ABSTRACT

Fusarium Head Blight (FHB) or scab, induced by *Gibberella zeae* (anamorph: *Fusarium graminearum*), is considered one of the most important wheat diseases in the southern region of Brazil and a challenge to researchers because of the conducive environment to the pathogen during plant anthesis. Control measures, such as crop rotation and fungicide control and resistance, have not been completely efficient. The more recent endemic occurrence took place in 1997, 1998, 2000, and 2001, years of high rainfall. The objective of this work is to gather information on resistance reaction of 25 wheat cultivars of Embrapa Trigo recommended in the State of Rio Grande do Sul. The wheat cultivars were exposed artificially to disease favorable conditions in the field. One hundred spikes were collected for evaluation at two growth stages: milk and ripening. The incidence, the severity, and the percentage of grain with symptoms of FHB were evaluated. The resistance reaction of the cultivars was based on the percent of scaby-infected grains. Embrapa Trigo released a number of Brazilian cultivars, such as BRS 177 (1999), BRS 179 (1999), BRS Timbaúva (2002), BRS Louro (2003), BRS Umbu (2003), BRS Camboim (2004), and BRS Tarumã (2004), that were considered moderately resistant to FHB in the field test.

MAS EFFICIENCY IN IMPROVING SCAB RESISTANCE IN SPRING WHEAT: A LOOK FROM THE REVERSE ANGLE

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OBJECTIVE

To survey SSR markers *Xgwm389, Xgwm493* and *Xgwm533* within a collection of conventionally bred spring experimental wheat lines, documenting resultant marker-phenotype relationships.

INTRODUCTION

Marker-assisted selection (MAS) is a promising yet unproven technology for breeding better scab resistance in wheat. Resistance derived from Sumai 3 has been utilized widely in North American wheat breeding efforts. A major resistance QTL, *Qfhs.ndsu-3BS*, was discovered within Sumai 3 (Waldron et al, 1999). SSR markers for this 3BSQTL have been identified, and their usefulness confirmed, in numerous mapping populations (Anderson et al, 2001; Angerer et al. 2002; Bourdoncle and Ohm 2002; Bowen et al. 2002; Buerstmayr et al. 2002; Chen et al. 2002; Gonzalez-Hernandez et al. 2002; Waldron et al. 1999; Xu et al. 2002; Zhou et al. 2002, 2003). Zhou et al. (2003) discussed the promises and limitations of using theses SSR markers in MAS. An interest in gauging the impact on agronomic and resistance characters associated with using these markers for selection led to the initiation of this study.

METHODS AND MATERIALS

Plant materials and field trials: Sixty-four experimental lines were selected for inclusion in this study from within the South Dakota State University spring wheat breeding program in 2002. Twenty-two of these lines ($F_{7:8}$, $F_{7:9}$, or $F_{7:10}$) were selected from our advanced yield trial, (AYT) while 42 lines ($F_{4:6}$ or $F_{4:7}$) originated from within our preliminary yield trial (PYT). Nineteen of the 42 entries were known to include Sumai 3 in their pedigrees (Table 1). 'Briggs', 'Sumai 3' and 'Wheaton' were included as checks in each field season. This 67-entry trial was conducted in a mist-irrigated nursery in Brookings, SD during the 2002, 2003, and 2004 growing seasons. Tests included two replications in a randomized complete block design. Plots were a single 1.67 m long ´ 0.33 m wide row. Disease was introduced by inoculating 20 to 25 heads / plot after emergence of anthers on spikes within the plot. Disease incidence (percent diseased heads within a plot) and severity (percent diseased spikelets / head) data were recorded for calculating disease index values (incidence ´ severity) each year. Type II FHB resistance was rated according to Hu et al. (2000). Grain yield (g/m^2), test weight (g/dl), and kernel tombstone ratings were collected on a single-plot basis after harvest in 2002 and 2003.

SSR assay: DNA was extracted from 0.25 g of young leaves from each entry using DNAzol ES solution (Molecular Research Center, Inc) according to the manufacturer's protocol. SSR primer sets gwm389, gwm493 and gwm533 were synthesized on the basis of sequence information (Röder et al. 1998). PCR amplification was carried out with slight modification according to Röder et al. (1998) and Anderson et al. (2001). PCR products were separated on 6% polyacrylamide sequencing gels and visualized with silver staining, following a protocol described by Yen et al. (2000). The size of an SSR allele was estimated by comparing the gel position of a bands density peak to a standard curve calculated using a 10-bp DNA ladder. At least three replications per PCR reaction were carried out to verify band size estimation. Where the estimated polymorphic alleles differed by fewer than 10 bp, the questionable PCR products were loaded

side by side on additional gels for more accurate comparisons.

Data analyses: ANOVA was carried out on disease incidence, severity, and index, as well as yield, test weight, and percent tombstone kernel ratings over years using the GLM procedure in SAS (SAS Institute, Cary, NC). Single-marker analysis was performed using the GLM procedure in SAS to test the significance of phenotypic differences associated with SSR genotypes. *F*-tests were carried out to compare lines with different SSR genotypes. Single-degree-of-freedom contrasts were also performed to test the significance of phenotypic trait differences between lines with and without Sumai 3 in their pedigree. Phenotypic differences were considered as significant, highly significant or very highly significant where $P < 0.05$, 0.01 or 0.001, respectively.

RESULTS AND DISCUSSION

The SSR allele patterns and mean performance of phenotypic traits for each entry were summarized over years and are presented in Table 1. Mean differences were either highly or very highly significantly between years for all traits. None of the lines were ranked as R in terms of FHB resistance, while 11 lines were ranked as MR, 41 as MS, and 12 as S. No entry possessed the same SSR genotype as Sumai 3. Only SD3776, (moderate FHB resistance) had all three Sumai 3 alleles associated with the 3BSQTL, and it was the only line that shared the *Xgwm389-135bp* allele with Sumai 3, suggesting a high recombination rate between the 3BSQTL and *Xgwm389-135bp*. Two lines, SD3777 and SD3784, had the same *Xgwm533-145bp* allele that Sumai 3 had, and were ranked as MS and MR, respectively. Thirty-three lines shared the *Xgwm493-190bp* alleles with Sumai 3. Generally, no significant differences were observed for any of the phenotypic traits among *Xgwm493* genotypes. However, lines with the *Xgwm493-190bp* allele differed significantly from those without this allele in terms of disease index, suggesting that this SSR marker may have some value in MAS for improving FHB resistance. Entries possessing different *Xgwm389* or *Xgwm533* alleles differed very significantly in terms of disease incidence. Entries with different *Xgwm533* alleles also differed significantly in test weight and yield. A clear distinction was not observed between the SSR genotypes and FHB resistance within lines having Sumai 3 as a part of their pedigree, nor among lines with and without an SSR allele common to Sumai 3. Highly significant differences existed for percent tombstone kernels between entries with and without Sumai 3 in their pedigree. Differences among these contrasting groups of entries were not statistically significant for the remaining traits, although differences among the means were noted. Specifically, entries with Sumai 3 in their pedigree had lower incidence, severity and thus disease index values ($P = 0.07$, 0.1 and 0.09, respectively) than those without.

The following conclusions may be drawn from this study. First, environment had a very strong impact on the performance of all the traits studied as evidenced by very significant year to year difference for all phenotypic traits. Second, differences in performance among entries were generally very significant for each trait. Third, Sumai 3 alleles made a large contribution to the improvement of percent tombstone kernel rating. They also slightly aided with the improvement of other resistance parameters, albeit significant differences were non identifiable. Lastly, MAS based solely on the SSR markers associated with the 3BSQTL might not be as reliable as desired for improving FHB resistance. It did, however, appear that line selection based on SSR genotypes seemed to have no detrimental effect on yield.

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Table 1. SSR allelic pattern and mean performance of the selected SD spring wheat during the years 2002, 2003 and 2004.

Entry	SSR allele patterns			Scab Index (I*S)	Grain Yield (g/m^2)	Test Weight (g/dl)	Kernel Tombstone (%)	Entry	SSR allele patterns			Scab Index (I*S)	Grain Yield (g/m^2)	Test Weight (g/dl)	Kernel Tombstone (%)
	533	493	389						533	493	389				
BRIGGS	A3	B1	M2	40.58	76.02	52.00	24.50	SD3754*	A3	C1	A2	41.51	115.74	52.45	9.50
SD3506	D3	C1	D2	42.94	106.45	52.74	10.75	SD3756*	K3	C1	C2	51.36	91.76	52.21	12.50
SD3533	A3	E1	B2	43.36	70.99	53.13	25.00	SD3757*	I3	A1	B2	56.04	72.11	49.48	12.50
SD3540	A3	B1	F2	31.35	101.34	53.98	7.50	SD3758*	A3	F1	L2	46.47	81.43	50.98	6.25
SD3546	A3	C1	B2	29.65	87.46	53.11	18.75	SD3759	A3	F1	F2	61.97	81.05	46.13	17.50
SD3618	A3	B1	C2	33.74	108.30	52.18	9.50	SD3760	A3	F1	D2	67.69	53.92	49.65	26.25
SD3623	A3	C1	C2	35.15	89.59	54.23	10.50	SD3763	I3	H1	D2	39.43	90.71	52.32	11.25
SD3635	D3	C1	C2	31.37	100.51	52.81	4.33	SD3765	A3	C1	J2	38.78	86.60	54.45	3.00
SD3641	I3	E1	G2	30.98	85.06	53.36	9.00	SD3768	A3	A1	D2	49.21	65.79	48.28	12.50
SD3668	A3	I1	A2	37.93	85.08	56.06	15.75	SD3769	A3	C1	D2	34.93	78.36	99.15	10.66
SD3672	A3	I1	J2	42.67	63.97	51.91	12.50	SD3771	F3	A1	D2	37.16	74.06	82.90	11.10
SD3687	A3	C1	J2	25.59	106.18	50.60	22.50	SD3773*	A3	C1	J2	36.94	78.36	65.96	9.65
SD3689	D3	C1	D2	34.12	59.18	56.22	8.25	SD3774*	E3	C1	B2	54.03	73.20	80.69	13.76
SD3694	A3	A1	A2	33.68	81.79	52.65	15.33	SD3776*	H3	C1	E2	26.37	52.96	78.59	11.58
SD3696	A3	B1	D2	29.53	74.06	60.01	4.25	SD3777*	C3	C1	M2	39.65	71.91	76.46	11.13
SD3698	G3	F1	J2	27.63	67.55	52.97	8.75	SD3778*	A3	C1	J2	28.74	59.85	81.56	10.74
SD3699	I3	G1	I2	40.19	70.98	53.74	24.50	SD3779	B3	A1	D2	58.61	80.09	74.22	14.90
SD3714*	A3	B1	D2	49.17	68.19	54.80	17.50	SD3780	B3	A1	D2	51.04	80.95	88.41	9.35
SD3719*	B3	C1	A2	28.21	108.85	52.73	4.25	SD3781	A3	F1	D2	31.59	68.89	66.91	10.47
SD3720*	A3	F1	K2	28.51	89.77	61.10	4.00	SD3782*	A3	C1	H2	26.85	75.35	80.77	10.74
SD3722*	A3	B1	D2	31.13	68.27	57.47	3.50	SD3784	C3	C1	D2	28.28	95.16	70.70	10.30
SD3728	A3	C1	B2	45.29	66.64	54.64	17.25	SD3786	G3	C1	J2	35.60	72.77	62.44	10.85
SD3730	A3	E1	A2	46.19	81.45	50.46	20.00	SD3788	A3	A1	D2	33.08	42.63	83.28	11.52
SD3736	A3	F1	A2	46.08	99.94	50.98	12.50	SD3791	A3	F1	J2	58.18	84.82	67.65	15.27
SD3737	A3	F1	A2	59.82	74.39	48.34	16.25	SD3793	A3	F1	D2	47.62	88.27	55.65	16.64
SD3739	A3	F1	D2	38.23	81.46	54.26	9.00	SD3794*	A3	A1	D2	34.68	62.86	64.68	9.47
SD3741	A3	C1	H2	53.16	73.23	49.37	21.67	SD3795*	A3	C1	D2	36.98	82.24	53.11	6.80
SD3743*	B3	E1	H2	40.35	83.94	52.56	6.00	SD3796*	A3	E1	D2	25.96	104.63	66.86	10.79
SD3744	A3	D1	D2	44.77	75.57	54.72	7.50	SD3798	D3	C1	D2	45.71	86.54	56.68	7.29
SD3745	A3	A1	D2	46.52	77.11	49.97	12.50	SD3799	I3	C1	A2	41.93	83.96	58.89	7.87
SD3746	A3	A1	D2	46.01	116.87	49.13	22.50	SD3800	D3	C1	B2	39.23	81.81	79.95	10.66
SD3747	A3	A1	D2	49.47	92.78	52.18	15.00	SUMAI 3	J3	C1	E2	12.36	148.12	64.87	1.00
SD3748	A3	D1	D2	50.69	74.44	47.44	28.75	WHEATON	A3	A1	A2	76.00	43.06	42.20	65.00
SD3751*	A3	C1	A2	50.83	57.97	52.62	11.25								

*: known to have Sumai 3 in the pedigree.

TOWARDS MAP-BASED CLONING OF THE *QFHS.NDSU-3BS* QTL THAT CONFERS RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT

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ABSTRACT

One objective of our research is the map-based cloning of a major QTL (*Qfhs.ndsu-3BS*) for Fusarium head blight (FHB) resistance in wheat. Two SSR markers (*Xgwm533* and *Xgwm493*) that flank *Qfhs.ndsu-3BS* were used to screen a mapping population of 3,155 plants derived from a single F_7 plant heterozygous for the *Qfhs.ndsu-3BS* region. A total of 382 recombinants were identified. To increase the DNA marker density of this QTL region, STS (sequence-tagged site) markers were developed from wheat ESTs that are homologous to rice genes on the sub-distal region of rice chromosome 1S. The STS markers and two more SSR markers were used to genotype the 382 recombinants. A high-resolution map was constructed that contains 16 markers covering a distance of 6.4 cM. The high-resolution map revealed a complex microsynteny among wheat, rice and barley at this QTL region. Homozygous recombinants were screened for FHB resistance and *Qfhs.ndsu-3BS* was placed to a less than 1 cM interval containing five DNA markers. We are phenotyping additional homozygous recombinants to further narrow down the marker interval containing this QTL. The five DNA markers were used to screen a Chinese Spring chromosome 3B BAC library. Each marker detected 5 to 6 positive BAC clones. Two BAC clones were detected by the same two DNA markers that are 0.3 cM apart. New DNA markers developed from the positive BAC clones are being used to close the physical gaps of the QTL region.

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CHARACTERIZATION OF THE RPL3 GENE FAMILY OF WHEAT

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ABSTRACT

The production of trichothecenes is an important virulence factor of *Fusarium graminearum* on wheat. Trichothecenes bind to ribosomes and act as inhibitors of eukaryotic protein biosynthesis, thereby most likely suppressing induced defense responses of the host plant. Mutants of *S. cerevisiae* showing semidominant resistance to the trichothecene trichodermin allowed the cloning of *TCM1* (trichodermin resistance), which was found to encode ribosomal protein L3 (*RPL3*) (Fried and Warner, 1981). Our group has recently identified several mutations in yeast *RPL3* which confer DON resistance (Mitterbauer *et al.*, 2004).

We have started the characterization of the *RPL3* gene family of wheat in order to find out whether alleles of *RPL3* exist in nature which cause different resistance properties against deoxynivalenol (DON). The *RPL3* gene family of wheat consists of six genes, three homeologs of the paralogous loci *RPL3A* and *RPL3B*. We developed gene specific primers and cloned and sequenced the genomic fragments and cDNAs from three different wheat cultivars with different *Fusarium* resistance (Frontana, the Sumai3 derivative CM82036, and the susceptible elite cultivar Remus). Sequence analysis revealed very high similarity between homeologs, and none of the amino-acid alterations leading to increased resistance in yeast were found in any of the cultivars. Three SNP-based markers could be developed which allowed the mapping of these *RPL3* genes by using two double haploid mapping populations. One of the genes maps close to a previously identified resistance QTL, fine mapping with a high resolution mapping population is ongoing.

In parallel also cDNAs of all homeologs from the resistant cultivar Frontana were cloned and expressed in toxin sensitive bakers yeast. All wheat cDNAs can complement the yeast *rpl3* gene disruption mutant. Small differences in the susceptibility against DON between the homeologs was observed. Despite the lack of mutations conferring high level resistance, we hypothesize that differential utilization of TaRPL3 isoforms with different properties could lead to differences in basal DON resistance.

ACKNOWLEDGEMENT

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DELETION OF A CHROMOSOME ARM ALTERED WHEAT RESISTANCE TO FUSARIUM HEAD BLIGHT IN CHINESE SPRING

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [telomorph=*Gibberella zeae* (Schw.)], is an important wheat disease worldwide. Production of deoxynivalenol (DON) by *F. graminearum* in infected wheat grain is a major safety concern for animal production and human health. To data, a major QTL on 3BS has been identified across various genetic backgrounds. Many other QTL have been reported, but their chromosome locations are inconsistent among different studies. Chinese Spring is a Chinese landrace with moderate resistance to FHB. A set of aneuploid genetic stocks have been developed and widely used in different genetic studies. In this study, a set of 34 ditelosomic lines derived from Chinese Spring was evaluated for Type II FHB resistance to determine effect of missing a chromosome arm on FHB resistance. All 34 ditelosomic lines derived from Chinese Spring were repeatedly evaluated for percentage of scabbed spikelets (PSS) in the greenhouse. Cultivars Sumai 3 and Clark were used as resistant and susceptible controls, respectively. Significant variation in amount of FHB infection was observed among the ditelosomic lines tested. PSS ranged from 17.6% to 94.6% for ditelosomic lines although PSS was 48.0% for Chinese Spring, suggesting that missing one chromosome arm can significantly alter the level of FHB resistance in Chinese Spring. Ditelosomic lines DT3BS, DT6DS, DT7AL and DT7BL demonstrated a significant decrease in PSS compared to that in Chinese Spring, while, DT1AL, DT1BL, DT1DL, DT1DS, DT3AL, DT3AS, DT3BL and DT6BL had significant higher PSS than that for Chinese Spring. The results suggest that several genes on different chromosome arms may involve in FHB resistance in Chinese Spring and they may either enhance or suppress FHB infection in Chinese Spring.

DIALLEL ANALYSIS OF HARD WINTER AND SPRING WHEAT FOR *FUSARIUM GRAMINEARUM* RESISTANCE

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ABSTRACT

Fusarium head blight (FHB), caused *by Fusarium graminearum*, is a major biotic constraint of wheat production in South Dakota. Breeding resistant varieties is the most efficient approach for combating this problem. Six genotypes consisting of susceptible winter wheat 'Nekota' and '2137', moderately susceptible winter wheat 'Harding', moderately resistant spring wheat 'ND2710' and 'BacUp' and resistant spring wheat 'Ning7840' were crossed in a partial diallel mating design to determine combining ability of FHB resistance. F_1 crosses were evaluated in the greenhouse, and F_2 crosses were screened in both greenhouse and mist-irrigated field conditions. One parent 'Nekota' was excluded from diallel mating design at the field condition because of few F_2 seed and poor plant stand. In the greenhouse, both F_1 and F_2 were artificially point inoculated at anthesis, whereas F_2 crosses in field condition were artificially inoculated by a combination of corn spawn spread at jointing stage and inoculum suspension spray at anthesis. Disease index percentage (incidence % * severity% / 100) of the crosses was analyzed using Griffing's method 4 and model 1. General combining ability was highly significant ($P<0.01$) in both greenhouse and field conditions, but specific combining ability was significant ($P<0.05$) only in F_2 crosses in greenhouse condition. The results showed that both additive and non-additive gene effects are involved in the inheritance of FHB resistance. The ratio of combining ability variance components [$(2s^2_{GCA}/2s^2_{GCA}+s^2_{SCA})$] ranged from 0.66 to 0.89 indicating that additive gene effects are more important than non-additive gene effects.

GRAINGENES 2.0: AN INTEGRATED RESOURCE FOR THE TRITICEAE

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ABSTRACT

GrainGenes (http://wheat.pw.usda.gov/GG2), the international database for genetic and genomic data about *Triticeae* species (e.g. wheat, barley, and rye) and Avena sativa (oat), was updated and extensively redesigned in 2004. The underlying database is now built on the MySQL relational database platform, and is fully integrated with the GrainGenes website into one resource. The new architecture provides marked improvements in data delivery with respect to speed, query power and user-friendliness. Pre-formatted "Quick Queries" from the traditional GrainGenes have been updated to interface with the new database, and additional advanced SQL and Batch Queries have been added. Over 1,100 chromosome maps from over 120 map studies can now be viewed via CMap, a comparative map display to identify and highlight common loci among multiple physical and genetic maps. The GrainGenes CMap server includes all maps in the GrainGenes collection, plus a separate database with Rudi Appels' Wheat Composite map compared to its component maps. A BLAST server offers custom databases such as mapped wheat ESTs, EST-SSRs, Barley1 GeneChip exemplars, and *Triticeae* repeat sequences. Other improvements include extended report pages, such as a new "marker" page that combines locus and probe data. Many of the improvements in GrainGenes 2.0 have been guided by comments and suggestions from our users, and we welcome further feedback as we continue to enhance its value as a resource for the grains research community. GrainGenes is a product of the USDA Agricultural Research Service (ARS).

EVALUATION OF *FUSARIUM* RESISTANT GERMPLASM INTRODUCED THROUGH THE USWBSI/ CIMMYT COLLABORATION

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OBJECTIVES

This paper reports on resistance levels in spring wheat germplasm introduced into the U.S. through a collaborative agreement between the U.S. Wheat and Barley Scab Initiative (USWBSI) and The International Maize and Wheat Improvement Center (CIMMYT) in Mexico. Specifically it reports on resistance in germplasm from Brazil, Argentina, Japan and CIMMYT.

INTRODUCTION

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), 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, (Wilcoxson et al., 1988; Bai and Shaner, 1994), rotations with corn (Windels and Kommedahl, 1984), the lack of effective cultural and/ or fungicide control (McMullen et al., 1997) and the lack of effective sources of genetic resistance. Host resistance has long been considered the most economical and effective means of control (Schroeder and Christensen, 1963; Martin and Johnston, 1982), but breeding has been hindered by a lack of effective resistance genes and by the complexity of the resistance in identified sources (Mesterházy, 1997). No source of complete resistance is known, and current sources provide only partial resistance, often in genetic backgrounds with inferior agronomic type. The identification of different sources of resistance and their incorporation into adapted wheat varieties is critical to the continued improvement of Fusarium head blight resistance in wheat.

In 2000, a collaborative agreement was initiated between the USWBSI and CIMMYT to promote the exchange of scab resistant germplasm among research programs globally. The overall goals were to acquire new sources of scab resistance in an effort to diversify the gene pool and facilitate the utilization of resistant germplasm in wheat. The collaboration also has enabled closer collaboration and exchange between the U.S. and CIMMYT. It was proposed that where CIMMYT lines combined scab resistance with other disease resistance genes necessary for economic production of wheat in the U.S. this collaboration would greatly accelerate the release of scab resistant cultivars for U.S. production.

To date, 313 lines have been introduced, quarantined, screened and distributed to interested scientists within the USWBSI. These lines have included lines from CIMMYT (117), Brazil (19), Argentina (107), Uruguay (5), Japan (15), China (30), Romania (7), and Hungary (13). An additional 186 spring wheats from CIMMYT are currently under quarantine. This paper reports on resistance levels of lines from South America, Japan and CIMMYT introduced in 2001.

MATERIALS AND METHODS

In 2001, 173 spring wheat lines including lines from Brazil, Argentina, and Japan were introduced into Missouri along with 32 spring wheats from CIMMYT. Lines were quarantined, increased, screened, and verified for type II resistance to *Fusarium graminearum*. Resistant ('Ernie') and susceptible (MO 94-317) check varieties were included in all screens.

Disease Evaluation: Type II disease evaluation was conducted in the greenhouse. At first anthesis, 8 plants per line were inoculated with 10µL of a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was placed in a single central floret using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously determined to be the most aggressive Missouri isolate on Missouri's most resistant cultivar, Ernie. Plants were incubated in a mist chamber (100% relative humidity) for 72 h post-inoculation to promote disease development and then returned to the greenhouse bench where disease development continued. Plants were rated at 21 d post-inoculation. Data collected included the number of spikelets on the inoculated head and disease spread defined as the number of diseased spikelets on the inoculated head. The Fusarium head blight index (FHBI) was determined as disease spread/total spikelets on the inoculated head. In addition to disease spread the rachis adjacent to the inoculated floret was inspected for disease symptoms and scored as either 0 (no disease) or 1 (disease symptoms). Lines having a mean disease spread of less than or equal to 2.5 spikelets in the inoculated head were retained for verification of disease resistance scores. Verification was conducted using similar protocols.

RESULTS AND DISCUSSION

Mean FHBI in the preliminary screen of 173 lines ranged from 3.6% to 79.4% compared with the resistant (18.0%) and susceptible (62.5%) check varieties while mean disease spread in the inoculated head ranged from 0.06 to 11.8 spikelets screened compared to 2.1 and 6.3 spikelets in the resistant and susceptible checks, respectively. Of all lines screened, one line from Argentina (Argentina 94) had no rachis involvement in any of the plants screened while an additional 10 lines had 6 or 7 of the eight plants screened with no rachis involvement. Lines with a mean disease spread of less than or equal to 2.5 spikelets were re-screened to verify disease resistance levels. Following verification, 65 lines had resistance levels better than the resistant check Ernie (Table 1 and Table 2). Although these sources of resistance appear to have high levels of type II resistance, evaluation of type I resistance is necessary to fully determine field performance. In addition, molecular genetic analysis including analysis with SSRs associated with known resistance alleles would help breeders make more informed decisions about which of these lines to include in crossing programs aimed at pyramiding different sources of resistance in U.S. winter and spring wheats. All lines are available to scientists within the USWBSI for further screening and/or inclusion in wheat research efforts.

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Table 1. Evaluation of type II resistance in spring wheat lines from Argentina that were introduced into the U.S. through the USWBSI/CIMMYT germplasm collaboration in 2001. Lines presented were screened using point-inoculations with *Fusarium graminearum* in 2002 and verified in 2003 at the Univ. of Missouri and represent lines with resistance levels better than 'Ernie', the soft red winter resistant check.

Designation	Cross	Disease spread†	FHBI‡
		No. of spikelets	%
Argentina 1	NING 8647	1.0	5.6
Argentina 3	WEAVER/3/CMH75A.66/CMH76.217//PVN	0.9	4.7
Argentina 5	COOPERACION MILLAN	1.4	9.0
Argentina 7	NANJING 8049/KAUZ	0.7	4.4
Argentina 8	F7 BULK PY NO. 5	0.6	3.6
Argentina 14	SHA8/GEN	0.9	5.4
Argentina 30	SHANGHAI 4	1.9	12.7
Argentina 31	PEKING 8	1.3	11.4
Argentina 35	WUHAN #3	0.8	4.6
Argentina 36	SHANGHAI 5	1.8	11.8
Argentina 38	NING 8611	1.1	6.2
Argentina 43	SUZHOE 1	0.7	4.6
Argentina 50	CI14227/TRM/MAD/3/FAN1	0.8	4.4
Argentina 52	H855/3/CC//JUSTIN/JAR	0.9	5.6
Argentina 55	PEL 73007	1.1	6.7
Argentina 56	YMI #6	1.6	9.8
Argentina 58	LAJ1409/LPI//PF7815/LAJ2079	1.1	7.4
Argentina 59	PF7815	1.3	8.3
Argentina 61	TP/NOBEOKA BOZU	1.4	7.6
Argentina 64	PEL 73081	1.9	10.5
Argentina 67	IAS53*2/TOKAI 66	1.3	8.8
Argentina 74	TP/NOBEOKA BOZU	0.9	6.1
Argentina 80	CEP8837	0.9	5.9
Argentina 82	LFN/II58.57//PRL/3/HAHN	1.1	5.2
Argentina 83	LAJ1409/LAJ2231//KLAT/PEL73001/3/LAJ1409/…..	0.7	4.4
Argentina 89	SUZHOE #9	1.0	5.8
Argentina 90	LAJ1409	0.9	6.4
Argentina 93	P81/TZPP*4//P68.16359	0.8	4.9
Argentina 94	TP/NOBEOKA BOZU	0.8	4.9
Argentina 101	V81608/3/CMH75A.66/CMH76.217//PVN	0.8	4.8
Argentina 103	LAJ1409//KLAT/SOREN/3/LRI/NOBEOKA BOZU	1.8	11.3
Argentina 104	KLAT/PEL74142/LRI/NYUBAY/3/KLAT/CEP75203//……	1.5	9.2
Ernie	RESISTANT CHECK	2.2	18.0
MO 94-317	SUSCEPTIBLE CHECK	6.3	62.5

† No. of spikelets showing disease symptoms 21 d following point-inoculation with *Fusarium graminearum*.
‡ Fusarium head blight index determined as the number of diseased spikelets/total spikelets on the inoculated head, expressed as a percentage.

Table 2. Evaluation of type II resistance in spring wheat lines from Brazil, Japan and CIMMYT that were introduced into the U.S. through the USWBSI/CIMMYT germplasm collaboration in 2001. Lines presented were screened using point-inoculations with *Fusarium graminearum* in 2002 and verified in 2003 at the Univ. of Missouri and represent lines with resistance levels better than 'Ernie', the soft red winter resistant check.

Designation	Cross	Disease spread†	FHBI‡
		No. of spikelets	%
Brazil 1	PF87512/CATBIRD	0.7	6.5
Brazil 2	PF87512/CATBIRD	0.7	5.7
Brazil 3	VEE'S'/JUN'S'//BOW'S'/3/BR23/EMB27	1.4	10.4
Brazil 5	OR1'S'//BR23/EMB27	0.9	9.6
Brazil 7	PR8722/3/TJB368.251/BUC'S'//BAU'S'/4/OR1	1.6	16.7
Brazil 8	PF87509//PF87512/B.BAGUAL	0.6	4.6
Brazil 9	OR1//CEP8749/OR1'S'	1.7	12.3
Brazil 10	CMH75A.270/5*MRNG//BAU'S'/3/BR32/0R1	0.9	5.9
Brazil 11	PF87509//PF87512/B.BAGUAL	0.6	4.4
Brazil 12	EMB27/M1029-89//BR23/EMB27	1.4	16.9
Brazil 15	CMH75A.270/5*MRNG//BAU'S'/3/BR32/0R1	0.6	4.6
Brazil 19	EMB27/KLEIN H3450 C3131	1.4	11.5
CIMMYT 4	DESC/3/ALD/PVN//YMI #6	1.4	8.0
CIMMYT 5	CBRD/KAUZ	1.6	8.8
CIMMYT 6	WUH1/VEE#5//MUNIA	1.5	8.3
CIMMYT 7	WUH1/VEE#5//CBRD	1.0	5.1
CIMMYT 8	WUH1/VEE#5//CBRD	1.8	10.4
CIMMYT 10	WUHAN #2	1.3	7.1
CIMMYT 11	SABUF	0.9	5.3
CIMMYT 13	SHA3/SERI//PSN/BOW	1.3	7.4
CIMMYT 17	BAU/MILAN	1.9	10.2
CIMMYT 20	XIANG82.2661/2*KAUZ	1.3	6.8
CIMMYT 21	ALD/PVN//YMI #6	1.7	9.7
CIMMYT 22	TINAMOU	1.1	6.2
CIMMYT 24	XIANG82.2661/2*KAUZ	1.4	6.5
CIMMYT 26	JUP73R/PVN	1.6	9.3
CIMMYT 30	WUH1/VEE#5//CBRD	0.8	4.7
Japan 1	SHIRO KOMUGI	1.1	8.0
Japan 5	OKINAWA ZAIRAI YUUBOU	1.7	18.2
Japan 8	ASO ZAIRAI (MUBOU)	0.8	4.4
Japan 9	OOITA KOMUGI	2.0	9.8
Japan 13	CHIKUZEN	1.0	5.6
Japan 15	SUMAI 3	1.0	5.9
Ernie	RESISTANT CHECK	2.2	18.0
MO 94-317	SUSCEPTIBLE CHECK	6.3	62.5

† No. of spikelets showing disease symptoms 21 d following point-inoculation with *Fusarium graminearum*.

‡ Fusarium head blight index determined as the number of diseased spikelets/total spikelets on the inoculated head, expressed as a percentage.

INHERITANCE OF FUSARIUM HEAD BLIGHT RESISTANCE IN THE US WHEAT CULTIVAR 'ERNIE'

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OBJECTIVES

This research was designed to confirm preliminary results of the inheritance of scab resistance in Ernie that were presented at the 2003 US Wheat and Barley Scab Initiative Forum. The objectives of this paper are to (1) update QTL associated with type II resistance in Ernie and (2) report on gene action associated with this source of resistance.

INTRODUCTION

Fusarium head blight (FHB), also called scab, caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)], is an important disease of (*Triticum aestivum, and T. durum*) and barley (*Hordeum vulgare L.*) in warm, humid areas of the world. Host plant resistance is considered the most economical solution to this disease; however, sources of resistance are limited as is knowledge of the genetics of resistance in many sources that are widely used in breeding programs. Research to date has primarily been focused on resistance in the Chinese cultivar Sumai 3 (Waldron et al., 1999; Anderson et al. 2001), its derivatives (Bai et al., 1999; Buerstmayr et al. 2002), the Brazilian cultivar Frontana (Steiner et al., 2003), as well as cultivars from Europe such as Fundulea 201R (Shen et al., 2003).

The soft red winter wheat Ernie, released in 1995 by the University of Missouri (McKendry et al., 1995) has a moderately high level of type II scab resistance and based on pedigree analysis, differs from Chinese and other know sources of resistance. It is an important source of resistance for US wheat breeding programs because it is adapted and has end-use quality essential to the US soft wheat market. The genetics of this source of resistance, however, have not been well studied.

MATERIALS AND METHODS

A set of 243 F_8 recombinant inbred lines (RILs) were developed for QTL analysis from the cross Ernie / MO 94-317. The experiment was arranged as a randomized complete block design replicated 3 times and grown in both 2002 and 2004. Eight plants per RIL per replication were phenotyped for resistance. For conventional analysis, 5 generations including the F_1 (Ernie/MO 94-317), reciprocal F_1 (MO 94-317/ Ernie), BC_1 (F_1/Ernie), BC_2 (F_1/MO 94-317), and the F_2 were developed. Plants were arranged in a completely randomized design and evaluated for resistance in 2003. The experiment was repeated twice.

Greenhouse evaluation of disease reactions to *F. graminearum* was done according to McKendry et al. (2002). Phenotypic data collected included the number of spikelets on the inoculated head and disease spread defined as the number of diseased spikelets on the inoculated head. The Fusarium head blight index (FHBI) was determined as disease spread/total spikelets on the inoculated head.

AFLP procedures followed manufacturer's recommendations from the AFLP System I Kit from Invitrogen (Carlsbad, CA). Sixty-four EcoRI/MseI primer pairs and 420 SSR primers were used to screen parents for polymorphisms. Sequence information of SSRs was from Roder et al. (1998) and Q. J. Song and P. Cregan USDA-ARS, Beltsvile, MD (personal communication). The chromosome locations of these SSR markers were from Röder et al. (1998) and J. Shi and R. Ward, Michigan State University (personal communication). Analyses of variance and QTL mul-

tiple regressions were done using SAS Version 8.0. MapMaker 3.0 was used to construct the linkage maps. Composite interval mapping was done using QTL Cartographer 1.16 model 6. Generation means analyses were conducted according to Mather and Jinks (1977).

RESULTS AND DISCUSSION

A total of 139 markers including 94 SSR and 45 AFLP markers were mapped on 19 chromosomes. Two chromosomes, 4D and 6D had only one marker. The order and distance of most mapped markers were consistent with the reference map (Röder et al., 1998; Shi and Ward, personal communication). Based on composite interval mapping at LOD =3.0, four QTL on chromosomes 2B, 3B, 4B and 5A were consistently identified in both years of the study (Table 1; Figure 1). A fifth QTL on 5DL associated with the SSR marker *Xgwm182* identified in 2002 was not present in 2004. All alleles were from Ernie and were associated with increased resistance. Ongoing research is aimed at saturating QTL regions on chromosomes 3B, 5A and 5D, and re-phenotyping recombinants in those regions in order to resolve differences in QTLs over the two years of study and to identify markers with closer linkages to QTL peak positions.

Conventional genetic analysis agreed closely with the results of the QTL analysis. The minimum number of effective genes conferring resistance to FHB was calculated from RIL variances for both disease spread and FHBI using the Cockerham's modification (Cockerham, 1983) of Wright's formula (Wright, 1968) in which the F_8 and F_9 generations are considered homozygous. Gene numbers conditioning disease spread were 4.3 and 4.2 for 2002 and 2004, respectively while gene numbers conditioning FHBI were 3.8 in both years. Results of generation means analysis (Table 2) indicated that both disease spread and FHBI, a genetic model containing additive, dominance and additive × dominance epistatic effects fit the observed data. Additive effects for reduced spread accounted for 95.7% of the observed variation while dominance effects accounted for 3.4% of the variation. Similar results were obtained for FHBI where additive effects accounted for 95.2% and dominance effects accounted for 4.2% of the variation. In both cases, the additive × dominance effect accounted for less than 1% of the variation and was not significant at P=0.05.

Generation variances provided estimates of the additive (D), dominance (H) and environmental (E) components of the F_2 phenotypic variance. Broad-sense heritabilities for disease spread and FHBI were 78.2% and 78.3% respectively while narrow-sense heritabilities were 51.3% and 55.4% for disease spread and FHBI, respectively. Broad-sense heritabilities determined from the combined analyses of variance of disease resistance in RILs were in good agreement with those determined from generation means analyses. Heritability for disease spread was 0.70 ± 0.06 while that for FHBI was 0.87±0.025.

Results of the inheritance study indicate that type II resistance in Ernie is heritable, has moderately high narrow-sense heritability and is conditioned by 4 genes that act in a primarily additive fashion. Results suggest that it should be possible to develop varieties with high levels of FHB resistance by selecting for transgressive segregates in crosses that combine resistance in Ernie with other complementary sources of resistance.

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Table 1. Quantitative trait loci (QTL) associated with Fusarium head blight index (disease spread/total spikelets in the inoculated head) in recombinant inbred lines from the soft red winter wheat cross Ernie/MO 93-317 from experiments conducted in 2002 and 2004.

Chromosome location†	Marker‡	QTL peak position§	LOD	R^2 (%)¶	Additive effect	Source of alleles
2002						
2B	*Xgwm 319*	118.5	4.0	5.4	-5.6	Ernie
3B	*Xgwm077*	98.8	3.0	5.1	-5.0	Ernie
4BL	*Xgwm495*	0.0	6.7	9.7	-6.7	Ernie
5A	*Xbarc165*	40.7	8.7	23.5	-10.1	Ernie
2004						
2B	*Xgwm271*	115.5	4.0	6.0	-5.3	Ernie
3B	*E8M1_1*	121.7	4.1	11.2	-6.4	Ernie
4BL	*Xgwm495*	0.0	3.5	5.7	-4.6	Ernie
5A	*Xbarc165*	44.7	3.1	7.5	-5.2	Ernie

† Locations determined based on linked SR markers.

‡ The closest marker to the QTL can be either left or right flanking.

§ Distance measured in centiMorgans (cM) from the terminal marker on the short arm of the chromosome.

¶ Percentage of the phenotypic variation explained by the QTL as determined by QTL cartographer.

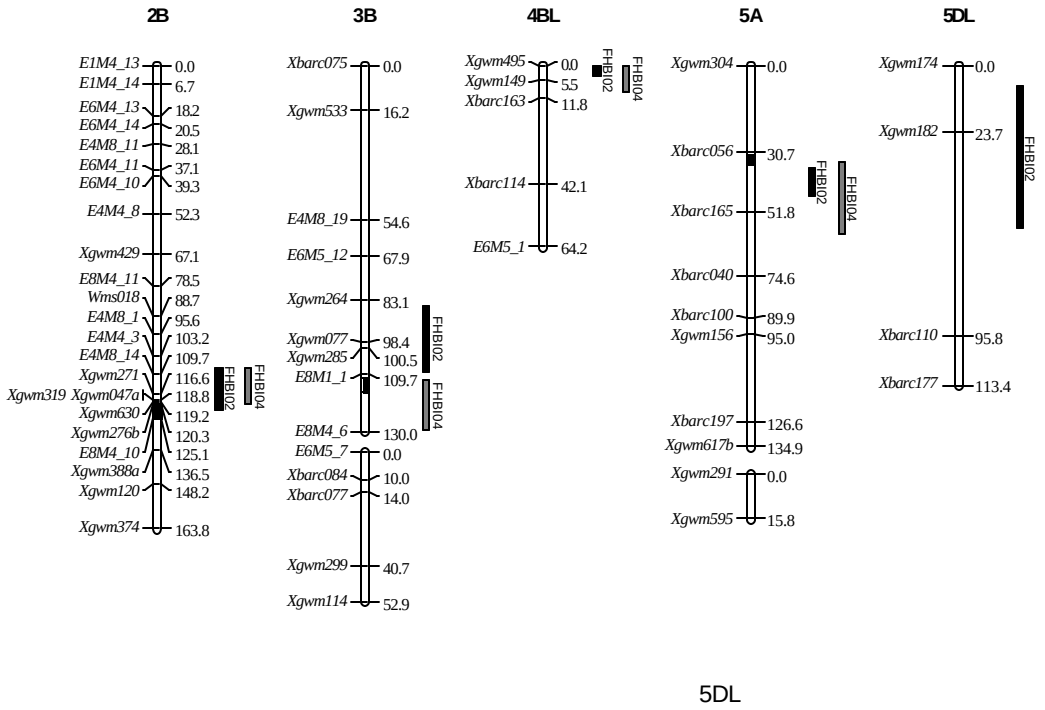

Figure 1. QTL associated with type II scab resistance in the soft red winter wheat cross Ernie x MO 94-317. Abbreviations are defined as follows: FHBIWW and FHBI = Fusarium head blight resistance index with and without wilted spikelets; SpreadWW and Spread = number of diseased spikelets with and without wilted spikelets, in the inoculated head

Table 2. Estimates (± SE) of genetic effects for disease spread and the Fusarium head blight index (FHBI) from 6 generations of the soft red winter wheat cross Ernie/MO 94-317 following greenhouse inoculation with *Fusarium graminearum*.

Parameter†	Disease spread	FHBI
m	6.78 ± 0.17	52.17 ± 1.00
[d]	5.13 ± 0.18	37.70 ± 1.01
[h]	-2.71 ± 0.39	-21.48 ± 2.41
[j]	-3.15 ± 1.02	-21.80 ± 6.52
$?^2$	4.32	2.52
P	0.120	0.280

† m = mid-parent value, [d] = the additive genetic effect, [h] = the dominance genetic effect, [j] = the additive by dominance interaction effect, $?^2$ is chi-squared value testing the goodness-of-fit of the genetic model to the data, and *P* is the probability associated with the $?^2$ statistic.

"STEELE-ND": A NEW HARD RED SPRING WHEAT CULTIVAR WITH NOVEL SOURCE OF RESISTANCE TO FUSARIUM HEAD BLIGHT

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OBJECTIVES

To develop new improved hard red spring wheat (HRSW) cultivar that combines novel source of resistance to Fusarium head blight (FHB) disease and superior grain yield and bread-making quality.

INTRODUCTION

Fusarium head blight, commonly known as scab, has been a serious threat to wheat production throughout the world (Schroeder and Christenson, 1963; Bai and Shaner, 1994; McMullen et al., 1997; Stack, 2003). In North America, FHB is caused mainly by *Fusarium graminearum* Schwabe [telomorph *Gibberella zeae* (Schwein.)] (Bai and Shaner, 1994; McMullen et al., 1997). Regionally, wheat scab has been a major disease for hard red spring wheat (HRSW) produced in North Dakota and neighboring states since 1993. Recent reports (Nganje et al., 2004) estimate combined direct and secondary economic losses caused by FHB for all crops were at $7.7 billion. Two states, ND and MN, account for about 68% ($5.2 billion) of the total dollar losses. Direct economic losses for wheat only were estimated to $2.492 billion from 1993 trough 2001 (Nganje et al., 2004). The use of genetically resistant cultivars is the most efficient and economical method of controlling this disease in HRSW. As a matter of fact, in 2002, 2003, and 2004, "Alsen", a moderate FHB resistance cultivar derived from the Chinese source "Sumai 3", released in 2000 by NDSU (with the support of the scab initiative funds) was planted on more than 2.1, 2.4, and 1.9 million acres representing 30.8, 37.4, and 28.9% of ND wheat acreages, respectively (N.D. Agricultural Statistics Service, USDA. 2002; 2003; 2004). The rapid increase in acreage planted to 'Alsen' indicates the desire of ND wheat growers to produce such HRSW cultivars. However, the use of Chinese material as source of resistance to FHB also brings with it the problem of genetic vulnerability risks that may be caused by the lack of genetic diversity for FHB resistance. Therefore, any new HRSW cultivar with FHB resistance different from Sumai 3 is warranted.

MATERIALS AND METHODS

Steele-ND was selected from the cross 'Parshall' /5/ 'Grandin' /3/IAS20*4/H567.71//'Amidon' /4/ Grandin*2/'Glupro' that was made in 1996. The F_1 was grown in the greenhouse and F_2 grown in the field at Casselton, ND in spring and summer of 1997. Two hundred spikes were selected from F_2 and advanced to F_3 in the greenhouse in fall of 1997 using single seed descent. Selection of spikes in the F_2 generation was based on agronomic appearance and reactions to FHB and foliar diseases. One spike from each F_3 plant was selected, harvested, threshed, and planted in one $F_{3:4}$ row plot in 1998 at the Casselton Experimental Station, ND. The selected $F_{3:4}$ plot of Steele-ND was harvested, threshed in bulk, and included in the Preliminary Yield trial (PYT) as an $F_{3:5}$ at two locations in the Summer of 1999. Ten $F_{3:5}$ selected spikes from the Casselton, ND PYT were harvested, threshed in bulk, and sent to Christchurch, New Zealand (NZ) in 1999-2000 for seed increase and generation advancement ($F_{3:6}$) in a four row 5 m long plot. $F_{3:7}$ seed from the NZ increase was used to establish the Advanced Yield Trial (AYT) at four locations in ND in 2000. Subsequently, the line was tested as ND 741 ($F_{3:8}$) in the ND Variety Trials (NDVT) in seven locations in ND from 2001 to 2003. Steele-ND was also tested in the HRSW Uniform Regional Nursery (URN) and Uniform Regional Scab Nursery (URSN) from 2001 to 2003 in North Dakota, Minnesota, South Dakota, Nebraska, Montana, Wyoming, Washington, and Manitoba, Canada. While the PYT were planted in two replicates at two locations, the AYT and NDVT

were planted in four replicates at four locations. The plot sizes of the PYT and AYT were 7 rows, 17 cm apart, and 3m long. The plot size of NDVT however, is larger than used PYT and AYT's but variable depending on the site.

RESULTS AND DISCUSSIONS

Reaction to FHB and leaf diseases - Based on 12 field site-years in ND mist-irrigated and artificially inoculated FHB, URN, and URSN nurseries conducted between 2001 and 2003 on Steele-ND is moderately resistant to FHB. Average FHB severity for Steele-ND was 35.5% comparable to Alsen (34.7%) but significantly ($p<0.01$) lower than the susceptible check '2398' (72.6%). Visual scabby kernels of Steele - ND (26%) was also very low ($p<0.01$) compared to the susceptible check 2398 (69%), but similar to Alsen (25%). Steele-ND does not include 'Sumai 3' in its pedigree and the source of resistance is being investigated. A population of 212 $F_{2:7}$ recombinant inbred lines (RIL) derived from the cross of FHB susceptible line, ND 735 with Steele-ND was developed for the purpose of mapping the FHB genes involved in Steele-ND. Preliminary results (Mergoum, unpublished data, 2004) on molecular marker *Xgwm533*, which maps to 3B, the location of QTL for genes for resistance coming from Sumai 3 (Anderson et al., 2001), showed that resistance in Steele-ND is different from Sumai3.

The seedling and adult plant screening tests conducted under greenhouse conditions from 2001-2003 showed that Steele-ND posses high level of resistance to pathotype THBL, the predominant race of leaf rust (caused by *Puccinia triticina* Eriks.) in the region. Steele-ND was evaluated from 2000 to 2003 at the USDA-ARS, Cereal Crop Research Unit, Fargo, ND for resistance to stem rust (caused by *Puccinia graminis* Per.:Pers. f. sp. *tritici* Eriks. & E. Henn) and was found to be highly resistant to pathotypes Pgt-QCCJ, -QTHJ, -RTQQ, -TMLK, -TPMK, and -HPHJ. Steele-ND was also screened in the greenhouse for Septoria nodorum [caused by *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano] and tan spot [caused by *Pyrenophora tritici-repentis* (Died.) Drechs]. On a scale of 1 to 5 where 1 is resistant and 5 susceptible, Steele-ND had average scores of 4 and 3 in reaction to Septoria nodorum and tan spot compared to 5 and 5 for the susceptible cultivar Alsen and 1 and 1 for the resistant check 'Erik', respectively.

Agronomic performance and quality parameters - Grain yield of Steele-ND (3958 kg ha^{-1}) over 23 site-years of testing in the NDVT and AYT was similar to 'Reeder' (3951 kg ha^{-1}) and Parshall (3843 kg ha^{-1}), but significantly ($p<0.05$) higher than Alsen (3716 kg ha^{-1}). In the URN trials conducted from 2001 to 2003 (47 site-years), Steele-ND yielded 3682 kg ha^{-1} compared to 3507, 3562, and 2647 kg ha^{-1} for 'Keene', 'Verde', and 'Chris', respectively (LSD0.05, 163 kg ha^{-1}). Mean grain volume weight of Steele-ND (770 kg m^{-3}) over 16 site-years in NDVT was similar to Reeder (769 kg m^{-3}) and Alsen (765 kg m^{-3}), but significantly ($p<0.05$) lower than 'Dapps' (799 kg m^{-3}) and Parshall (780 kg m^{-3}). In the URN trials however, Steele-ND averaged significantly ($p<0.01$) higher grain volume weight (768 kg m^{3}) than Chris (731 kg m^{-3}), Verde (754 kg m^{-3}), and Keene (754 kg m^{-3}). Similarly, grain protein of Steele-ND (158 g kg^{-1}) was comparable to Reeder (157 g kg^{-1}) and Parshall (160 g kg^{-1}), but lower ($p<0.05$) than Alsen (163 g kg^{-1}) and Dapps (162 g kg^{-1}).

Flour yield for Steele-ND from 19 trials averaged 703 g kg^{-1} compared to 692, 691, and 681 g kg^{-1} for Alsen, Parshall, and Reeder, respectively (LSD0.05, 15 g kg^{-1}). Water absorption was 66.6%, significantly $p<0.05$) higher than Reeder (64.3%) and Parshall (64.7%), but not different from Alsen (65.3%). Mixogram mix time (after 3 hrs fermentation) was 2.25 min, greater ($p<0.05$) than Reeder (2.00 min), similar to Parshall (2.30 min) and less than Alsen (2.40 min). The mixing tolerance of Steele-ND (18.2 min) was longer ($p<0.05$) than Reeder (16.4 min) and comparable to Alsen (19.5 min) and less than Parshall (20.1 min). Loaf volume was 1126 mL, comparable to Parshall (1126 mL) and Alsen (1110 mL), but superior ($p<0.05$) to Reeder (1084 mL).

Plant height of Steele-ND (82 cm) is similar to 'Gunner', 5 cm taller than 'Alsen', and 3 cm shorter than Parshall in 19 site-years of NDVT. Steele-ND heads on average (60 d after planting) 1 d later than Alsen and 1 d earlier than Gunner. Steele-ND has

moderate resistance to grain shattering, comparable to Alsen, and has medium straw strength that is similar to Gunner.

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Table 1. Fusarium head blight (FHB) severity and Visual scabby kernels (VSK); leaf rust (LR), stem rust (SR), tan spot (TS), and Septoria nodorum (SN) reactions of hard red spring wheat Steele-ND, Alsen and 2398 grown under filed and greenhouse conditions, Fargo, ND 2001-2003.

Genotype	FHB Severity (%)	FHB VSK (%)	LR*	SR*	TS (1-5)	SN (1-5)
Steele-ND	35.5	26	R	R	4	3
Alsen	34.7	25	MS	R	5	5
2398	72.6	69	R	R	-	-

*R=Resistant and MS=Moderate susceptible

Table 2. Agronomic and quality performances of hard red spring wheat Steele-ND, Alsen, Reeder, and Parshall grown in North Dakota during the period of 2000-2003.

Cultivar	Agronomic traits*			Quality parameters**						
	GY Kg/ha	PH cm	DH d	GVW Kg/m^3	GP g/kg	FE g/kg	WA %	MT min	MT L min	LV ml
Steele-ND	3958	82	60	770	158	703	66.6	2.25	18.2	1126
Reeder	3951	78	58	769	167	681	64.3	2.00	16.4	1084
Alsen	3716	77	59	765	163	692	65.3	2.40	19.5	1110
Parshall	381	85	61	780	160	691	64.7	2.30	20.1	1126

*GY=Grain yield; PH=Plant height; DH=Days to heading.

** GVW=Grain volume weight; GP=Grain protein; FE= Flour extraction; WA=Water absorption; MT=Mixing time; MTL=Mixing tolerance; LV=Loaf volume.

BREEDING STRATEGIES AND THEIR RESULTS AGAINST FHB IN WHEAT

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OBJECTIVE

Compare the usefulness of different genetic approaches and evaluate effective selection methods to increase FHB resistance in wheat.

INTRODUCTION

The curiosity of the FHB is that genetic background and symptom development can be very far because disease development is strongly influenced by morphological factors (Mesterházy 1987), aggressiveness of inoculum (Mesterházy 1983, 1995), duration of wet period of the head, the complicated genetic background and the long flowering period of the wheat genotypes. During the disease development different resistance components influence symptom severity and other traits like FDK, yield loss, toxin contamination and others (Mesterházy 1995). They often overlap each other. The result is far diverging results in resistance tests between years, locations and other conditions. The phenotyping of DH populations for medium and lower effective QTLs shows clearly this (Mesterházy and Buerstmayr, unpublished, Chen et al. 2003). For this reason the determination of resistance needs careful work. For this several methodical rules should be applied (Mesterházy 1997). As higher resistance results mostly in lower toxin values (mostly DON content, Mesterházy et al. 2002), to increase food and feed safety means first of all to increase resistance.

Actually we can use different genetic strategies to increase resistance: A/ The spring wheat Sumey-3 QTLs from different crosses is extensively used and generally secures rather high resistance level. The 3BS QTL can be identified now in MAS as a service for breeders of US. The problem is here that DH lines with 3BS show highly significant differences. Therefore not every plant with 3BS QTL will have high resistance. Adapted Nobeoka Bozu lines are only in Hungary (Mesterházy et al. 1999) and their resistance is comparable to that of the Sumey-3, but QTLs for this cultivar have not been identified until now. Evaluations of DH populations are in progress. B/ We can use more adapted plant material with known less effective QTLs like from Frontana or Arina. C/ based on our long year's observations we can use genotype with superior genotypes in crossing programs like cvs Renan, Bence, etc. D/ We should check the FHB resistance of genotypes from crosses made not for FHB resistance as the experience shows that also here superior genotypes might be identified.

MATERIALS AND METHODS

In this paper the results of the 2002 and 2003 years will be summarized. All inoculations were made with two *Fusarium graminearum* and two *F. culmorum* isolates as described by Mesterházy (1995). Here the mean reactions to the isolates will be listed.

RESULTS AND DISCUSSION

In the past 30 years we had tested all possibilities. The most intensive work was made with Sumey3 and Nobeoka Bozu (A). Frontana and Arina were not used as the spring wheat cvs in group A provided a much higher resistance so their domestication did not seem to be necessary (B). Case C was represented by several crosses, but D was searched more intensively as the whole advanced material of the institute together with candidates for cultivar and released cultivars were screened since 20 years with about 1500 advanced lines. Depending of the year 20 30 % of the geno-

types tested had superior resistance to the check cvs. Table 1 and 2 presents the group of genotypes bred for FHB resistance. In Table 2 only the group means will be presented. All Nobeoka Bozu progenies are winter wheats with good agronomic properties. Among Sumey-3 progenies also spring wheats are present mostly from the cross Sgv/NB//MM/Sum3, but the lines from the other crosses are winter wheats. The resistance is highest in this group. However, the very low FHB values are not always accompanied by excellent FDK or DON data. The entries 163 and 210 have high FDK and DON ratios. This is the phenomenon late blighting. All resistance sources have DON content at about 10 ppm.

From the winter wheat crosses we have lines with higher susceptibility. FDK and DON means are four fold higher. The next group, where through large scale screening tests plus variants were found, has the same performance than the lines from the winter wheat crosses for FHB except the DON data that are lower. The susceptible control cultivars have the worst data in all respect. The situation is similar also for 2003.

Fig. 1 shows the FHB-FDK regression form 2002. Numerous genotypes with low or lower FHB have high FDK values. At about 20 % FHB FDK data are between 13 and 70 %. Similar data are present for FHB-DON (Fig.2) where at 30 % FHB DON varied between 6,2 and 101 ppm; and for FDK-DON (not shown). So we select first to low FHB severity; than FDK should be checked and discard the highly infected ones. At last, a DON test can discard the entries with higher DON contamination.

The data show that strategically the highly resistant spring wheats give the highest resistance level. The use of Nobeoka Bozu results in a similar resistance as Sumey-3, therefore to decrease possible disadvantages of only use of Sumey-3, could be highly important worldwide. We have these lines. By their use in winter wheat winter hardiness problems might arise. Except several genotypes the yielding ability of such genotypes is lower than that of the control cultivars. It means that the third generation materials will be most useful for cultivar breeding. When superior winter wheats are used for crossing, the resistance achieved is similar we find at screening large scale materials from non FHB programs. Their best genotypes have similar FHB resistance than the more susceptible genotypes of the first group. We think that this resistance level is sufficient to combat medium level epidemics mostly present in Hungary with additional fungicide treatment when necessary. For higher level of epidemics highly effective QTLs are necessary to secure good or excellent food safety. Such genotypes are needed for organic farming. It is important that the winter wheat in Europe contains considerable resistance to FHB and can decrease infection severity by two third compared to the susceptible popular cultivars. Variety offices have the task to ban the way of the susceptible cultivars into the commercial production and withdraw the highly susceptible ones as soon as possible.

Screening experience teaches us that for large scale screening a variant of the spraying inoculum or deployment of infected corn supplied with mist irrigation is the most effective. For this reason resistance sources for Type I are as important as Type II. The use of other resistance types like resistance to kernel infection and DON are highly important and helpful. It is suggested to use different genetic approaches at the same time to increase efficacy of the breeding work.

We have to evaluate or adapt breeding technologies that allow effective screening of segregating populations. They differ in efficacy, exactness, but all make possible large scale screening in these populations. This is even more important as effect of smaller and medium effective QTLs cannot be forecasted exactly. Therefore the resistance differences can be identified only in the nursery. As breeding is a continuous work, misidentification of the resistance can be improved in the next generation when susceptible plants have lower infection value (escape or other reasons). When the case is the opposite, e. g. moderate resistant plants with high FHB values (for example longer mist irrigation for early genotypes) can be discarded and lost.

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Fig. 1. Relationship between FHB and FDK at 237 wheat genotypes, 2002

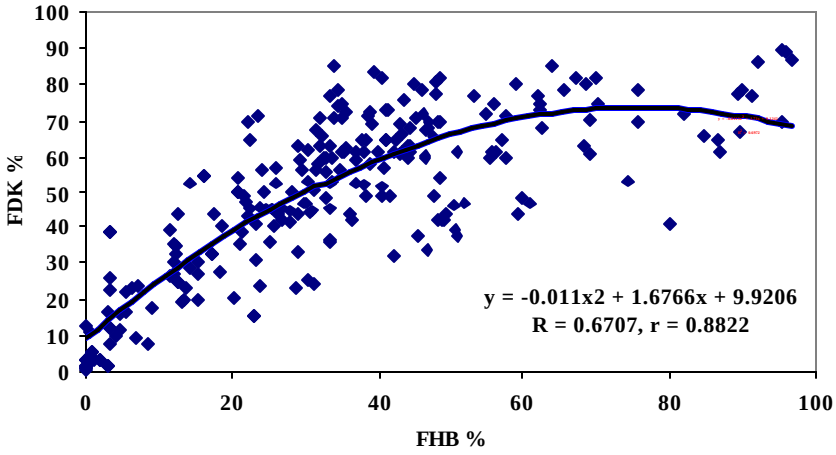

Fig. 2. Regression for FHB and DON, 2003

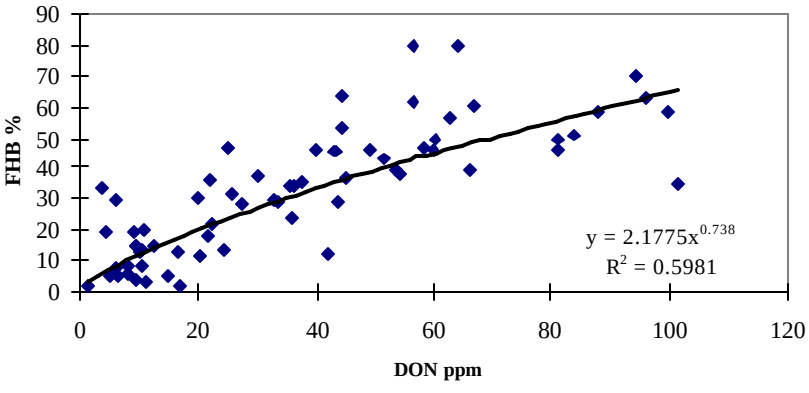

Table 1. FHB resistance of genotypes originating from different breeding approaches, 2002.

Plot No.	Genotype	FHB %	FDK %	DON ppm	Rel yield %
	Resistance sources and their progenies				
454	Sgv/NB//MM/Sum3	0.00	0.67	5.20	75.68
276	Sgv / NB // MM / Sum3	0.00	1.00	1.90	-
278	Sgv / NB // MM / Sum3	0.00	1.67	2.30	-
277	Sgv / NB // MM / Sum3	0.00	3.50	3.40	-
150	Wuhan 6B	0.03	12.56	6.60	92.16
156	Sumey3	0.54	11.22	7.40	104.12
317	Zu // Ré / NB	0.55	2.83	8.10	76.81
292	Sgv / NB // MM / Sum3	0.72	5.33	7.90	-
195	Zu // Ré / NB	0.99	3.11	3.90	95.49
286	Sgv / NB // MM / Sum3	2.03	3.33	3.90	-
183	Sum3/81.61//Ko	3.06	16.67	8.20	56.90
289	Sgv / NB // MM / Sum3	3.11	1.67	11.60	-
179	Sum3/81.61//Ko	3.15	7.61	5.30	64.23
163	Sgv/NB//MM/Sum3	3.18	26.00	13.50	68.90
210	St 902 /3/ Sgv /NB // MM / Sum 3	3.22	38.33	21.40	-
209	St 902 /3/ Sgv /NB // MM / Sum 3	3.36	12.33	8.70	-
176	RST/NB	4.12	9.67	8.30	69.73
159	Nobeoka Bozu	4.68	11.56	4.50	66.78
172	RSt//MM/NB	5.40	16.50	6.00	60.58
169	Sgv/NB//MM/Sum3	5.50	21.78	13.40	74.67
175	RST/NB	6.86	9.17	5.20	67.33
282	Sgv/NB//MM/Sum3	8.53	7.67	5.70	76.62
239	Frontana	7.00	23.56	7.00	57.04
	Mean	2.87	10.77	7.36	73.80
	Genotypes from WW crosses				
301	84.42 / 85.50	11.32	26.33	13.20	69.75
143	85.92 // Ko / In	12.63	25.00	13.30	-
189	BeSK48.21	16.20	55.00	35.10	-
196	Ttj/F379	17.55	43.67	28.80	52.92
177	RSt	18.35	27.56	28.90	44.53
53	80.1.61 // Rst / NB	21.11	35.00	20.40	55.32
52	Ke / SO 89.807	21.33	38.67	20.20	49.34
442	Ttj / RC 103	25.95	53.00	17.40	55.80
299	Zu / 85.50	30.21	62.11	23.10	49.38
115	Várkony	27.74	44.44	36.00	57.37
		22.49	45.64	26.27	62.06

Table 1. cont.

	Cultivars from non FHB programs				
306	B 1201	18.08	47.72	22.31	57.15
99	Tiszatáj	23.47	71.67	19.00	60.19
123	Smaragd	25.42	45.00	16.00	-
91	Attila	25.53	39.89	10.10	68.49
82	Tenger	30.43	44.17	27.40	58.23
90	Héja	38.19	48.67	19.10	51.37
		26.85	49.52	18.99	59.09
	Popular susceptible control varieties				
13	Zugoly	44.57	80.00	51.20	32.88
77	Élet	45.91	72.22	45.60	48.26
107	Miska	47.89	77.50	68.60	43.56
104	Favorit	48.07	80.83	53.00	44.21
78	Kalász	48.42	70.00	82.30	35.87
85	Verecke	59.14	80.00	75.00	37.70
	Mean	49.00	76.76	62.63	40.41
	LSD 5 %	3.13	6.99	20.34	7.20

Table 2. FHB resistance of genotypes originating from different breeding approaches, 2003.

		Group means			
Plot No.	**Genotypes**	**FHB %**	**FDK %**	**DON ppm**	**Rel.yield %**
	Resistance sources and their progenies	12.00	19.40	9.10	64.00
	Genotypes from WW crosses	26.50	48.80	26.90	44.60
	Cultivars from non FHB programs	35.80	48.10	34.60	31.00
	Popular susceptible control cultivars	55.23	65.78	75.28	29.67

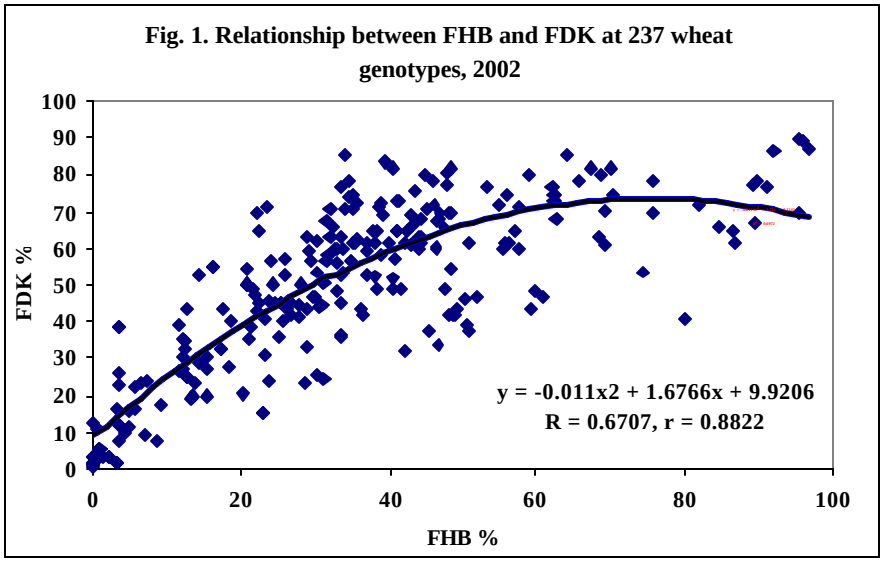
Fig. 1. Relationship between FHB and FDK at 237 wheat genotypes, 2002
FDK %
FHB %
y = -0.011x2 + 1.6766x + 9.9206
R = 0.6707, r = 0.8822
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100

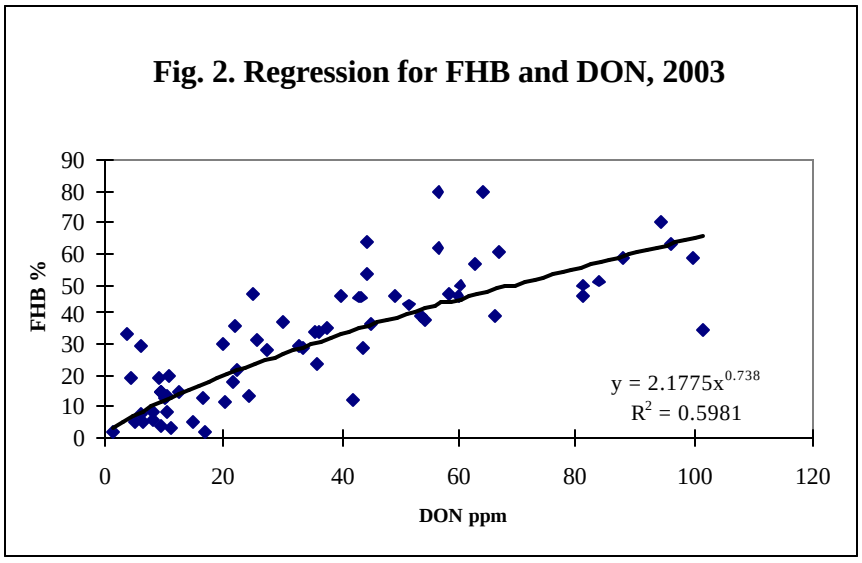
Fig. 2. Regression for FHB and DON, 2003
FHB %
DON ppm
y = 2.1775x^0.738
R2 = 0.5981
0
10
20
30
40
50
60
70
80
90
0
20
40
60
80
100
120

RESISTANCE OF GENOTYPES OF THE UNIFORM SOUTHERN SOFT RED WINTER WHEAT FHB NURSERY AGAINST OUR ISOLATES OF *FUSARIUM*

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ObJECTIVES

Objectives of the current study were to describe FHB reaction of the nursery against two-two isolates of *F. graminearum* and *F. culmorum*. Additional task was to characterize the genotypes by different field traits like yield, resistance to other diseases and quality.

INTRODUCTION

The USSRWW is an important ring test to evaluate FHB resistance of new breeding lines. In 2004 38 lines were evaluated and we added five good ARGE lines from the last year nursery. As results show, the epidemic in this year was stronger than in the last year.

MATERIALS AND METHODS

The experimental design corresponds to Mesterhazy et al. (1999). In each cultivar a 5 m^2 plot was sown, in each plot 4 (2+2) isolates were used for *F. graminearum* and *F. culmorum* in three replicates. A replicate meant 15-20 heads sprayed by the inoculum and covered 48 hrs by polyethylene bags. Additional mist irrigation has not been given. Following harvest 10 heads/head of group were separated, threshed carefully. The grain was measured and FDK was evaluated as most important resistance trait for FHB besides DON response. Also plots were harvested and NIR quality test was made. During vegetation period powdery mildew, leaf rust, yellow rust and leaf spots were evaluated. The data are comparable except the several genotypes inoculated at 01.06.2004 (480, 482, 489, 504) as here the bag coverage lasted three days, we received on Thursday 100 mm rain and we could not remove bags only one day later.

RESULTS AND CONCLUSION

Table 1 presents the FDK values as most important parameter.

The genotypes provide a wide range from 4.92 to 67.50. It seems that the materials with good resistance increased from 2003. It is important to compare *F. graminearum* isolates with the *F. culmorum* isolates. In this case isolate 3 and 4 caused very low symptom severity and only the most susceptible genotypes provided significant FDK values. 1 with

In Table 2, the other FHB and field data will be shown. The ANOVA shows highly significant genotype effect and the difference between isolates is also significant. Interaction is low, but significant; the source is the different aggressiveness behavior.

The tendency is similar for the other FHB traits we have seen for FDK. The correlations are very close between reactions to the different traits meaning that low FHB values normally mean low yield loss and low FDK values. There are several diverging genotypes like the entry 503 and 481 with higher FDK values than usual at this FHB value. At 490 we have the opposite situation, less FDK was found than accepted. The correlation between yield an FHB traits are at 0.12-0.15, not significant. For this reason, when further test will bring similar results, the higher resistance should not mean automatically low yielding ability. Several lines have excellent overall resistance, but others are highly susceptible to yellow rust ((Cooker), leaf rust or powdery mildew. Wet gluten is except several is over 28 %, but gluten quality was not measured. It seems that resistance of the lines tested is much better than in 2003 (Murphy et al. 2003).

ACKNOWLEDGEMENTS

Authors are indebted for support to OTKA project TS040887 and D38486, FVM 38194/2003 for technology development as well as.

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Murpy, J. P., Navarro, R. A. and D. A. van Sanford, 2003. Uniform Southern Soft Resd winter Wheat FHB Screening Nursery . 2003 Nursery Report. North Carolina State Univ., Dept. Crop Sci. 28 pp.

Table 1. FDK evaluation (%) of the harvested plant material, 2004.

Plot No.	Genotype	Isolates				Mean
		12377 Fg	40 FG	12375 Fc	12551 Fc	
487	ARGE 97-1033-10-2	16.67	2.67	0.00	0.33	4.92
486	ARGE 97-1043-6a-5	18.33	4.00	0.00	0.00	5.58
511	NCO1-27308	11.67	4.67	0.00	6.67	5.75
492	VAO3W -646	23.33	5.67	2.67	1.00	8.17
512	NCO1-27809	7.67	11.00	0.00	16.67	8.83
278	ARGE 97-1047-4-2	22.50	17.50	0.00	0.00	10.00
477	B 011117	25.00	20.00	0.33	0.00	11.33
502	BERETTA	30.00	21.67	0.33	0.33	13.08
279	ARGE 97-1048-3-6	27.50	27.50	0.00	1.00	14.00
491	VAO3W -647	36.67	15.00	3.33	1.67	14.17
493	VAO3W -671	33.33	25.00	0.00	1.00	14.83
494	VAO3W -672	40.00	21.67	0.33	0.00	15.50
488	ARGE 971064-13-5	43.33	18.33	1.33	0.00	15.75
490	VAOOW -526	30.00	31.67	2.33	2.00	16.50
501	MD 27-37	36.67	31.67	0.33	1.33	17.50
276	ARGE 97-1033-3-5	50.00	17.50	3.50	3.50	18.63
507	F 96035G11-2	40.00	36.67	1.00	1.33	19.75
485	ARGE 97-1022-5-1	36.67	43.33	1.00	0.00	20.25
509	F 98198G2-1	60.00	30.33	0.00	0.00	22.58
499	GA 951216-2E26	46.67	40.00	3.33	3.33	23.33
506	D 99-5528	56.67	38.33	0.00	2.00	24.25
277	ARGE 97-1042-4-5	55.00	40.00	3.00	2.50	25.13
483	AR 857-1-2	53.33	46.67	0.00	0.67	25.17
505	DOO*6874	56.67	45.00	3.33	2.00	26.75
275	AR 857-1-1	70.00	35.00	0.00	3.00	27.00
496	GA 951079-2E31	63.33	43.33	1.33	2.00	27.50
508	F 96502G4-104	50.00	56.67	2.67	1.67	27.75
503	DOO 6383	76.67	38.33	0.00	0.33	28.83

Table 1 cont.

Plot No.	Genotype	Isolates				Mean
		12377 Fg	40 FG	12375 Fc	12551 Fc	
503	DOO 6383	76.67	38.33	0.00	0.33	28.83
481	B 010098	53.33	56.67	3.00	3.67	29.17
500	GA 951079-2A25	65.00	50.00	1.67	1.33	29.50
478	B 990081	60.00	46.67	9.00	3.33	29.75
484	AR 93019-2-1	60.00	56.67	1.33	4.67	30.67
497	GA 95652-2E56	73.33	53.33	4.67	4.00	33.83
479	B 006624	70.00	36.67	17.33	16.00	35.00
475	ERNIE	70.00	70.00	1.67	4.00	36.42
510	NCO1-26765	68.33	60.00	20.00	6.67	38.75
495	VAO3W -652	60.00	65.00	1.00	33.33	39.83
489	ARGE 97-1008-3-3	60.00	46.67	21.67	43.33	42.92
498	GA 951216-2E14	83.33	56.67	31.67	25.00	49.17
504	DOO*6847	83.33	70.00	36.67	60.00	62.50
476	COKER 9835	95.00	88.33	21.67	53.33	64.58
480	B006693	83.33	73.33	30.00	83.33	67.50
482	PAT	90.00	80.00	43.33	56.67	67.50
Mean		50.99	39.05	6.39	10.53	26.74
LSD 5 %						1.04

ANOVA

Source of var.	*SS*	*df*	*MS*	*F*	*F crit.*
Genotype A	134410.26	42	3200.24***	50.51	1.42
Isolates B	182713.73	3	60904.57***	961.33	2.63
AxB	49230.73	126	390.72***	6.17	1.26
Within	21794.00	344	63.35		
Total	388148.72	515			

*** P= 0.001

Table 2. FHB performance and other traits at the Uniform Southern Soft Red Winter Wheat FHB Nursery, 2004 (Data:means for four issolates)

Plot No.	Genotype	Traits			
		FHB %	FDK %	Yd loss%	Yield kg/plot
487	ARGE 97-1033-10-2	1.97	4.92	11.81	2.35
486	ARGE 97-1043-6a-5	2.05	5.58	22.31	2.76
507	F 96035G11-2	3.38	19.75	21.34	2.51
511	NCO1-27308	3.57	5.75	20.30	3.70
485	ARGE 97-1022-5-1	4.38	20.25	20.80	3.10
477	B 011117	4.40	11.33	17.93	3.70
493	VAO3W -671	4.61	14.83	20.20	3.07
279	ARGE 97-1048-3-6	4.92	14.00	21.24	3.41
502	BERETTA	6.72	13.08	16.15	4.13
492	VAO3W -646	7.73	8.17	10.84	3.13
508	F 96502G4-104	8.37	27.75	24.49	3.38
481	B 010098	8.60	29.17	37.11	1.71
488	ARGE 971064-13-5	8.75	15.75	19.04	3.00
503	DOO 6383	9.08	28.83	24.27	3.51
491	VAO3W -647	9.82	14.17	21.95	3.94
512	NCO1-27809	10.32	8.83	17.16	4.08
506	D 99-5528	10.82	24.25	28.47	3.07
509	F 98198G2-1	10.94	22.58	25.71	2.93
497	GA 95652-2E56	11.03	33.83	27.67	3.83
501	MD 27-37	11.69	17.50	19.10	3.16
483	AR 857-1-2	12.13	25.17	32.29	2.98
505	DOO*6874	12.19	26.75	30.78	3.61
494	VAO3W -672	12.94	15.50	29.43	3.22
278	ARGE 97-1047-4-2	13.35	10.00	23.37	3.66
499	GA 951216-2E26	13.88	23.33	35.16	3.87
276	ARGE 97-1033-3-5	14.06	18.63	28.30	2.83
484	AR 93019-2-1	15.38	30.67	37.08	3.48
490	VAOOW -526	15.41	16.50	31.13	4.23
275	AR 857-1-1	15.81	27.00	28.23	3.16
496	GA 951079-2E31	16.82	27.50	18.23	3.46
478	B 990081	17.22	29.75	42.21	4.56
475	ERNIE	17.49	36.42	46.77	3.48
500	GA 951079-2A25	19.13	29.50	11.13	3.40
510	NCO1-26765	21.19	38.75	28.08	3.19
479	B 006624	24.11	35.00	46.00	3.54

Table 2. cont.

Plot No.	Genotype	Traits			
		FHB %	FDK %	Yd loss%	Yield kg/plot
277	ARGE 97-1042-4-5	24.58	25.13	23.00	2.66
498	GA 951216-2E14	25.15	49.17	38.59	3.96
495	VAO3W -652	25.33	39.83	39.02	3.55
476	COKER 9835	40.57	64.58	66.60	3.90
489	ARGE 97-1008-3-3	43.71	42.92	53.28	2.46
482	PAT	61.56	67.50	63.29	3.59
504	DOO*6847	62.05	62.50	62.42	3.64
480	B006693	65.28	67.50	56.49	3.19
Mean		17.03	26.74	30.20	3.35
LSD 5 %		1.04	6.36	6.89	‘-

Correlations between responses to traits

Traits	FHB %	FDK %	Yield loss%
FDK %	0.8915***		
Yield loss%	0.8348***	0. 8717***	

*** P= 0.001

QUANTITATIVE-GENETIC ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANCE AND DON CONTENT IN EUROPEAN WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is caused in Germany by *Fusarium graminearum*, *F. culmorum* and some minor important species. Winter wheat is the most important crop grown on 3.2 Million hectar. For deoxynivalenol (DON) an advisory level of 0.5 mg kg^{-1} in cleaned cereals has been passed by the German Government in 2004. Several European winter wheat varieties have been described as resistant to FHB. For estimating selection gain, we want to analyse (1) genotypic variation in five populations of European winter wheats for FHB rating, (2) heritability in large-scale testing, (3) covariation between FHB rating and DON content in one population. Five $F_{2:4}$ (1^{st} year) and $F_{2:5}$ (2^{nd} year) populations derived from single crosses among resistant (Piko, Arina), moderately resistant (Ambras, Pegassos) and highly susceptible (Ronos, Kontrast) parents were tested at three to five environments (=location x year combinations). Ninety-five progeny per population were grown in drilled two-row microplots in three replicates and inoculated by *F. culmorum*. We rated FHB severity on a 1-9 scale (1 = no symptoms, 9 = >95%, respectively) and analysed DON by an immunoassay. Mean disease severity across populations was medium to high. The parental means generally resembled the means of their respective progeny. Significant ($P<0.01$) genotypic and genotype x environment interaction variances of similar size were detected. Heritability was medium to high. All populations showed a quantitative distribution for FHB rating. Significant transgressive segregants in both directions were found in the Arina x Piko and Pegassos x Ambras populations, although the parents were quite similar. Mean DON content in the Arina x Kontrast population ranged from 22 to 87 mg kg^{-1}, heritability was 0.81. High coefficients of phenotypic and genotypic correlations ($r=0.85$ and 1.0, resp.) were observed between FHB rating and DON content with the parents representing the extremes of the distributions for both traits. In conclusion, high entry-mean heritabilities and large genetic variation within populations, even when crossing parents were rather similar, should lead to a high expected selection response for FHB rating in multi-environmental tests. Similar parental and progeny means indicate an oligo-/polygenic inheritance with mainly additive gene action. A cconsiderable indirect selection gain should be realized for low DON content by selecting for less FHB symptoms. Based on these results it should be feasible to reduce DON content in the grain and to increase FHB resistance level by recurrent selection within European genotypes as a promising alternative for the use of non-adapted germplasm.

CONTRIBUTIONS OF THE ARKANSAS WHEAT PROGRAM TOWARD THE DEVELOPMENT OF FHB-RESISTANT SOFT RED WINTER WHEATS

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OBJECTIVES

To develop FHB-resistant soft red winter wheat cultivars adapted to the Midsouth, to develop FHB-resistant lines suitable for use as parents in breeding programs, and to assist other winter wheat breeding programs with selecting lines for resistance to FHB and other diseases.

INTRODUCTION

Wheat cultivars resistant to Fusarium head blight (FHB) are likely to be an important component of any integrated management strategy for FHB. Before FHB-resistant cultivars will be accepted by growers in the Midsouth, FHB resistance will need to be incorporated into agronomically suitable, high-yielding cultivars with adequate resistance to other limiting diseases. The Arkansas program has taken a short-term approach of crossing sources of resistance to adapted cultivars in order to develop FHB-resistant cultivars for the Midsouth as quickly as possible and a long-term approach of parent building, that is, pyramiding resistance genes in agronomically suitable backgrounds. To facilitate the development of winter wheat cultivars resistant to FHB, the Arkansas program also evaluates advanced breeding lines from all winter wheat programs involved in developing FHB-resistant cultivars, early-generation populations and lines from breeding programs in the Midsouth, and likely sources of resistance in both winter and spring backgrounds.

MATERIALS AND METHODS

The Arkansas breeding program has been actively involved in developing wheat using scab-resistant sources since the 1991 FHB epidemic in Arkansas. Originally, the resistance genes were from CIMMYT (derived from Chinese and South American lines) and Eastern Europe (Romania and Yugoslavia). Locally adapted lines from those crosses have now been used as parents for currently segregating populations. In 2000, a major effort to pyramid resistance using F3 Arkansas breeding lines was undertaken. About 250 crosses were made using F3 Arkansas lines derived from P88288C1 (type I); P92823A1 (type II resistance from Ning 7840); Ernie (type II), Roane (types IV & V), and Patton (type II). These populations have been advanced using a bulk breeding procedure under inoculated field conditions.

The Arkansas Germplasm Enhancement Program began making crosses for developing parental lines with resistance to FHB, leaf rust, stripe rust, and Septoria leaf blotch in 1997. Segregating populations were first selected for adaptation, yield potential, resistance to foliar diseases, and visual grain quality. Selected lines from the most promising populations were further selected for FHB resistance under high disease pressure in inoculated, misted nurseries at two locations during each season. Lines with low levels of FHB based on visual ratings were harvested and further selected for low levels of scabby seed and high visual grain quality.

Selected lines eventually were evaluated for type II resistance in the greenhouse, but these greenhouse tests were not part of the selection process. A recurrent selection project for FHB resistance was begun in 2000 to provide a continuous source of lines with improved agronomic and resistance traits. A male-sterile population is being used to facilitate annual intercrossing among the most resistant lines from the Northern and Southern Uniform Winter Wheat FHB nurseries. Potential sources of FHB resistance are evaluated in FHB field nurseries each year to identify FHB-resistant lines in good agronomic backgrounds. To assist winter wheat breeding programs with the development of FHB-resistant cultivars, the Northern and Southern Uniform Winter Wheat FHB Nurseries and the FHB nursery from the Arkansas breeding program are evaluated for FHB resistance in two inoculated, misted field tests and in the greenhouse for type II resistance.

RESULTS AND DISCUSSION

In 2004, eight lines were identified in an inoculated yield trial at Stuttgart, AR, that had higher yields and lower FHB scores than the check 'Pat' (Table 1). Six of these lines were entered in the Uniform Southern Scab Nursery. These entries have resistance derived from Ning 8026 and P88288C1-6-1-2. AR93027-3-2 was tested in the Uniform Eastern Soft Red Winter Wheat Nursery in anticipation of possible release. It will be tested for a second year in the Arkansas State Variety Test in 2004-05. It will also be tested in the official state variety tests in Wisconsin, where seed was requested due to its scab resistance and yield.

Thirteen advanced lines from 11 sources of FHB resistance (Table 2) have been selected for distribution to breeders for use as parents to develop FHB-resistant cultivars. Ten of these lines have been evaluated in the Southern Uniform Winter Wheat FHB Nursery in 2003 or 2004, and most ranked among the top five entries for several measures of FHB resistance. These lines also have reasonably good yield and test weight and adequate resistance to contemporary races of stripe rust, leaf rust, and leaf blotch. Most of the lines also have resistance to spindle streak mosaic and/or soilborne mosaic. The resistant and susceptible check cultivars used in 2004 had lower than expected ratings for FHB severity, scabby seed, and DON (Table 2). These abnormal results may have been caused by planting the checks as single-row plots (to save space) bordered by rows of tall triticale (for markers) while lines for evaluation were planted in 2-row and 3-row plots at Fayetteville and Kibler, respectively. Evaluations in Arkansas of entries in the Northern and Southern Uniform Winter Wheat FHB Nurseries for resistance to FHB and other diseases has contributed to the development of FHB-resistant winter wheat cultivars.

ACKNOWLEDGMENT

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Table 1. Performance of checks and experimental lines at Stuttgart and Kibler, AR, in 2004.

Entry	Yield (bu/A)	Test wt (lb/bu)	Lodging (%)	FHB severity[1]	Diseased leaves[2]
AR97124-4-1	87.3	59.1	25	26	35
AR97124-4-2	80.6	59.7	5	30	40
AR97002-10-1	76.3	58.0	25	40	45
AR97007-8-1	74.5	56.6	5	30	68
AR93095-4-1	74.2	57.4	10	31	55
AR97070-7-1	73.2	58.4	35	45	40
AR97007-12-1	72.9	56.3	0	30	60
AR97124-4-3	72.2	59.3	5	30	45
AR93035-4-1	72.0	57.8	10	26	58
AR97007-7-2	71.9	57.1	5	45	60
Pat	71.4	58.3	0	42	55
AR97078-2-1	70.7	60.5	0	40	74
AR97007-16-1	70.7	56.0	5	40	76
AR97048-1-1	69.9	58.6	15	26	78
AR97002-2-1	69.7	58.6	15	22	45
AR922-5-1	69.5	58.4	5	30	89
AR97048-2-1	68.9	58.4	25	31	71
AR97070-8-1	68.3	59.4	30	35	50
AR97079-9-1	68.1	59.4	50	55	94
AR97007-8-2	67.8	56.6	5	35	65
AR97048-8-1	67.6	58.1	10	30	55
AR93108-3-2	67.5	58.7	5	35	83
AR93035-4-2	67.5	59.1	5	26	45
AR97007-8-3	67.5	56.7	5	45	74
AR97048-4-1	67.3	59.1	15	26	69
AR97002-10-2	67.1	58.7	0	35	35
AR97079-7-1	66.9	60.8	20	35	94
AR910	66.4	59.0	3	28	52
AR97002-3-2	66.2	56.9	30	40	55
Sabbe	66.1	57.1	5	48	37
AR97048-7-2	66.0	56.7	10	30	50
AR97147-4-3	65.9	61.0	5	30	55
AR97007-4-1	65.2	57.9	10	30	78
AR97079-6-1	64.4	60.4	5	22	45
AR97007-7-1	64.2	56.4	5	30	78
AR97002-3-3	63.4	57.0	30	35	45
AR97147-4-1	63.3	58.5	5	30	55
AR97147-4-2	63.2	59.4	10	30	50
AR97124-7-1	62.8	58.0	5	30	45
AR97002-2-2	62.8	58.0	15	22	55

[1]Percentage of diseased florets at Kibler. Mean of 4 reps.

[2]Percentage of foliage diseased at Kibler. Diseases were Septoria leaf blotch, stripe rust, and leaf rust in this order. Mean of 4 reps.

Table 2. Agronomic and disease ratings for F8, BCF7, and TCF7 lines developed as FHB-resistant parents, 2004.

ARGE No.	Parentage	Yield[1] (bu/A)	Test wgt.[1] (lb/bu	Spindle streak[2] (0-9)	Soilborne + spindle streak[3] (0-9)	Stripe rust (%) Fayetteville[4]	Stripe rust (%) Peru[5]	% Leaf area diseased[6]	FHB severity[7] Fayetteville	FHB severity Kibler[7]	FHB type 2[8]	Lodging[9] (0-9)	Scabby seed[10] (%)	DON[10] (ppm)
97-1022-5-1	Mason/Catbird (G49)	82.9	57.2	0.0	2.7	2	30	39	16	30	45	0.0	8.7	2.3
97-1042-4-5	Mason/Catbird (G93)	69.9	58.6	3.0	3.7	0	0	28	4	26	23	1.7	2.7	0.9
97-1043-6a-5	Mason/Catbird (G95)	76.6	58.0	1.0	2.3	0	TR	31	3	30	12	0.0	2.0	0.9
97-1033-3-5	Freedom/Catbird (G82)	74.9	58.4	1.0	4.7	18	TR	25	1	15	9	0.0	1.0	0.4
97-1033-10-2	Freedom/Catbird (G82)	77.4	58.3	3.3	6.3	37	TR	32	4	15	10	0.3	1.3	0.7
97-1048-3-6	Mason//Sha 3/Catbird	68.6	58.1	1.7	0.7	14	40	35	3	35	7	0.0	2.3	0.4
97-1064-11-5	Mason//Freedom/Super Zlatna	75.4	57.2	1.7	0.3	4	50	40	8	26	13	1.3	2.7	1.1
97-1064-13-5	Mason//Freedom/Super Zlatna	79.1	55.8	4.3	1.7	2	50	40	8	35	27	0.0	1.7	0.7
97-1038-3-5	Mason*2//Sha3/Super Kauz	77.5	58.7	2.3	0.0	1	50	42	10	26	10	0.7	0.7	0.6
97-1008-3-3	P2684/Er-Mai 9	73.5	59.0	0.3	0.3	14	20	40	5	30	21	0.3	4.7	1.2
97-1010-3-5	Mason/Yu-Mai 7	65.7	59.0	4.0	5.0	3	50	69	7	35	18	1.0	2.3	1.0
97-1060-5-5	Mason/3/Freedom//Clark*4/N7840	65.5	58.7	1.0	0.0	3	40	32	15	40	45	2.7	1.3	1.4
97-1047-4-2	P2684/3/N7840//Parula/Veery#6	77.7	58.2	2.0	0.3	2	70	39	12	30	19	0.0	1.0	0.7
	Ernie resistant FHB check	-	-	-	-	-	-	85	5	9	10	-	0.7	0.4
	Pioneer 2684 susceptible FHB check	-	-	-	-	-	-	94	10	30	52.0	-	5.0	1.5

[1] Mean of 3 reps each at Stuttgart and Marianna. Means of checks (Terral TV8466 and Croplan Genetics 514) were 94.4 bu/A and 57.0 lb/bu.

[2] Mean of 3 reps at Clay Co. grower's field. Ratings =3 were susceptible.

[3] Mean of 3 reps at Northeast Research and Extension Center, Keiser. Ratings =6.5 were susceptible.

[4] Mean of 7 reps (2 experiments) at Fayetteville. Susceptible checks were =70%.

[5] Rating for 1 rep in collaboration with Mohan Kolhi of CIMMYT. Susceptible lines were 100%

[6] Mean of 7 reps (2 experiments) at Kibler. Diseases were Septoria leaf blotch, stripe rust, and leaf rust in this order. Susceptible checks were =90%.

[7] Mean of 4 reps. Visual estimate of the percentage of florets diseased.

[8] Mean of 3 reps with 5 to 8 heads/rep inoculated in center floret. Visual estimate of the percentage of florets diseased.

[9] Mean of 3 reps at Stuttgart. 0 = no lodging.

[10] Mean of 4 reps at Fayetteville. DON analysis by Pat Hart's lab.

PLEIOTROPIC DRUG RESISTANCE IN WHEAT: CHARACTERIZATION OF PDR-TYPE ABC TRANSPORTER GENES

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ABSTRACT

In the yeast *Saccharomyces cerevisiae* basal resistance to trichothecenes like deoxynivalenol (DON) is mediated by *PDR*-type ABC transporters (Mitterbauer & Adam, 2002). These plasma membrane-localized proteins confer pleiotropic drug resistance (*PDR*) by removing toxic substances ("molecular efflux pumps") using the energy of ATP hydrolysis. Using a seed germination assay we obtained evidence that pleiotropic drug resistance could also be a relevant mechanism of trichothecene resistance in wheat. *PDR*-type ABC transporter genes are present in plant genomes as large gene families. According to our annotation the number of rice *PDR* genes is about 25 (including possible pseudogenes). Consequently, hexaploid wheat (AABBDD) may contain dozens of *PDR* genes. We set out for the development of molecular markers for this large gene family for use in marker-assisted plant breeding programs. Since we expect the highest degree of polymorphism within non-coding regions (introns), we first had a closer look at the gene structue of the predicted transporter genes of *A. thaliana* and *O. sativa.* The position of introns is well conserved, which should also be true for wheat. Based on *Triticum aestivum* ESTs with homology to *PDR*-type ABC transporter genes 70 primer pairs were designed which allowed us to amplify the corresponding intron spanning regions from genomic DNA of wheat. Primers were used to obtain amplicons from parental wheat cultivars Remus (highly *Fusarium* susceptible), Frontana and CM-82036, for which phenotypically characterized doubled haploid populations exist. Polymorphic amplicons were revealed by SSCP analysis (single-strand conformational polymorphism), digestion with restiction enzymes (CAPS, cleaved amplified polymorphic sequence) or by sequencing (SNP, single nucleotide polymorphism). First results of marker development and technical difficulties caused by the large size of the *PDR*-type ABC transporter gene family of wheat are discussed.

ACKNOWLEDGEMENT

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2003-04 UNIFORM SOUTHERN SOFT RED WINTER WHEAT FUSARIUM HEAD BLIGHT SCREENING NURSERY

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ABSTRACT

The 2003-04 Uniform Southern Soft Red Winter Wheat Fusarium Head Blight (FHB) Screening Nursery comprised 39 advanced generation breeding lines and two check cultivars. Cooperators in the United States submitting entries included six public (Univ. of Arkansas, Univ. of Georgia, Louisiana State Univ., Univ. of Maryland, N.C. State Univ., and Virginia Tech) and two private (Syngenta Seeds and AgriPro) institutions. Three entries were submitted from the Agricultural Research Development Institute, Fundulea, Romania. Ten cooperators in the United States, Hungary and Romania returned field and/or greenhouse data for the annual report. Copies of the nursery report will be available at the International Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

MAPPING OF QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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OBJECTIVES

Mapping of QTL for FHB resistance type II in a mapping population from a cross between Wangshuibai (resistant)/Falat (susceptible) using SSR markers

INTRODUCTION

Molecular markers have been used to estimate number and location of genes involved in FHB resistance and could complement classical plant breeding. Waldron et al. (1999) identified five QTLs for FHB resistance by analyzing RFLPs in a Sumai 3/Stoa recombinant inbred lines population (112 F_5- derived RILs). Two major QTL were identified on 3BS of Sumai 3 and 2AL of Stoa. The best RFLF markers in 3BS region explained 15.4% of the phenotypic variation. Zhou *et al*. (2003) verified the effects of 3BS QTL and the predictive value of SSR markers linked to the QTL in a F_2 mapping population (Ning 7840/ Wheaton) and a $F_{3:4}$ population of Ning 7840/IL89-7978. Our objective was mapping of QTLs for FHB resistance Type II in a F_2 mapping population from a cross between Wangshuibai (resistant)/Falat (susceptible) using SSR markers.

MATERIALS AND METHODS

Plant material - Wangshuibai is a FHB resistant spring wheat cultivar from China. It has high resistance to spread of scab within an inoculated spike. Falat is an Iranian spring wheat and highly susceptible to spread of scab within the spike. Two hundred fifty random F_2 kernels were planted in large plastic containers. The containers were arranged randomly on benches in the greenhouse. The soil texture was sandy loam. The plants were grown with a 16 h photoperiod, watered as needed and fertilized twice. 196 F_2 plants were used for marker analysis.

FHB evaluation - Thirty plants of each parent (Wangshuibai and Falat) and 213 F_2 plants were tested for spread of FHB within a spike in greenhouse. The inoculum of F. graminearum contained a mixture of five isolates. At anthesis 3 spikes from each F_2 plant were inoculated with a 10 microlitre droplet (20,000 conidial/ml) of conidial suspension placed directly into a single floret of a spikelet near the center of the spike, following procedures described by Anderson et al (2001). This method bypasses primary infection and targets Type II resistance (Waldron et al. 1999). A gentle overhead mist was applied and plants were covered with a humid plastic bag for three days after inoculation. Three weeks after inoculation, percentage of scabbed spikelets (PSS) on inoculated spikes was determined as disease severity. Average PSS from 3 inoculated spikes per plant was recorded for each F_2 plant.

Genotyping with SSR markers - Parents and two resistant and susceptible bulks were screened for polymorphism with 341 SSR markers, 166 from Roder et al. (1998) and 175 from Cregan et al. (2001). Primers that showed polymorphism between two parents, as well as between the two bulks were used to screen individuals of the two bulks. If there was a significant difference in allele frequency between the individual lines in the R and S bulks, the whole population was screened for the marker.

Statistical analysis - Simple regression was used to identify markers significantly associated with FHB in the population. A Linkage map was constructed using Map Manager QTX (Manly et al. 1999) using Kosambi mapping function (Kosambi 1944). Multiple regression and interval analysis were conducted using the computer program QGENE (Nelson 1997). A LOD threshold of 3 was selected for interval mapping analysis.

RESULTS AND DISCUSSION

FHB evaluation - The F_2 lines displayed a continuous variation for FHB infection severity. FHB severity in the F_2 lines ranged from 4.5% to 100%, showing large phenotypic variation in the population. Disease severity of the two parents was 14.2% for 'Wangshuibai' and 88.5% for 'Falat' (data not shown). In this study we provided favorable moisture and temperature conditions in a controlled greenhouse to minimize environmental variation.

QTL analysis - Simple regression analysis detected 10 markers significantly associated with FHB resistance at a = 0.05. A multiple regression model was used which included all of the markers significantly (a < 0.001) associated with FHB resistance (table 1). This model explained 25% of the phenotypic variation. The analysis showed that the QTLs associated with the closest markers Gwm 533 on 3BS, Barc 15 on 2AL and Gwm 369 on 3AS decreased FHB severity by 7.3%, 7% and 4.7%, respectively (table 1). Based on interval analysis, we identified three QTL regions on these chromosomes that accounted for 33% of the phenotypic variation (Fig. 2). The most likely QTL position on chromosome 2AL was in the *Xbarc 15 – Xbarc 353.2* interval with a high LOD score (7.85). This major QTL explained 15% of the phenotypic variation for FHB resistance. A QTL of similar chromosomal location was reported by Waldron et al. (1999), Anderson et al. (2001) and Gervais et al. (2003). The location of the QTL on chromosome 3BS was at the interval of *Xgwm 533 and Xgwm 389* (LOD = 5.22). Our results concerning the 3BS QTL are in agreement with Anderson et al (2001), Gonzalez-Hernandez et al. (2002). The third QTL was identified on 3AS in the *Xgwm 369 – Xgwm 2* interval with a LOD of 5.42. A QTL on chromosome 3A with small effect was reported by Gervais et al. (2003) on resistant variety Renan. The QTLs on chromosomes 3BS and 3AS explained 12% and 6% of the variation respectively.

Zhou et al. (2003) reported that marker assisted selection in the F_2 can be as effective as that in homozygous generations when codominant markers are used. Therefore marker assisted selection for the major QTLs in F_2 or other early generations and selecting homozygous individuals can accelerate the breeding practice and significantly increase selection accuracy. Providing DH lines to verify the identified QTLs in different environments is currently underway.

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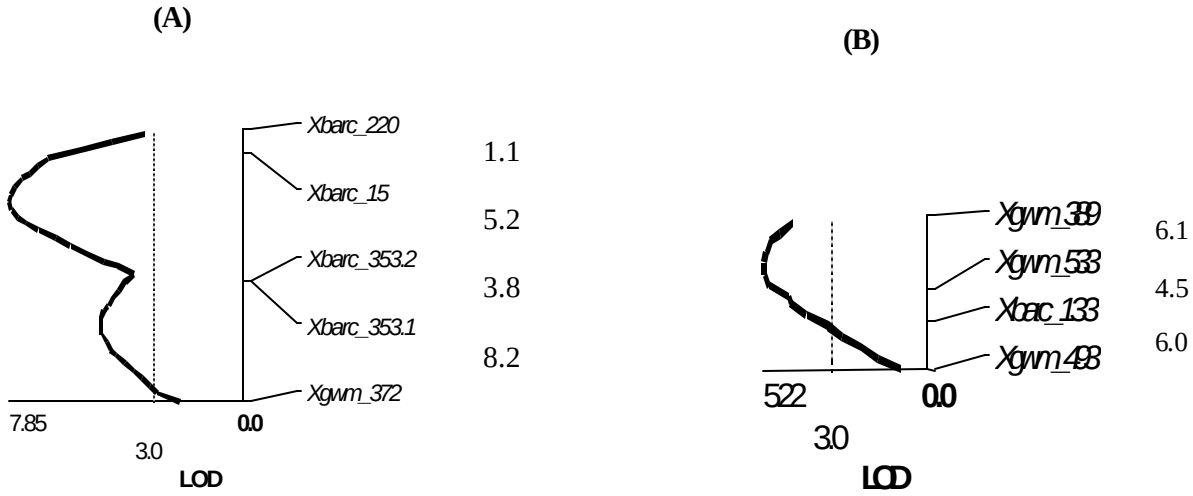
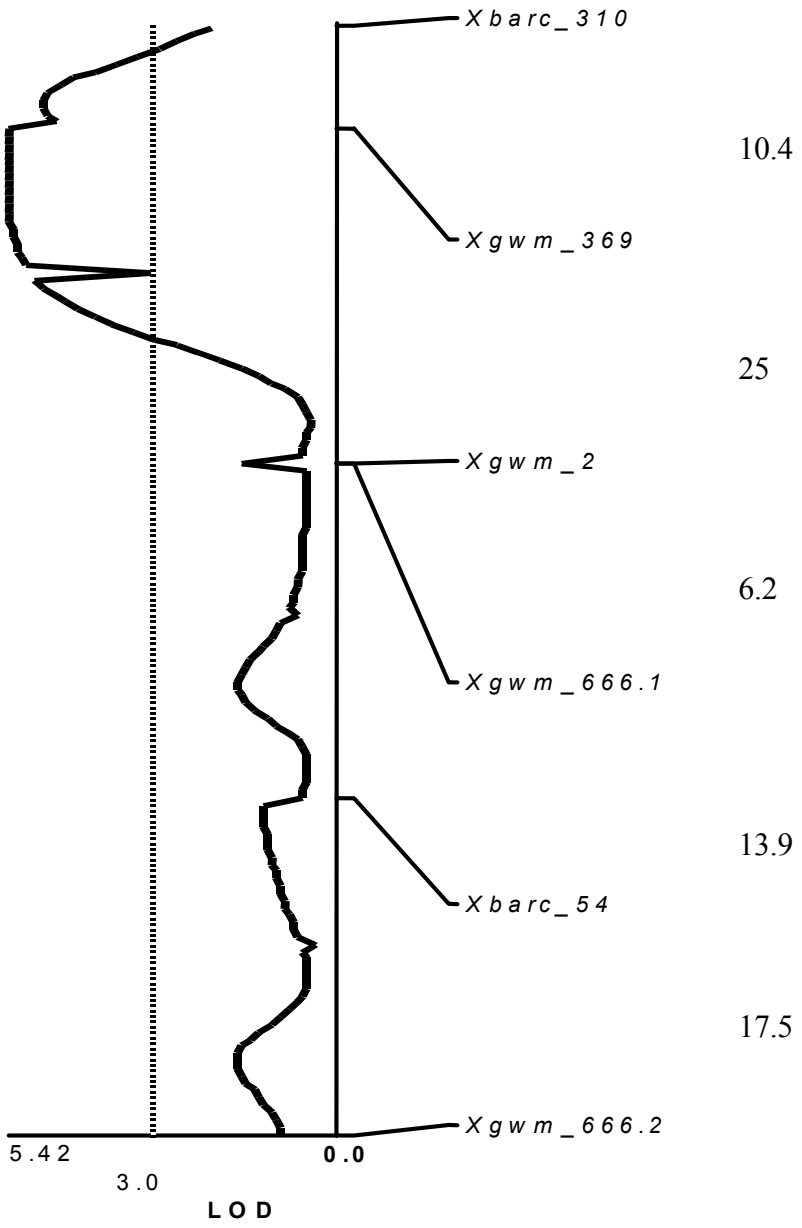
Table 1. Multiple regression model of three markers for estimating phenotypic effects in the F_2 mapping population of Wangshuibai/Falat ($R^2 = 0.25$)

Marker	Chromosome	Estimate*	Standard error	*P*
Gwm 533	3BS	7.3	2.4	<0.001
Barc 15	2AL	7	2.9	<0.001
Gwm 369	3AS	4.7	2.5	0.01

*Regression coefficients in the multiple regression model.





(C)

Figure 1. Interval analysis of QTLs for Fusarium head blight resistance on linkage group corresponding to chromosomes 2A (A), 3B (B) and 3A (C). The map distances (units shown between marker loci) were derived using Map Manager QTX.

VARIETAL DIFFERENCES TO FUSARIUM HEAD BLIGHT IN BRAZILIAN TRITICALE

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ABSTRACT

Triticale (X *Triticosecale* Wittmack) is an important crop in the winter season in southern Brazil. The area sown to triticale has stabilized near 130,000 ha for the last three years. The grain yield average ranged from 1,600 to 2,100 kg ha^{-1} over the last decade. Notwithstanding, yield potential of triticale cultivars obtained in research trials was higher than 6,000 kg ha^{-1} at several locations. These results are mainly due to the selection of superior genotypes, adapted to the specific environment of production, and the development of better management practices, such as the use of adequate amounts of fertilizers, crop rotation, and pest and weed control. One of the greatest limitations to triticale production in Brazil is associated to heavy rains during flowering and maturation, which induce the occurrence of foliage and spike diseases, mainly Fusarium Head Blight (FHB) or scab, induced by *Gibberella zeae* (anamorph: *Fusarium graminearum*). This fungus causes yield reduction, lower grain quality, and can limit grain feed utilization for monogastric animals, such as poultry and swine, due to the synthesis of mycotoxins in the grains. The objective of this study was to identify susceptibility to FHB in triticale cultivars in Brazil. To evaluate such susceptibility, 13 genotypes were sown at two or more seeding times during the recommended period in Passo Fundo, RS, between 1999 and 2003. At full flowering, the central floret of 30 spikes in each plot was inoculated with 0.02 mL of a solution containing $5x10^5$ propagules of *F. graminearum*. The maturing spikes were evaluated using the following scale: 10= disease did not spread beyond the infected spikelet; 30= disease spreaded to no more than three spikelets; 50= disease spreaded to less than half of the spike; 70= disease spreaded to less than three quarters of the spike; and 90= disease spreaded all over the spike and to the peduncle. The disease indices were represented by the average scores over sowing times and were analyzed. Observing the highest disease index, all the genotypes could be classified as susceptible or very susceptible to scab.

EVALUATION OF TETRAPLOID WHEAT GERMPLASM FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Sources of resistance to Fusarium head blight (FHB) have been identified and utilized in breeding for FHB resistance in common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD). However, sources of effective FHB resistance are limited in durum wheat (*T. turgidum* L. ssp. *durum*, $2n = 4x = 28$, AABB). Attempts to transfer resistance from hexaploid wheat to durum wheat have met with minimal success. The objective of this study is to identify novel sources of FHB resistance usable for enhancing resistance of durum wheat to FHB. We systematically evaluated 185 accessions of five subspecies under *T. turgidum* for resistance to spread of FHB infection (Type II resistance) in one greenhouse season. These subspecies include Persian wheat (*T. turgidum* L. ssp. *carthlicum)*, cultivated emmer wheat (*T. turgidum* L. ssp. *dicoccum*), Polish wheat (*T. turgidum* L. ssp. *polonicum*), oriental wheat (*T. turgidum* L. ssp. *turanicum*), and poulard wheat (*T. turgidum* L. ssp. *turgidum*). Preliminary results from this study indicated that four accessions of cultivated emmer wheat and six accessions of Persian wheat had a similar level of resistance as 'Alsen', a 'Sumai 3' derived hard red spring wheat cultivar in North Dakota. Further evaluations are being conducted to confirm FHB resistance of these cultivated tetraploid wheat accessions in the greenhouse and field. These accessions could serve as novel sources of resistance to develop durum wheat cultivars resistant to FHB.

FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT-ALIEN SPECIES DERIVATIVES

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ABSTRACT

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* Schwabe, is a destructive disease of wheat (*Triticum* L.) in humid growth conditions throughout the world. Genetic resistance of the host plant is considered the most effective and sustainable means of defense against FHB; however, only limited sources of resistance are available in wheat. Relatives of wheat have proven to be an invaluable gene pool for wheat improvement. The objective of this study was to explore relatives of wheat for FHB resistance. We evaluated 293 lines derived from the crosses of wheat with its relatives for resistance to spread of FHB infection over two greenhouse seasons. Of these 293 derivatives, 66 were susceptible, 153 appeared moderately resistant, and 74 lines exhibited a level of resistance comparable to *T. aestivum* cv. Sumai 3, the most widely used source of resistance to FHB. Alien species involved in development of these derivatives include *T. tauschii* (Coss.) Schmal., *Roegneria kamoji* C. Koch, *R. ciliaris* (Trin.) Nevski, *Leymus racemosus* Lam., *Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey, *Th. elongatum* (Host) D.R. Dewey, *Th. junceum* (L.) Love, *Th. intermedium* (Host) Barkworth & D.R. Dewey, *Dasypyrum villosa* L., *Secale cereale* L., and oat *(Avena sativa* L.). The wheat-alien species derivatives identified as resistant to FHB include wheat-alien species amphiploids, synthetic hexaploid wheat lines, and wheat-alien species substitution and translocation lines. These derivatives could serve as novel sources to enhance resistance of wheat to FHB.

A GENOMICS APPROACH TOWARDS UNDERSTANDING THE DEFENSE RESPONSE OF WHEAT AND MAIZE AGAINST *FUSARIUM GRAMINEARUM* INFECTION

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ABSTRACT

Using a combination of genomic and proteomic approaches, we are investigating the response of wheat and maize reproductive tissues to infection by *Fusarium graminearum* (*Fg*). In the first phase of the research program, cDNA libraries were constructed from *Fg*-challenged reproductive tissues (silk and kernel for maize, heads for wheat) of tolerant and susceptible wheat and maize varieties. Thousands of clones were randomly sequenced from those libraries to build a database of sequences from genes specifically expressed during the early phase of *Fg* infection. Along with contributions from other genomics projects at our Centre, cDNA clones were used to design and produce a 6.6K unigene maize and a 5 K unigene small grain cereal (wheat and rye) cDNA microarray.

Time-course microarray hybridization experiments (6, 24 and 48 hrs in maize; 0, 24, 48, 96 hrs in wheat) have been conducted with the cDNA arrays, comparing mock-inoculated and *Fg*-inoculated tissues from the susceptible varieties B73 (maize, silk and kernel) and Roblin (wheat, florets). Many differences could be observed in the response of wheat and maize to *Fg* infection. For example, the RNA level of many genes belonging to isoprenoid biosynthetic pathways were strongly induced or up regulated in both infected-maize silk and kernel tissues (observed with array, Northern, DD-RT-PCR and cDNA library abundance). In contrast, in wheat, homologs of those genes either showed no change in expression level following infection, or could not be found in our EST collection (suggesting low abundance). Some isoprenoids, known as phytoalexins, can act as antimicrobial compounds synthesized in response to pathogen attack and have been associated with disease resistance in many plant species. There were also many quantitative and qualitative differences in the PR (pathogenesis-related) genes affected by *Fg* infection in maize vs wheat, especially for the PR2 (1,3 B-glucanases) and PR3 (chitinases) families. PR genes are considered indicators of the defense response mounted by the plant and many have been shown to exhibit antifungal activity. As expected, the expression level of many genes involved in photosynthesis (eg CAB, RUBISCO SS, Oxygen-evolving enhancer protein, carbonic anhydrase) was drastically reduced in infected wheat tissues while no major change was observed in the non-phytosynthetic maize tissues.

Proteomic analyses are being conducted on the same maize tissue samples used for gene expression profiling via microarrays. The complex mixture of proteins isolated from kernel and silk tissues of susceptible (B73) and resistant (CO441) maize inbreds were separated by high-resolution two-dimensional gel electrophoresis (2D). Differential analysis of the 2D gels provided information on protein expression profiles of resistant and susceptible plants, and also how the protein expression patterns were altered by *Fg* infection. Selected up regulated and novel proteins were in-gel digested with trypsin and analyzed by MALDI-TOF/MS or by QTOF-MS.

The resulting peptide ion mass lists were searched against proteins predicted from proteomic and genomic databases resulting in the identification of several *Fg*-induced proteins. The identified proteins include zeamatin, endochitinase and several other pathogenesis-related (PR) proteins, as well as carbonyl reductase and a protein similar to profilin (G-actin binding protein). We are also using ICAT (isotope-coded affinity tag) to quantitatively compare cysteine-containing protein expression between mock and *Fg*-inoculated maize and have identified at least 20 proteins up regulated in the resistant CO441 inbred upon *Fg* infection.

WHEAT RESISTANCE TO SEEDLING AND HEAD BLIGHT IN SLOVAKIA

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OBJECTIVES

The aim of contribution was to find the wheat cvs which simultaneously express the same type resistance or susceptibility in both stages sensitive to blight (seedling and spike) caused by main pathogen *Fusarium culmorum*.

INTRODUCTION

Fifteen *Fusarium* species we identified in Slovakia in/on wheat. The main species included in head (FHB) and seedling blight (FSB) are *F. culmorum* (W. G. Smith) Sacc and *F. graminearum* Schwabe. Recently, using manual Nelson et al. (1983) F. *cerealis* and *F. sambucinum* we isolated from diseased caryopsis. Others *Fusarium (oxysporum, avenaceum, moniliforme* and *nivale)* are sporadically present in soil and ears of diseased plants. The additional species we recorded on spikes stem, leaves, (*solani, equiseti, sambucinum, poae, acuminatum, sporotrichioides, tricinctum, sulphureum*) namely in years none appropriate for disease.

The most of mentioned species are seed transmitted and are able to produce toxic metabolites, which probably play a role in the aggressiveness of the pathogen and promote disease development and colonization (Chelkowski 1994) in next vegetation period through the seed. The best solution of this problem is to select cultivars with genotypes resistant to the both diseases and athogenes´metabolites.

MATERIALS AND METHODS

The susceptibility of 23 winter wheat cultivars authorized in Slovakia to the fungus *F. culmorum*. We evaluated in field (FHB) and in greenhouse (FSB) experiment during vegetative period of 1997-1999, based on methodological investigations (Perkowski et al. 1996) in spike. In the evaluation of wheat resistance in FSB after seeds infection the production parameters together with the visual symptoms. The average of three years of experiment we present as a percentage of damage to the control as a 100 %. Representative cultivars after one year evaluation (Pavlová and Šrobárová 1997) resistant genotype (Hana) or moderately resistant with the same one ancestor (Samanta) we infected (Perkowski et al.2002) with three isolates of *F. culmorum* different in pathogenicity in experiments for relationship to the toxic metabolites (III. type of resistance).

Design of the greenhause experiment. Pots with 40 cm diameter were filled up to 2/3 by a mixture of sterile peat and garden soil in 1:1 proportion and watered. On the soil surface 20 g of wheat inoculum was added, covered by 2 cm layer of soil and moistured. Later into each pot 25 seeds were sowed and covered by 1cm layer of soil. Seeds were sawed after sterilization by 1% NaOCl. Plants were evaluated in the phase of three leaves by 5-point scale (Wildermuth and McNamara 1994) and production parameters.

Design of the field experiment For multiplication of fungi, strains of *F.culmorum* were grown for 21 days on Potato-Dextrose-Broth from Sigma (St.Louis, USA), used at 20g per L, at 25 ^{O}C with a 12 hours light period. Conidia and the airal mycelium were scalped by razor blade from 50 Petri dishes (diameter 100 mm), homogenized with 2500 mL of distilled water

using ETA grinder. The average number of conidia was $5x10^5$ per mL, established by Burker chamber.

The cultivars of winter wheat were sown on experimental fields of the institute in Slovakia in 4 variants (control and inoculated) in 3 replicates each. Seeds of cultivars were sown on the end of September or beginning of October, 1996 – 1998 and had grown separately on 6 m^2 (2x3 m^2) experimental plots bordered with rows and paths.

Inoculation. Each wheat head (500 per replicate) was inoculated 4 days after anthesis (stage 10.5 on the Feekes scale), with 1 mL of mycelium and spores suspension mixture – in anthesis on second part of June 1997-1999. The inoculation was done early in the morning with a manual (100 mL) sprayer. Control variants were treated the same way but instead of inoculation suspension 1 mL of distilled water was applied. The treated spikes were covered for 24 hours with plastic bags.

Evaluation. Ten heads per replicate were collected and infestation was evaluated visually by: VSS – visual symptoms score (5-point scale) according to CheBkowski (1994) including percentage of bleaches spikes (PBS). Heads were trashed manually and the following yield factors were estimated: grain number per spike (GNS), number of scabby kernels per spike (GNS-D); weight of kernels per spike (GWS); thousand-kernel weight (TKW).

Chemical analysis. Wheat kernels were analysed for presence of the group B trichothecenes: deoxynivalenol (DON), nivalenol (NIV) according to Perkowski (1993).

Samples were extracted with acetonitrile/water (82:18) and cleaned – up on a charcoal column [Celite 545/ charcoal Darco G/60/activated alumina neutral 3:9:5 (w/w/w)].Trichothecene toxins were detected and quantified by gas chromatography – mass spectrometry with a GC/MS apparatus HP 6890 in a Selected Ion Mode (SIM) after derivatization with trimethylsilyl derivatives. The detection limit of the complete method was 5-10 $mg.kg^{-1}$ with recovery rates 78-91 %.

Statistical analysis. The variability of yield traits was evaluated by analysis of variance. The dependence among the observed parameters was checked by correlation analysis. Computer software Statistica for Windows was used in statistical calculations.

RESULTS AND DISCUSSION

Resistance to pathogen. No cvs was without infection, the most resistant in spikes were cultivars Hana, Blava, Ilona with 0- 40 % TKW reduction and other parameters, namely AUDPC (the I. and II type of resistance sensu Schroeder and Christensen 1963). Cultivars Butin, Regina, Barbara represent (Tab.1) the most susceptible cvs (TKW depression up to 80 %). Moderately resistant cultivars (represented by cv Samanta) lost their productivity up to 60%. In the set of tested cultivars, we have identified genotypes **resistant (Tab.2) to the** *F. culmorum* in the phase of germination (Tab.2) as well as in heads (Hana, Samanta, Blava) and the susceptible ones were Butin, Barbara, moderately resistant Samanta. The resistant cvs. posses the stem rust resistance genes (Bartoš et al., 1994) and common ancestor „Mironovská“or other Russian breeding source.

Resistance to the toxic metabolites. Samanta – a cultivar more susceptible to scab than Hana - exhibited, tendency of DON concentration decrease with simultaneous increase of NIV level after 21 days, what was caused by I1 *F.culmorum* isolate used in inoculation. No such results were considering in Hana cultivar.When I2 and I3 *F. culmorum* isolates were used the process of trichothecenes formation was similar to that reported by Miller and Young (1985). Hana posse's ability to degrade DON (Fig.1 in kernels - III type of resistance (Mesterhazy, 1995) and tolerance to high concentration of DON with lower loosing yields parameters as Samanta (TKW) in spite of visual symptoms. It means Hana posses no resistance to the pathogen penetration and its spreading in first phase after inoculation, but tolerance to DON. Samanta is in the same position in NIV synthesis, but posses no tolerance to DON, because of high decreasing of yield parameters (Tab.2) -TKW compare to control, but it posses a resistance to the pathogen penetration and

spreading. It means according to the Schröeder and Christensen (1963) Samanta posses some degree I.and II.type of resistance.

Profile and concentration of analysed toxins depended on the F. *culmorum* isolate used in inoculation and on the term of harvest (Tab.3., Fig.1).The higher level of the toxic metabolites accumulation was found for less resistant genotype Samanta. . Several authors (Miller and Young, 1985) have demonstrated a close linear relationship between seed infection and toxin concentration levels, recent data (Mesterhazy, 1995, Lemmens et al. 1997; Perkowski et al., 1996) proved a significant correlation among DON content and yield traits.

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Table 1. The response of winter wheat cultivars after spike inoculation by *F. culmorum*: Parameters- average of three years of experiments.

cultivar	Area under disease progress curve	disease severity	Reduction in % from control (100 %)		
			TKW	number of seeds per head	weight of seeds from one head
Barbara	420,10	3,46	22,17	83,07	35,50
Blava	300,79	3,00	67,46	98,46	68,82
Bruta	355,29	3,22	40,30	70,26	28,47
Danubia	377,90	3,48	34,74	68,74	23,52
Hana	288,58	2,54	64,66	82,23	45,42
Ilona	336,29	2,96	41,30	74,24	30,76
Iris	325,47	3,06	26,47	52,97	14,01
Košutka	289,85	2,78	68,74	74,50	42,01
Lívia	331,20	3,18	37,75	65,15	24,66
Regia	280,36	2,82	47,81	68,16	31,34
Regina	348,84	3,28	25,73	49,81	12,80
Samanta	368,21	3,02	44,43	60,24	26,97
Selekta	380,37	3,50	30,66	65,45	20,68
Senta	385,65	3,78	31,20	60,30	18,66
Simona	395,04	3,32	36,85	56,49	19,41
Sofia	395,70	3,46	51,56	88,99	44,27
Sparta	397,49	3,66	32,26	60,08	19,31
Torysa	350,00	2,80	60,00	72,59	36,68
Vega	405,17	3,08	45,21	51,34	23,64
Viginta	384,71	3,54	32,77	64,30	21,03
Vlada	338,40	3,64	41,71	77,05	31,85
Zdar	407,52	3,24	37,92	64,55	24,61
Butin	432,34	3,98	25,46	54,52	15,03

Table 2. The response of winter wheat cultivars after inoculation of seedlings by *F. culmorum*: average of three years of investigation.

cultivar	Area under disease progress curve	disease severity	Reduction in % from control (100)		
			seedlings length	fresh seedlings weight	dry seedlings weight
Barbara	287,5	2,87	88,74	78,92	80,55
Blava	170,0	1,60	92,26	90,17	96,24
Bruta	285,0	2,85	88,42	81,72	89,47
Danubia	265,0	2,65	89,73	85,78	91,68
Hana	107,5	1,07	96,40	98,42	96,57
Ilona	230,0	2,47	94,20	95,91	95,11
Iris	235,0	2,15	99,55	90,10	95,31
Košutka	127,5	1,30	84,31	76,61	95,02
Lívia	245,0	3,05	83,54	71,91	88,72
Regia	310,0	3,10	75,00	60,18	71,20
Regina	265,5	2,62	89,23	84,70	84,80
Samanta	252,5	2,52	85,74	73,68	84,38
Selekta	97,5	0,97	97,20	94,10	97,77
Senta	222,5	2,22	90,43	93,54	93,75
Simona	197,5	1,97	82,94	65,07	94,42
Sofia	180,0	1,77	80,52	72,22	93,60
Sparta	130,0	1,30	86,02	77,43	95,63
Torysa	135,0	1,35	85,41	83,98	97,50
Vega	212,5	2,05	96,58	92,98	95,80
Viginta	255,0	2,40	92,67	92,70	90,63
Vlada	165,5	1,65	79,00	71,86	93,64
Zdar	290,0	2,90	82,12	63,81	71,62
Butin	195,0	1,90	93,78	83,37	93,42

Table 3. Correlation coefficients between mean values of all observed criteria in selected winter wheat cultivars infected by three isolates of *Fusarium culmorum.*

	Hana/ Samanta	Hana I_1 / I_2	Hana I_1 / I_3	Hana I_2 / I_3	Samanta I_1 / I_2	Samanta I_1 / I_3	Samanta I_2 / I_3
VSS	$0,337^{-}$	$0,187^{-}$	$0,519^{-}$	$0,183^{-}$	$0,169^{-}$	$0,400^{-}$	$0,480^{-}$
PBS	$0,417^{-}$	$0,184^{-}$	$0,553^{-}$	$0,108^{-}$	$0,678^{+}$	$0,360^{-}$	$0,736^{+}$
GNS	$0,271^{-}$	$0,107^{-}$	$0,180^{-}$	$0,086^{-}$	$0,231^{-}$	$0,191^{-}$	$0,452^{-}$
GNS-D	$0,600^{+}$	$0,752^{++}$	$0,442^{-}$	$0,747^{+}$	$0,891^{++}$	$0,971^{++}$	$0,932^{++}$
GWS	$0,748^{+}$	$0,964^{++}$	$0,989^{++}$	$0,983^{++}$	$0,921^{++}$	$0,842^{++}$	$0,829^{++}$
TKW	$0,753^{++}$	$0,903^{++}$	$0,959^{++}$	$0,978^{++}$	$0,917^{++}$	$0,913^{++}$	$0,842^{++}$
DON	$0,138^{-}$	$0,070^{-}$	$0,532^{-}$	$0,424^{-}$	$0,111^{-}$	$0,120^{-}$	$0,781^{++}$
NIV	$0,167^{-}$	$0,043^{-}$	$0,159^{-}$	$0,174^{-}$	$0,058^{-}$		

VSS - visual symptom score, TKW - thousand kernels weight,
PBS - percentage of bleached spikes, GNS - grain number per spike,
GNS-D - grain number per spike diseased GWS - grain weight per spike
DON - deoxynivalenol NIV - nivalenol

SIMILARITY OF FUSARIUM HEAD BLIGHT RESISTANCE RATINGS COLLECTED OVER MULTIPLE YEARS

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OBJECTIVES

This study was initiated with the objective of measuring test-to-test similarities associated with three FHB resistance parameters collected by the South Dakota State University spring wheat breeding program. An additional objective was to explore the impact of various test year and replications within year combinations on the standard deviation of disease index values.

INTRODUCTION

Where increased resistance to FHB, caused by *Fusarium graminearum*, is a major goal, field-based screening tests are vitally important endeavors. Accurate assessments of resistance allow for the most gain to be made from selection. Reproducible disease assessments likely provide the most accurate assessment of a genotypes true resistance value. Groth et al (1999) performed a study to measure test-to-test similarities associated with FHB screening endeavors. They reported that coefficients of determination (r^2) associated with disease index values ranged from 0.59 to 0.78. Their conclusion was that 59 – 78% of the variation observed in one year could be reproduced in subsequent testing years. Campbell and Lipps (1999) used variance component estimates to evaluate the effect of various numbers of test environments, replications, and heads evaluated per entry on the standard error of an entry mean. Along with concluding that FHB resistance was as environmentally sensitive as grain yield, they reported that the most practical and economically feasible gains in selection efficiency would be made through increasing the number of replications within a screening program. Additional testing environments also provide an excellent means of reducing standard errors, however, this is not economically feasible within our program where we operate a single mist-irrigated field screening nursery each summer. Preliminary and advanced breeding lines are currently screened for FHB resistance, along with check entries, in three replication tests where 20 heads per entry per replication are evaluated. Although additional testing environments within years are not feasible in our program, several evaluation years can be used to reduce the standard deviation of disease index values for an entry by combining entries that are common over years into a single multi-year analysis. This study was initiated with the objective of measuring test-to-test similarities associated with three FHB resistance parameters collected by the South Dakota State University spring wheat breeding program. An additional objective was to explore the impact of various test year and replications within year combinations on the standard deviation of disease index values

MATERIALS AND METHODS

Field Screening

An explanation of germplasm, field screening procedures, and methods of resistance data collection are presented by Liu et al (2004).

Data Analysis

Resistance Parameter Estimation and Correlation Over Years - Although disease severity ratings were collected in order to obtain index scores, they were excluded from our current analyses. Mean disease incidence, index, and percent tombstone kernel ratings were analyzed within years using the GLM procedure in SAS (SAS Institute, Cary, NC). In addition to means, significance levels associated with ANOVA and phenotypic ranges were calculated. Pearson's product-moment, as well as Spearman's rank-order correlation coefficients were calculated to compare the

similarity of resistance parameter means obtained over years using the CORR procedure in SAS.

Expected Standard Deviation of an Entry Mean - The expected variance of an entry mean (Var_{entry}) from a series of replicated tests over years is expressed as follows:

$$Var_{entry} = \sigma^2_{EY}/Y + \sigma^2_e/RY$$

where the variance components in the numerator were derived from expected mean squares of a fixed-effect analysis of variance conducted over years (Liang et al, 1966). Years and replications were represented by Y and R respectively. Expected variance of an entry mean was predicted by substituting observed values for the variance components into the above equation. Several values representing various numbers of test years and replications were substituted into the denominator as a means of predicting the effect of additional test years and replications within tests. The square root of resultant expected values from these calculations were then plotted as the standard deviation of an entry.

RESULTS

Means and phenotypic ranges, as well as within-test significance levels, for the three resistance parameters are presented in Table 1. The level of disease incidence was significantly different among entries only in 2004. Likewise, significantly different percent tombstone kernel ratings were observed in 2003 and 2004. Disease index values were, however, significantly different within each of the three test years.

Both Pearson's and Spearman's year-to-year correlation coefficients for disease incidence hovered near zero, and were non significant except for the comparison of incidence levels collected in 2003 and 2004 (Table 2). In this instance, both coefficients were positive and significantly different than zero (Table 2).

Pearson's and Spearman's year-to-year correlation coefficients for disease index values were again most significant for the comparison of 2003 with 2004 (Table 3). Large changes in index rank prevented Spearman's year-to-year correlation coefficients from being significant in all but the 2003 to 2004 comparison.

Correlation coefficients for percent tombstone kernel ratings were only significant for the 2003 to 2004 comparison (Table 4).

Standard deviations for disease index values were derived from the expected variance of an entry mean. These values were plotted and are presented visually in Figure 1.

DISCUSSION

Our results indicate that disease index values appear as the most repeatable parameter from year to year (Table 2). Additionally, index scores were significantly different ($P<0.001$) within each test year. Unfortunately, changes in entry rank order from 2002 to 2004, prevented our rank order correlations from being significant among all three comparisons. Interestingly, coefficients of correlation for all three resistance parameters were most significant with the comparison of 2003 to 2004. It seems that comparable environmental conditions within these test years may have elevated the similarity between tests. Additional test years will help to further address this issue, however, it appears that our collection and usage of disease index scores is repeatable within the program. During the first few years of testing, expected standard deviations associated with disease index scores appear to be reduced most dramatically with the addition of reps within test years. In the case where a preliminary yield trial entry is eventually released as a variety, we will possess 6 to 7 years worth of resistance data. Our analysis suggests that by combining data of the fully-advanced entry with that from other common test entries into a single multi-year analysis, the standard deviation of disease index values should be less than five.

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Table 1. Means, phenotypic ranges, and within-test significance levels for three FHB resistance parameters collected over three test years.

Year		Disease Incidence	Disease Index	Tombstone Kernels
2002	Mean	99.92	43.08***	17.36
	Range	95.00 - 100.00	26.75 – 75.50	5.00 – 45.00
2003	Mean	99.52	43.22***	8.56***
	Range	89.40 – 100.00	14.95 – 75.00	1.00 – 65.00
2004	Mean	95.00**	35.97***	21.23***
	Range	60.00 – 100.00	5.40 – 77.00	4.00 – 75.00

*, **, *** Significant at 0.05, 0.01, and 0.001 levels respectively.

Table 2. Pearson's product-moment (above the diagonal) and Spearman's rank-order (below the diagonal) correlation coefficients associated with yearly disease incidence ratings

	2002 Incidence	2003 Incidence	2004 Incidence
2002 Incidence	-	-0.027	0.006
2003 Incidence	-0.031	-	0.264*
2004 Incidence	0.073	0.297*	-

*, **, *** Significant at 0.05, 0.01, and 0.001 levels respectively.

Table 3. Pearson's product-moment (above the diagonal) and Spearman's rank-order (below the diagonal) correlation coefficients associated with yearly disease index ratings

	2002 Index	2003 Index	2004 Index
2002 Index	-	0.271*	0.302*
2003 Index	0.242*	-	0.671***
2004 Index	0.229	0.643***	-

*, **, *** Significant at 0.05, 0.01, and 0.001 levels respectively.

Table 4. Pearson's product-moment (above the diagonal) and Spearman's rank-order (below the diagonal) correlation coefficients associated with yearly percent tombstone kernel ratings

	2002 Tombstone	2003 Tombstone	2004 Tombstone
2002 Tombstone	-	0.069	0.063
2003 Tombstone	0.045	-	0.569***
2004 Tombstone	0.086	0.565***	-

*, **, *** Significant at 0.05, 0.01, and 0.001 levels respectively.

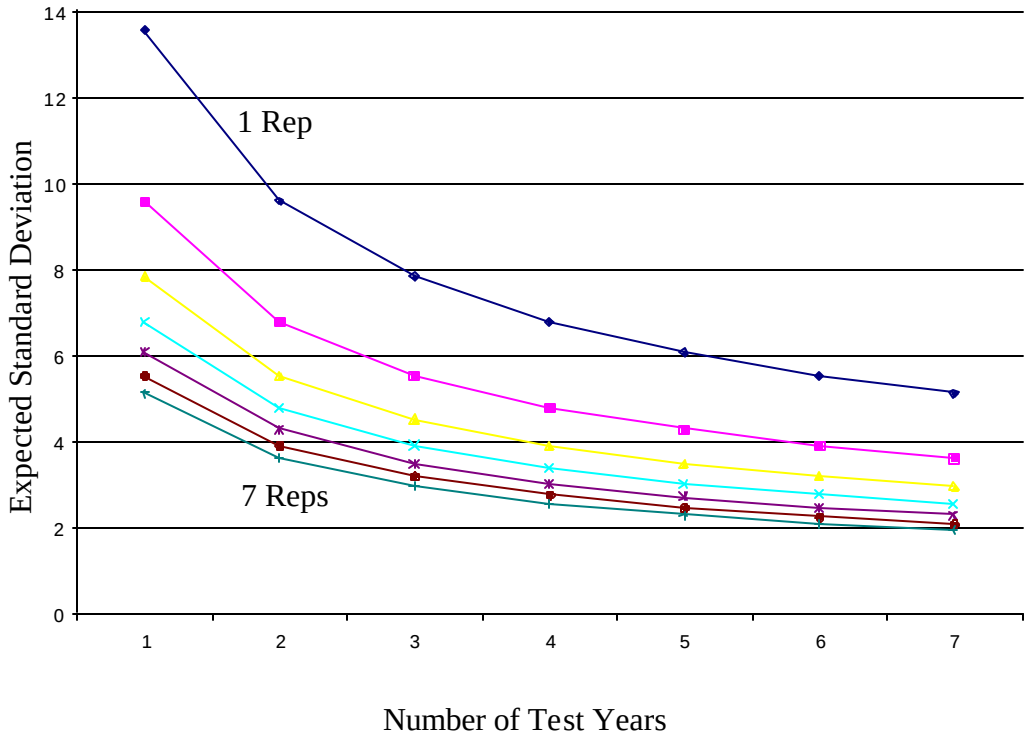

Figure 1. Expected standard deviation of an entry mean for FHB disease index ratings when tested over various year and replications within year combinations.

CHARACTERIZATION OF BARLEY GENOTYPES FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN URUGUAY

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ABSTRACT

Barley grain yield losses caused by Fusarium head blight (FHB) in Uruguay during the epidemic years 2001 and 2002 have been estimated in 58% and 23%, respectively. FHB is also considered important because of the production of mycotoxins of which deoxynivalenol (DON) is the most prevalent and responsible for reducing the trading value of the grain which is basically an export commodity. Emphasis has been placed on breeding for FHB resistance as this remains the most desirable management option for this disease. Since 1996, INIA has been screening barley genotypes for FHB resistance. In 2003, 28 barley genotypes were screened for resistance in La Estanzuela in three trials. Two trials, planted on July 24 and August 14 were conducted under greenhouse conditions to assess types of resistance I and II. Spikes were inoculated at heading with a solution of *F. graminearum* macroconidia (5 x 10^4 spores per ml) from a bulk of isolates by spray or point inoculation. FHB incidence and severity were evaluated at 7, 14, and 21 days after inoculation (dai). Area under disease progress curves (AUDPC) were calculated from the percentage of infected spikelets of each spike in point inoculated plants in order to assess resistance type II. One field trial was conducted where genotypes were planted in plots with two replicates, inoculated with *F. graminearum* colonized corn grains two weeks prior to heading and maintained under mist-irrigation. Each genotype was evaluated at Zadoks growth stage 80-83 for FHB incidence and severity on 10 spikes, spike morphology (absence/presence and size of sterile spikelets, spike nodding, and spike density), and after harvest for the percentage of scabby grains and deoxynivalenol (DON) content. Early maturity genotypes (early heading) had greater levels of resistance types I and II than intermediate and late maturity genotypes. Influence of spike morphological traits for the genotypes tested was not clear. Best genotypes for all determinations in all trials were resistance source Zau 2, Uruguayan line CLE 231, and ICARDA/CIMMYT line Gob/Humai10/3/Mpyt169.1Y/Laurel/Olmo/4/Canela. Genotypes like Imperial and Zhedar 1 that have been reported as resistance sources in other parts of the world performed poorly. Results from this study are encouraging and raised the need to include Uruguayan *F. graminearum* isolates in global studies of the pathogen variability.

IMMUNOLOGICAL ESTIMATION OF EXOANTIGEN CONTENT IN SMALL GRAIN CEREALS FOR ASSESSMENT OF RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB) is a widespread and destructive disease of wheat and other small grain cereals. Especially the contamination of grains with the mycotoxin deoxynivalenol (DON) is harmful for animals and man. The best way to prevent wheat or barley from being affected by FHB is to develop resistant cultivars. The resistance is quantitatively inherited and a large number of samples have to been evaluated. For quantification of the disease severity of FHB in breeding material a fast, economical and reliable method is essential for resistance evaluation and selection. Immunological methods appear to be particularly suitable for such an approach. Altogether 12 polyclonal antisera prepared to antigens from *Fusarium*-species were tested in various immunological detection systems. In Western blotting experiments, specific glycoprotein bands with molecular weights of the two immunodominant antigens above 63 kD were detected in artificially *Fusarium* infested wheat, rye, triticale and barley grains. The antiserum assessment resulted in one antiserum that was appropriate for the detection of *Fusarium*-exoantigens (ExAg) in cereal grains by an indirect ELISA-format, the plate trapped antigen (PTA-ELISA), though discrimination between various *Fusarium*-species was not possible. The polyclonal antibody detection system was optimized for different test parameters and a standard protocol was elaborated. The examination of wheat grain samples originating from field experiments located in Böhnshausen revealed a high coefficient of correlation (r = 0.94) between visual FHB rating and ExAg amount expressed as optical density units (OD at $E_{405\ nm}$). The PTA-ELISA was also applied for the detection of ExAg in barley grains. The correlation between number of infected grains and OD values ranged between r = 0,8 to 0,91 in two experiments. A moderate disease severity resulted in an ExAg value of 0.87 OD in wheat samples from 113 genotypes, which were analysed for their symptoms, ExAg and DON content in the grain after artificial inoculation with a highly aggressive isolate of *F. culmorum* in three location-by-year combinations. DON content ranged from 12.0 to 105.2 mg/kg and genotypic and genotype-by-environment interaction variances were significant (P=0.01). Coefficient of phenotypic correlation between DON content (analysed by a commercially available immunoassay) and ExAg content was r=0.86. The correlation between DON content and symptom rating in wheat was r=0.77. It has been shown, that an increase of FHB disease severity is associated with an increase in fungus colonization expressed as higher amounts of ExAg and higher DON contents in artificially infected cereals. First results showed that under natural infection conditions, however, a lower correlation between FHB rating and ExAg content was observed and widely differing amounts of DON were detected. To increase the test sensitivity and specificity, monoclonal antibodies (MAbs) have been developed to *F. culmorum* surface washings. At present two selected MAbs are being evaluated for their usability to detect ExAg in wheat grains in a more sensitive test format by biotin labelled MAbs.

ASSESSMENT OF THE BREEDING VALUE OF QTLS FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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ABSTRACT

Quantitative trait loci (QTL) for Fusarium head blight (FHB) resistance have been located on chromosome 5AS of 'Wuhan 3' and 5BL of 'Fujian 5114'. The 5AS QTL has been found in 'Sumai 3'-derived materials. In order to confirm and characterize the resistance produced by these QTL, near-isogenic lines (NIL) from several genetic backgrounds were developed. Additionally, 35 molecular markers in these genomic regions were screened in order to develop more precise molecular maps of these areas. Microsatellite markers flanking the QTL regions were selected to develop four QTL-NIL pairs from two different genetic backgrounds for the 5AS QTL region and seven QTL-NIL pairs from two different genetic backgrounds for the 5AS QTL region. Each pair was tested in one point-inoculation experiment and at two field FHB screening sites during 2004. Overall, significant disease reduction effects ($P<0.05$) were observed following field screening among the 5AS NIL for visually scabby kernels (VSK), 30-spike seed weight, disease severity, and DON. Significant disease reduction was observed only for VSK for the 5BL NIL pairs. Individually, one out of four pairs for 5AS displayed significant reduction (36%) in disease severity at both sites, while an additional pair displayed a 34% reduction in disease severity at one site. One out of seven pairs for 5BL displayed significant reduction (24%) in disease severity at one site. No pairs displayed significant differences at both sites, although one site was affected by root rot. There was no significant difference in FHB resistance in the greenhouse point-inoculation experiment for either 5AS or 5BL. Previous research indicates that the 5AS QTL is related to resistance to initial infection; therefore, we did not expect to see significant effects.

ACKNOWLEDGEMENT

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DEVELOPMENT OF NEAR-ISOGENIC LINES FOR VALIDATION OF QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

Marker assisted-selection (MAS) seems a promising tool to develop regionally adapted and FHB resistant wheat cultivars. Two major QTL were previously found on chromosomes 3B and 5A in the highly resistant spring wheat line CM-82036 (Buerstmayr et al. 2002, 2003) and these regions are well covered with SSR markers. The objectives of this work are: 1) quantifying the effectiveness of MAS by using linked SSR markers, 2) evaluating the effects of two QTL: *Qfhs.ndsu-3BS* and *Qfhs.ifa-5A* in near-isogenic lines in different genetic backgrounds (highly susceptible to moderately resistant), and 3) developing adapted winter wheat lines with all possible combinations of two QTL for FHB resistance derived from CM-82036. To achieve these goals CM-82036 as donor plant was crossed with 15 winter wheat lines or cultivars (recurrent parents) differing in their genetic background. F1 plants were back-crossed with their recurrent parents and approximately 30 BC1 plants per cross were genotyped with flanking SSR markers: *Gwm389*, *Gwm493*, and *Gwm533* for the 3B QTL and *Gwm156*, *Gwm293* for the 5A QTL. Lines which had both QTLs in heterozygous condition were back-crossed to the BC_2. Selected BC_2F_1 plants were selfed and screened again with the same SSR markers to select BC_2F_2 plants homozygous for all four possible combinations of the two QTL (resistant alleles on 3B and on 5A from CM-82036, only the 3B resistant allele, only the 5A resistant allele, no resistant allele). The progeny of these plants (BC2F2:3) will be evaluated in replicated and artificially inoculated experiments for FHB resistance in 2004/05.

ACKNOWLEDGMENTS

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THE DEVELOPMENT OF FUSARIUM HEAD BLIGHT (FHB) TOLERANT VARIETIES OF WHEAT IN NEBRASKA FROM 2001 TO 2004

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ABSTRACT

Although FHB is a periodic disease in Nebraska, there are years when we estimate that high moisture can lead to infection of approximately 1,000,000 wheat acres by FHB. As humans consume virtually all of this wheat and over one half is exported, safe, healthy grain is critical. The primary objective of this project is to identify and develop elite winter wheat varieties that are tolerant to FHB, using conventional breeding methods. The second objective is to screen elite hard winter wheat lines including the Regional Germplasm Observation Nursery (RGON) so that this information can be shared with breeders and growers in the southern Great Plains. Currently, 41 F_2 populations, 34 F_3 populations,1000 head rows, 34 F_5 lines, and 2 F_6 lines, with diverse sources of FHB tolerance some including Sumai 3 derivatives, are being advanced in the breeding program. Among these an FHB tolerant line, NE01643 is being considered for possible release in two years, and an additional 6 lines with high FHB tolerance are also being considered for possible release. Thirty four new crosses using germplasm from the RGON nursery, with above average level of *Fusarium* head blight tolerance have been made.

MOLECULAR MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE LOCI IN TWO EUROPEAN WINTER WHEAT POPULATIONS

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ABSTRACT

Objective of this investigation was the identification of quantitative trait loci (QTL) associated with FHB resistance in two winter wheat recombinant inbred line (RIL) populations. The mapping populations were developed by crossing 1) the resistant German cultivar Dream with the susceptible British cultivar Lynx and 2) the resistant line G16-92 with the susceptible British cultivar Hussar.

The 145 $F_{7:8}$ and 136 $F_{8:9}$ RILs of the Dream/Lynx and the G16-92/Hussar population were genotyped using AFLP and SSR markers to construct genetic maps and evaluated in the field for reaction to spray inoculation with a *Fusarium culmorum* spore suspension. Field trials were performed at four environments in two replications in Germany. For the detection of resistance QTLs composite interval mapping (CIM) with a LOD threshold of 3.7 was applied for the FHB severity means across the environments.

Three FHB resistance QTLs were identified in the population Dream/Lynx on the chromosomes 6AL, 1B and 7BS explaining 17%, 15% and 17% of the phenotypic variance. In the G16-92/Hussar population two QTLs associated with FHB resistance were located on the chromosomes 1AS and 2BL explaining 144%, and 17% of the phenotypic variance.

The QTL alleles conferring resistance on 6AL and 7BS originated from Dream and on 2BL from G16-92. The resistance QTL on chromosome 1B is probably associated with the T1BL.1RS rye translocation of Lynx. For the QTL on 1AS the susceptible parent Hussar contributed the resistance allele. The resistance QTLs on 6AL and 1AS partly overlapped with QTLs for plant height and the resistance QTL on 7BS coincided with a QTL for heading date.

The resistance QTLs on 6AL and 7BS identified in the Dream/Lynx population and the QTL on 2BL of the G16-92/Husssar population are linked with the SSR markers GWM82, GWM46 and GWM47. In order to verify the association of the three SSR markers with FHB resistance a population with an independent genetic background was developed by a four-fold crossing approach. Resistant lines of both mapping populations were crossed with two highly susceptible winter wheat cultivars. The F_1-progeny of about 600 lines was classified with the three SSR markers and disease severity of the lines was evaluated in field trials after spray inoculation with a suspension of *Fusarium culmorum* spores in four environments. Examining the marker classes and the phenotypic data revealed a shift towards resistance of lines carrying all three markers linked to the corresponding resistance QTLs compared to lines without these markers.

EVALUATION OF BARLEY ACCESSIONS FOR FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

Malting and feed barley are affected by different *Fusarium* species, which might produce toxins and may result in rejection or discount at the elevator. Based on previous studies in which thousands of barley lines were screened, the worldwide distribution of resistance sources in barley were identified. Over 550 barley accessions were selected from regions known to be sources of resistant germplasm and evaluated for resistance to FHB caused by *F. graminearum.* Seeds were obtained from IPK gene bank in Gatersleben, Germany and Austrian and Swiss gene banks. Resistant (Chevron, CIho 4196) and susceptible (Stander, ICB 111809) checks were included in all experiments. The experiments were carried out in field plots and arranged in a randomized complete block design with three replicates. At the late-milk to early-dough stage, the spikes were spray-inoculated at dusk with a macroconidial suspension of *F. graminearum*, (20.000 macroconidia/ml in 2003 and 10.000 macroconidia/ml in 2004), which was repeated two days later. For disease evaluation, the average percentage of infected kernels/spike (on 5-10 randomly chosen spikes) was assessed on each accession 14 and 21 days after inoculation. In addition, several morphological criteria were determined in 2003. In 2003, the resistant checks Chevron and CIho 4196 had severities between 7% and 27%, while both susceptible checks had infection values up to 34% and the local checks Barke and Fontana reached up to 36%. In 2004 the disease development was enhanced due to long rainfall periods compared to 2003, which is indicated by slightly higher FHB severities for the resistant checks (18 – 24%) and clearly demonstrated by FHB severities on susceptible checks (43 – 57%) despite 50% reduced inoculum concentration. Five winter and four spring barley accessions exhibited resistance against *F. graminearum.* The most resistant two-and six-rowed spring barley accessions originated from Switzerland, Germany, Austria and Denmark, while the most resistant winter barley accessions are of Austrian, Hungarian, Romanian, Swiss and Japanese origin and are all two-rowed. The six-rowed spring barley accessions showed resistance level comparable to Chevron in 2004. The screening of 26 accessions from Austrian collections and 22 accessions from Swiss collection yielded in addition three two-rowed accessions after field screening in 2003 and 2004 and verification under controlled conditions. The spring and winter barley accessions exhibiting resistance and moderate resistance were characterized by rough awns, white to yellow lemma and aleuron color, intermediate spike density, long rachilla hair, small tendency to neck breakage and to lodging, except for winter barley. Although these criteria are not suitable as reliable selection criteria, they do provide hints for potential passive resistance mechanism, linkage or co-founding factors for resistance and susceptibility. However, before these resistance sources can be utilized confidently in breeding programs, multi-location and multi-season verification is required. Due to the high variability of this plant-pathogen-system between years and locations, confirmation of resistance under controlled conditions is absolutely necessary.

NOVEL TOOLS FOR DEVELOPING FUSARIUM RESISTANT AND TOXIN FREE WHEAT FOR EUROPE

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ABSTRACT

Wheat is Europe's most important cereal crop, cultivated on 16 Million ha, yielding 88 Million metric tons per year. The wheat quality and consumer safety is threatened by Fusarium Head Blight (FHB) caused i.e. by *Fusarium culmorum, F. graminearum* and *F. avenaceum*. Most of the wheat varieties grown in the EU today are susceptible to this disease. Crop management and chemical control measures to prevent the disease and associated mycotoxin contamination are either not available or not feasible. The development and cultivation of resistant varieties is the most reliable and environmentally sound means to combat this disease.

The project aims to reduce mycotoxin contamination in wheat at the start of the production chain by means of improved FHB resistance. To improve the efficiency of selection for resistant lines, artificial infection in mist irrigated fields and under controlled conditions are performed [(Workpackage 1 (WP1)]. By QTL mapping of segregating populations, high resolution mapping populations and selective genotyping of resistant germplasm will enable us to develop molecular markers for FHB resistance (WP 2). In WP3 the search for candidate resistance genes is undertaken.

Breeding companies within the consortium are already profiting from advancements in nursery establishment, phenotyping and genotyping. Some examples of promising results are given below.

In WP1 the genotypes having two major QTL on chromosome 5B and 3A are more resistant then the group having a single QTL or the group containing no major QTL. In the traits where the differences are significant between groups we found that the major QTL influence very similar traits. Regarding DON accumulation, data show good correspondence with FHB and Fusarium damaged kernels. Field and greenhouse data from Hungary and UK, respectively, revealed minor QTL with smaller effects towards FHB resistance on chromosomes 7A, 2A, 2B, 3B and 6B, but might be influenced by different environments. Therefore, an increased uniformity in field experiments and phenotyping is mandatory.

In WP2 a significant amount of the mapping work has already been achieved in mapping populations and for screening nursery lines AFLP and SSR markers were successfully applied. Progress is made in high-resolution mapping after sufficient recombinant lines were identified.

In WP3 progress is being made in analyzing the role of specific genes in wheat and in the development of PCR-based markers to differentiate forms of these genes. We have also made progress in identifying novel candidate genes for marker development. Primers based on EST sequences are being used to amplify fragments that are checked for cultivar specific differences.

More details are presented at the Project website (http://www.boku.ac.at/fucomyr). Further, several projects receive and will receive funding from National Science Foundations by following up on findings generated by our project. Several peer-review publications are generated by the project and more are under preparation.

MAPPING OF *FUSARIUM* RESISTANCE GENES IN THE WHEAT GENOTYPES 'ARINA' AND 'NK93604'

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ABSTRACT

Fusarium head blight (FHB) of wheat (*Triticum aestivum* L.) is a devastating disease in wheat production worldwide. Breeding for FHB resistant spring wheat adapted to the Norwegian climatic conditions is one of the top priorities in the country. This study is undertaken to map and characterize quantitative traits loci (QTL) for resistance to FHB in promising sources of resistance. The investigation was performed on 93 double haploid (DH) lines derived from a cross between the cv. 'Arina' (a Swiss winter wheat) and NK93604 (Norwegian spring wheat breeding line). Both parents have moderate levels of Type I and II resistance for FHB. The DH population and parental lines were examined for disease reaction by spraying a macroconidial suspension of Fusarium culmorum in field for 3 years (2001-2003) using the plastic bag method of Mesterhazy (1995). Seed samples from 2002 experiments were used for deoxynivalenol (DON) determination (the fluoroquant method) in CIMMYT. The parents and DH lines were genotyped using fluorescent amplified fragment length polymorphsm (fAFLP) and simple sequence repeat (SSR) markers using ABI 377 DNA Sequencer. A total of 471 markers (290 fAFLP and 181 SSRs) were used for mapping and QTL analysis.

The DH lines segregated transgressively for FHB, with highly significant differences. A number of lines consistently superior or inferior to the parent(s) were identified. Correlations for FHB scores between years were around 0.75. There was also highly significant variation for DON content, the correlation with FHB in 2002 and the means of years ranging between 0.64-0.75. A total of 316 loci were mapped and the preliminary map spans 2,598 cM in 38 linkage groups. We were able to assign 22 groups into their respective chromosomes but the others remain to be defined. QTL analyses were done in two ways: using the simple interval mapping (SIM) from PLAB-QTL programme, and Partial Least Squares Regression (PLSR), a method developed from Principal Component Analysis by Bjørnstad et al. From SIM, there appeared to be three major QTL associated with FHB resistance: on chromosomes 2D, 1B (both from 'Arina') and 7A (from 'NK93604'), that explained 30.7% (adjusted R^2) of the mean phenotypic variation for disease severity over three years. This degree of explained variance for FHB is comparable to other studies in this disease. An examination of the 7 most resistant and 7 most susceptible lines showed consistent results, whereas intermediate phenotypes were less consistent. The QTL on 1B and 7A were consistent in all the three years, the 2D one only in 2001 and in the three year mean and are different from other studies that used Arina as a parent. For DON, three QTL (on 2B, 4D and undesignated, all from 'Arina') were detected that together accounted for 21.8% of the phenotypic variation. PLS revealed the same QTL for FHB as above, but in addition a clear QTL on 4B. The level of variance explained after cross validation was 38%. The QTL for both FHB and DON, however, remain to be confirmed by determining the unassigned linkages groups by incorporating more SSR and restriction fragment length polymorphism (RFLP) anchoring markers. In order to increase accuracy of the maps and linkage groups, the DH lines are currently being genotyped using Diversity Array Technology (DArT). The QTLs mapped in this cross will be compared and discussed with other studies in 'Arina' and other resistance sources.

EVALUATION OF TYPE I AND TYPE II RESISTANCE TO *FUSARIUM GRAMINEARUM*

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ABSTRACT

Effective resistance to Fusarium head blight will probably require both type II and type I resistance. The objectives of this study were to determine the best conditions for evaluation of type I resistance and to determine if the two types of resistance are correlated. We measured both types of resistance in a group of lines previously selected for type II resistance. The experiments were conducted in the greenhouse, using conidial inoculum produced in Mung bean extract medium. To measure type I resistance we sprayed fully flowering heads with a water suspension of conidia. To measure type II resistance, we inoculated a single, well-developed floret near the top of the head with 10 mðL of spore suspension. After inoculation by either method, heads were covered with a clear polyethylene bag for 48 h to provide moisture for infection. We assessed for type I resistance 5 d after inoculation, so that any type II resistance a line possessed would not confound measurement of type I resistance. We assessed type II resistance as the number of blighted spikelets 20 d after inoculation. There were highly significant differences in type II resistance among lines. The correlation for type II resistance between repeated experiments was 0.72. There were likewise highly significant differences among lines for type I resistance, with about a 4-fold difference in the percentage of blighted spikelets 5 d after inoculation. However, expression of type I resistance was not as repeatable as type II resistance. The correlation between repeated experiments was only 0.44. In neither experiment was there a correlation between type I and type II resistance. A marker analysis conducted by Dr. G Bai revealed that most of these lines do not carry the major resistance QTL on 3BS. To investigate the effect of inoculation variables on the expression of type I resistance, we inoculated a subset of lines from the experiment described above by spraying them with either 20,000 or 40,000 spores/ml, on one or both sides of the head, at early flowering (GS 10.51) or full flowering (GS 10.53). The effect of each variable was highly significant. There were significant interactions between line and each of the inoculation variables, indicating that the relative degree of type I resistance shown by a line depended on the conditions of the test. For the variables tested, an inoculum concentration of 20,000 spores/ml applied at GS 10.53 to both sides of the head was the most reliable means to evaluate type I resistance. We also compared type I with type II resistance in a recombinant inbred population derived from Clark (susceptible) ´ Chokwang (moderately resistant). The population was tested twice for type II resistance and 3 times for type I resistance. Family means for type II resistance ranged from 3 blighted spikelets to all spikelets blighted. The correlation between the repeated type II tests was 0.51. Among the 3 evaluations of type I resistance, line means ranged from 3% to 58%, 86%, or 50% of the spikelets blighted. The correlations of type I resistance between experiments were low. There was no correlation between type I and type II resistance in this population. The 3 RILs with a consistently high degree of type I resistance had little type II resistance. Chokwang has a minor gene for resistance at 3BS, but marker analysis indicates more of its resistance is at a locus on 5D.

MARKER-ASSISTED CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN A *LOPHOPYRUM*-DERIVED WHEAT LINE

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ABSTRACT

The closely-related, by backcrossing to 'Thatcher', wheat substitution lines K2620 (7D/7el$_2$) resistant to Fusarium head blight (FHB) and K11463 (7D/7el$_1$) susceptible to FHB, were crossed to produce an F_2 mapping population segregating for FHB resistance. The F_2 plants were evaluated for type II resistance in a greenhouse. Significant genetic variation for FHB resistance was observed in the population and between the two parent lines. DNA markers, including SSR, EST, and EST-CAPS were screened with the parents and four translocation lines. Markers polymorphic between the two parents and also present in the correspondent translocation lines were considered as markers located on chromosome 7el. A preliminary map, based on the genotyping and phenotyping of 277 F_2 plants, was constructed with 12 markers with a total distance of 105 cM. A major QTL associated with FHB resistance was detected in the distal part of chromosome 7el$_2$. The most closely linked markers for the QTL are psr121-Dpn II and Cfa2240. Psr121-Dpn II is a dominant marker and Cfa2240 is co-dominant. They respectively account for 13% and 25% of the phenotypic variation in this population. We also suggest that line KS10-2, a translocation line previously reported as containing the whole short arm and proximal part of the long arm of 7el$_2$ by RFLP analysis, may actually have the whole long arm because all the 12 markers on the long arm have the same genotype in KS10-2 as in K2620, its donor parent.

A HIGH DENSITY LINKAGE MAP OF WHEAT

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ABSTRACT

We display a preliminary high density linkage map of wheat with 1728 AFLP, RFLP, SSR, and other loci constructed from published and unpublished (new Xbarc loci) data for the ITMI (Opata/W7984) mapping population. Framework maps of all 21 chromosomes with 586 loci and combined genome length of 3956.9 cM were generated with Mapmaker. The framework maps were then used to inform an iterative process in which Joinmap was used to generate a final map of 1728 loci with a total length of 2640 cM. Of particular interest is the increased resolution of the identity and order of loci at the distal ends of one or more arms of 11 chromosomes.

FHB RESISTANCE IN SOFT RED WINTER WHEAT ADAPTED TO THE EASTERN US

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ABSTRACT

The Ohio State University wheat breeding program has evaluated several thousand soft red winter wheat (SRWW) breeding lines for scab resistance in an inoculated and listed disease nursery from 2002 to 2004. Most lines are derived from crosses between adapted parents that are not necessarily designed to improve scab resistance. Each year multiple lines per cross are compared to a moderately resistant (Freedom) and susceptible (Pioneer 2545) check. We have summarized FHB index (% scabby florets) data from lines in their first year of testing (YR1) and those in the second year of testing (YR2). Resistance to FHB in the YR1 test is often a primary criteria for selecting entries for the YR2 tests. Data from consecutive years was used to assess the FHB resistance of YR2 entries while one year of data was used for the YR1 entries. Lines were considered to have moderate resistance if their index value was d•Freedom's while lines were considered susceptible if their index value was e•Pioneer 2545's.

When summed over years, moderately resistant lines were common as 58.4% of the 320 YR2 entries and 43.7% of the 1366 YR1 entries had index values d•Freedom. In contrast, only 0.6% of the YR2 entries and 4.3% of the YR1 entries were as susceptible as Pioneer 2545. Strong resistance was rare as only 2.8% of the YR2 and 11.6% of the YR2 entries were significantly more resistance than Freedom. The percentages varied by test (YR1 vs YR2) and years perhaps due selection pressure, and variation for pedigree and disease pressure between years. Moderate resistance was derived from many crosses. Across years, moderately resistant lines were derived from 61.8% of the 144 YR2 crosses and from 65.7% of the 271 YR1 crosses. Susceptibility was noted in just 1.4% of the YR2 pedigrees and only 11.8% of the YR1 pedigrees.

The main conclusions are 1) there is considerable genetic variation for scab resistance in adapted SRWW germplasm, 2) moderate resistance in common, though resistance or susceptibility is not, 3) recombination within SRWW can generate improved resistance, 4) recurrent selection should improve scab resistance of adapted SRWW as moderate resistance appears to come from many sources, and 5) recurrent selection is needed to increase the probability of resistance.

GENETICS OF FHB RESISTANCE IN THE SOFT RED WINTER WHEAT CULTIVAR "FREEDOM"

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ABSTRACT

There is considerable resistance to Fusarium Head Blight in soft red winter wheat (SRWW) adapted to the eastern USA. Little is know of the genetics of this resistance. One source of resistance in SRWW is the moderately resistant cultivar "Freedom". A mapping study in the cross Freedom x Ning 7840 suggested that Freedom has an allele for FHB resistance on chromosome 2AS, an allele with an effect similar to the well known allele on 3BS from Sumai 3 and Ning 7840. We have initiated studies to confirm this finding. In a study of continual selection for FHB resistance in a RIL population from the cross ZM10782/Freedom//30584-37-2/VA91-54-219, 19 lines were selected for excellent FHB resistance. These lines had an average FHB index of 6.1, vs 20.7 for Freedom. Of the 19 lines, 83% had Freedom marker alleles at the 2AS marker (Xgwm296) originally used to map the Freedom allele for FHB resistance. A similar skewing was noted for 3BS markers from the Chinese line ZM10782. The results indicate that 2AS from Freedom and 3BS from ZM10782 where select for during the phenotypic evaluation and selection. The 3BS marker haplotype of ZM10782 matches that of Ning 7840. We have also investigated the 2AS region in a RIL population from the cross of Freedom x OH546 (moderately susceptible). RILs from this population have been screened in the green-house and field for FHB resistance. Considerable segregation and transgressive segregants have been noted. Results from this mapping will be presented.

REPORT ON THE 2003-04 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERY (NUWWSN)

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OBJECTIVE

Evaluate soft winter wheat lines for resistance to FHB in a multi-site uniform nursery.

INTRODUCTION

Each year the USWBSI funds coordination of a uniform nursery to assess FHB resistance in soft winter what lines adapted to the northern US.

MATERIALS AND METHODS

The 2003-04 NUWWSN evaluated 50 breeding lines and six checks from 12 breeding programs (Table 1). Data was collected from 15 field trials in the US and Canada and four greenhouse tests. The field trials used various methods of inoculation and rating, though most use mist irrigation and Fusarium infected corn kernels as inoculum. Most cooperators reported incidence (INC as %) and severity (SEV as %) collected on 20 heads per replication. INC and SEV were used to calculate index (IND=INC*SEV/100). Some also collected data on kernel rating (KR, visual estimate of % FDK) percent scabby seed (PSS) based on seed weight and DON in ppm. An ISK rating was calculated as ISK = (0.3xINC)+(0.3xSEV)+(0.4x%SS) and ranges from 0-100. The greenhouse tests were conducted using single floret inoculation and all reported severity (GHSEV as %). For this report, data is averaged over all locations and the genotype x location interaction was used as the error to calculate an LSD (0.05). For each trait, each entry was compared to the entry with the lowest mean, and the entry with the highest mean using the $LSD_{0.05}$.

RESULTS AND DISCUSSION

A brief summary of the NUWWSN trial is presented here. The full version of the 2003-04 NUWWSN report will be available at the 2004 USWBSI forum and at the USWBSI web site (http://www.scabusa.org/). Based on proportion of total sum of squares (TSS) accounted for by genotype by environment interaction (GEI) effects, GEI was an important source of variation for SEV (21.3% of TSS), IND (16.5%), and GHSEV (43.5%). The data summarized over all locations the data for all lines is presented in Table 2. The data for the best and worst lines is summarized in Table 3.

Table 1. Entries in the 2003-2004 Northern Uniform Winter Wheat Scab Nursery (NUWWSN).

ENTRY	NAME	PEDIGREE
1	PIONEER 2545	Check
2	ERNIE	Check
3	FREEDOM	Check
4	IL97-6755	Check
5	PATTERSON	Check
6	TRUMAN	Check
7	97397J1-4-1-4-7	96204-A1-12//GOLDFIELD/92823A1-11
8	981238A1-1-44-1	ERNIE//91193D1-10/X117
9	981312A1-6-2-2	GOLDFIELD/X117//ROANE/92145A2-4-6
10	981517A1-1-5-2	GOLDFIELD/201R
11	992128A2-4-1	PATTERSON/201R//91202D1-1/3/INW9811/X117//PATTERSON
12	VAN98W-342	CK983//GA-ANDY/VA90-21-20 (79IWWRN67//CK65-20/ATR) ,F13
13	VA03W-630	VR95B717/PION2684,F4:6
14	VA03W-633	VA96W-234//VR95B717/VA96W-234,BC1F5
15	VA03W-644	Roane//W14/Roane,BC1F5
16	VA03W-674	PC-7(CHILL"S"/YM16:SCAB-RES)/3/92-51-39// CK9803/RCT/4/93-52-55 (MSY*3/ BALKAN//SAL),F9
17	IL96-24851-1	IL90-6364//IL90-9464/Ning 7640
18	IL99-27048	IL90-6364/Pioneer brand 2571
19	IL00-8061	P81138I1-16-5-50/Foster//IL93-2485
20	IL00-1665	IL91-13114/Y88-3a// Foster//Pontiac
21	IL99-20756	P813I1-16-2-1-2-3-3/Foster//IL93-2489
22	KY97C-0151-1	ROANE/KY9787C-42-8-5
23	KY96C-0895-1	JACKSON/COKER 9803//2552
24	KS00HW175-4	ARL/89H20
25	KS950409-P-4	HBK0935W-24/JGR'S'//HEYNE
26	MD27-37	LOV29/TYLER//RCT*2/GAINES/CKR9835
27	MO010925	MO 94-182/Ernie
28	MO010789	Coker 9474/Clemens
29	MO010574	MO 94-103/Pioneer 2552
30	MO010719	MO 12278/Pioneer 2552
31	MO011130	MO 94-046/Pioneer XW535
32	NY88046-8138	Susquehanna/Harus
33	Caledonia Resel-T	Reselection from Caledonia
34	NY91028-9073	Harus/4/CS/A.Curvif//Glenn/3/Ald/Pvn(M-30)
35	NY91028SP-9245W	NY91028SP-9245WHarus/4/CS/A.Curvif//Glenn/3/Ald/Pvn(M-30)
36	NY89025-9111W	88076(PF84432/Augusta)/FL302
37	OH743	OH529/OH506
38	OH751	10584-0801/COKER 9663
39	OH776	OH513/OH515
40	OH788	PIONEER2571/OH483
41	OH790	PIONEER2571/OH483
42	X00-1051	T814//MO11769/LX8728D
43	X00-1058	T814//MO11769/LX8728D
44	Y00-3044	XY90-1B//LB291/PS8424
45	E2057	D2295/GR942
46	E2038	PIONEER 2555/D1098
47	E2048	D3583/TAM 101
48	E2037	PIONEER 2737W/D1098
49	E0009	NY82-105-2/CAYUGA
50	RCATL33	(Ruby/Frontana #1 x AC Ron//WEKO609H3 x AC Ron)
51	RCATL10	(AC Ron x SVP72017//Balkan)
52	RCATL24	(Ena x AC Ron//Ruby/Frontana #1)
53	RCATL12	(Fundulea x AC Morley)
54	RCAT L2	(Ruby/Frontana #1 x 2737W//Balkan)
55	WESLEY	KS831036-3//COLT/CODY
56	NE98466	KS89H50-4/NE90518(-BRL//SXL/BENN)

Table 2. Average FHB trait values for entries in the 2003-2004 NUWWSN. The number of times an entry was not significantly different from the lowest mean in a column is shown in the "#l" column, while the "#h" column shows the number of times an entry was not significantly different from the highest mean in the column. Heading date (HD, julian days) and height (HGT, inches) were not included in the #l or #h statistics.

ENTRY	NAME	INC	SEV	IND	GHSEV	KR	PSS	ISK	DON	#l	#h	HD	HGT
1	PIONEER 2545	80.7 h	53.9 h	43.5 h	32.7	50.9 h	48.2 h	42.0 h	11.3 h	0	7	141	33
2	ERNIE	60.2	33.0	24.4	13.9 l	29.4 l	18.6 l	18.6 l	8.4 l	5	0	139 l	30 l
3	FREEDOM	62.9	39.4	26.3	12.2 l	41.9	35.9	32.2	5.8 l	2	0	142	35
4	IL97-6755	47.0 l	21.1 l	18.2 l	9.8 l	24.4 l	17.9 l	12.0 l	3.7 l	8	0	140	38
5	PATTERSON	69.7	44.4 h	37.3 h	44.7 h	38.1 l	22.9 l	26.6	6.4 l	3	3	140	35
6	TRUMAN	48.3 l	20.4 l	13.0 l	8.2 l	28.6 l	22.6 l	14.0 l	5.1 l	8	0	145	35
7	97397J1-4-1-4-7	59.1	28.6 l	21.5	10.3 l	33.1 l	24.6 l	18.8 l	5.9 l	6	0	139 l	32
8	981238A1-1-44-1	63.4	36.6	27.3	22.6 l	45.6	25.0 l	24.5	4.9 l	3	0	139 l	32
9	981312A1-6-2-2	62.3	32.8	24.1	17.6 l	37.5 l	27.8	21.3 l	5.1 l	4	0	142	31 l
10	981517A1-1-5-2	56.5 l	29.0 l	20.6 l	10.8 l	38.2 l	21.9 l	18.5 l	7.6 l	8	0	142	32
11	992128A2-4-1	79.0 h	46.9 h	38.9 h	24.9	52.3 h	30.9	35.2 h	6.5 l	1	5	139 l	32
12	VAN98W-342	76.6 h	47.5 h	35.4	25.1	48.9 h	40.4 h	41.6 h	8.7 l	1	5	141	29 l
13	VA03W-630	74.0 h	51.0 h	40.2 h	49.2 h	47.2 h	36.1	35.9 h	12.1 h	0	7	143	32
14	VA03W-633	71.7 h	43.2 h	32.0	26.8	40.3 l	34.6	33.7	10.5 h	1	3	141	30 l
15	VA03W-644	66.5	35.6	25.3	15.2 l	39.7 l	22.9 l	22.7 l	6.8 l	5	0	140	30 l
16	VA03W-674	67.7	45.7 h	33.7	34.2	43.6	30.4	29.6	11.8 h	0	2	139 l	30 l
17	IL96-24851-1	60.8	28.9 l	21.2	9.9 l	40.8 l	25.8 l	19.9 l	4.6 l	6	0	142	32
18	IL99-27048	60.0	32.7	26.7	19.1 l	31.9 l	19.3 l	22.8 l	5.8 l	5	0	138 l	34
19	IL00-8061	54.5 l	25.5 l	18.3 l	18.1 l	33.3 l	17.0 l	15.2 l	6.9 l	8	0	141	36
20	IL00-1665	61.2	34.7	24.0	23.3 l	42.7	27.1 l	24.9	6.8 l	3	0	141	33
21	IL99-20756	59.2	31.4 l	25.0	11.9 l	26.4 l	13.8 l	17.2 l	7.3 l	6	0	138 l	34
22	KY97C-0151-1	72.1 h	46.4 h	36.7 h	32.3	46.7 h	32.7	32.4	7.5 l	1	4	141	35
23	KY96C-0895-1	58.5	34.7	24.2	27.9	36.5 l	27.1 l	23.8 l	8.2 l	4	0	144	37
24	KS00HW175-4	63.7	39.9	28.1	22.6 l	42.9	37.1	30.5	9.9 l	2	0	143	35
25	KS950409-P-4	70.2	42.8	32.5	18.8 l	49.3 h	43.5 h	34.2	11.8 h	1	3	142	34
26	MD27-37	66.3	41.4	34.0	25.0	37.9 l	25.3 l	28.5	5.5 l	3	0	139 l	32
27	MO010925	61.3	37.7	27.6	29.5	40.9 l	30.7	26.1	4.9 l	2	0	142	35
28	MO010789	58.1	38.1	26.0	37.5 h	34.9 l	28.2	29.2	10.3	1	1	143	37
29	MO010574	67.1	37.2	27.8	19.4 l	38.9 l	28.8	25.5	10.4 h	2	1	142	37
30	MO010719	58.4	31.0 l	23.2	14.5 l	40.6 l	29.0	21.8 l	10.4 h	4	1	141	40
31	MO011130	73.4 h	45.4 h	37.6 h	32.6	49.3 h	38.7	37.8 h	12.9 h	0	6	142	38
32	NY88046-8138	67.2	36.3	26.6	31.9	53.9 h	51.5 h	30.7	13.5 h	0	3	147 h	36
33	Caledonia Resel-T	72.1 h	37.2	25.8	32.6	49.5 h	46.3 h	34.0	15.2 h	0	4	147 h	36
34	NY91028-9073	60.1	28.6 l	20.1 l	23.7 l	54.4 h	50.6 h	28.4	16.9 h	3	3	148 h	35
35	NY91028SP-9245W	59.4	27.8 l	19.4 l	35.6 h	47.6 h	41.0 h	22.0 l	16.8 h	3	4	148 h	35
36	NY89025-9111W	62.2	32.6	23.2	35.4 h	51.3 h	43.6 h	27.7	15.1 h	0	4	148 h	37
	AVERAGE	63.7	36.7	27.1	25.2	42.8	32.0	27.0	9.0			142	34
	MINIMUM	47.0	20.4	13.0	8.2	24.4	13.8	12.0	3.7			138	29
	MAXIMUM	80.7	53.9	43.5	51.0	56.7	51.5	42.0	16.9			148	41
	LSD (0.05)	10.1	11.2	7.6	16.6	16.6	13.4	11.8	6.5			2	2
	# LOCATIONS	11	11	14	4	3	5	5	3			9	4

l, h indicate means that are not significantly different from the lowest or highest mean in that column, respectively.

Table 2. (continued)

ENTRY	NAME	INC	SEV	IND	GHSEV	KR	PSS	ISK	DON	#l	#h	HD	HGT
37	OH743	64.5	40.1	30.6	24.1 l	44.3	34.8	29.1	7.5 l	2	0	143	36
38	OH751	70.5	39.9	29.8	25.1	41.6	28.5	29.6	8.2 l	1	0	142	35
39	OH776	68.6	43.4 h	34.0	35.1 h	44.8	31.9	26.0	10.1 l	1	2	141	34
40	OH788	64.9	45.6 h	35.7	50.1 h	52.6 h	38.8	33.9	9.4 l	1	3	140	34
41	OH790	71.5 h	43.2 h	35.4	43.7 h	50.1 h	38.1	35.9 h	7.5 l	1	5	141	34
42	X00-1051	57.7	34.1	22.0	18.0 l	36.2 l	23.1 l	23.0 l	4.9 l	5	0	142	33
43	X00-1058	65.7	42.5	27.8	12.0 l	44.8	34.0	30.8	9.4 l	2	0	144	33
44	Y00-3044	65.9	49.1 h	29.2	30.0	56.7 h	45.8 h	38.7 h	10.3	0	4	145	33
45	E2057	56.4 l	29.0 l	19.7 l	22.4 l	39.1 l	40.5 h	22.5 l	11.5 h	6	2	146 h	32
46	E2038	58.3	29.2 l	18.8 l	13.4 l	42.1	27.4	20.8 l	12.2 h	4	1	147 h	34
47	E2048	57.4	29.0 l	19.7 l	26.7	47.6 h	39.7	23.8 l	10.2 l	4	1	146 h	38
48	E2037	59.4	34.2	21.6	23.0 l	53.8 h	38.4	29.6	11.6 h	1	2	147 h	35
49	E0009	55.0 l	26.4 l	18.9 l	27.8	45.8	30.4	20.3 l	8.7 l	5	0	148 h	38
50	RCATL33	57.0 l	30.7 l	23.0	51.0 h	53.1 h	28.5	25.0	7.4 l	3	2	140	41 h
51	RCATL10	67.5	36.5	26.5	31.2	52.7 h	43.0 h	32.7	9.8 l	1	2	145	39
52	RCATL24	49.7 l	22.8 l	16.2 l	33.9	41.6	30.5	16.4 l	11.8 h	4	1	147 h	42 h
53	RCATL12	58.3	35.2	24.5	26.1	48.2 h	44.5 h	27.5	9.0 l	1	2	144	40
54	RCAT L2	62.7	39.0	28.5	23.6 l	39.3 l	25.1 l	24.0	8.7 l	4	0	140	40
55	WESLEY	77.2 h	54.1 h	41.7 h	34.2	63.1 h	53.2 h	46.6 h	6.6 l	1	6	143	33
56	NE98466	69.4	44.3 h	33.4	31.0	47.8 h	36.5	35.6 h	5.5 l	1	3	142	37
	AVERAGE	63.7	36.7	27.1	25.2	42.8	32.0	27.0	9.0			142	34
	MINIMUM	47.0	20.4	13.0	8.2	24.4	13.8	12.0	3.7			138	29
	MAXIMUM	80.7	53.9	43.5	51.0	56.7	51.5	42.0	16.9			148	41
	LSD (0.05)	10.1	11.2	7.6	16.6	16.6	13.4	11.8	6.5			2	2
	# LOCATIONS	11	11	14	4	3	5	5	3			9	4

l, h indicate means that are not significantly different from the lowest or highest mean in that column, respectively

MOLECULAR BREEDING OF WHEAT AND INTROGRESSION OF FHB RESISTANCE QTL

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ABSTRACT

During the last 6-7 years, several publications have reported on the map location of QTL associated with Fusarium head blight (FHB) resistance. Many of these findings have been validated in similar mapping studies conducted at Agriculture and Agri-Food Canada which lead to the development of a large-scale molecular breeding effort to introgress FHB resistance QTL into Canadian wheat. Beginning in 2002, resistant and elite susceptible parent lines were identified and characterized genotypically. This established which QTL were in each donor line and which crosses were most appropriate to track the QTL introgression. The project included three sources of FHB resistance (Sumai 3, Wuhan, Nyubai) used in four initial crosses (streams) to different elite susceptible wheat. Each stream went through two cycles of backcrossing then selfing to derive BC_2F_2 plants. Molecular breeding selected for FHB resistance QTL and approximately 70 background genetic loci to accelerate genome restoration. In total, >12,300 half seeds were genotyped generating >64,000 microsatellite allele datapoints which resulted in selection of 48 homozygous BC_2F_2 plants for stream intercrossing. The intercrossing assembled novel FHB resistance gene pyramids from the different sources of resistance. Doubled haploid (DH) lines from the intercross F1's have been produced and seed increases are planned for the winter of 2004/05. Our expectation is to test >4,000 DH lines in replicated FHB nurseries in 2005. In 2004, a single FHB nursery with three replications was planted in Ottawa, ON with 127 entries of BC_2F_3 lines derived from the project carrying 0-3 FHB resistant QTL. Heavy infection levels were established and all entries were evaluated for 1) FHB index, 2) % Fusarium damaged kernels and 3) DON analysis. A comparison between sister (BC_2F_3) lines carrying zero FHB resistance QTL and 1-3 FHB resistance QTL showed significantly lower infection and FDK in lines carrying FHB resistant QTL. The DON analysis will also be presented when completed. Genetic background was also noted to affect the expression of FHB resistance QTL. A detailed description of the molecular breeding and genetic aspects of the project will be presented along with the preliminary findings from the 2004 FHB nursery.

THE RESPONSE OF WHEAT SEEDLING TO THE METABOLITES OF *FUSARIUM CULMORUM*

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OBJECTIVES

The aim of this contribution was to relate the possible response of wheat seedlings to the crude metabolites and trichothecene of *F. culmorum* with their resistance to root rot caused by pathogen.

INTRODUCTION

The most frequent species *F. culmorum* and *F.graminearum* have so far been identified in plant and soil on wheat in Slovakia. Because certain fungal strains are able to synthesize a number of toxic metabolites (Jacobellis and Bottalico, 1981), it is possible that several different mycotoxins will be present in a single plant. In Slovakia zearalenone, deoxynivalenol, only trace of T-toxin and diacetoxyscirpenol, were identified in wheat kernels (Šrobárová and Bachan 1994, Perkowski et al., 2002) and through the seed may be transmitted into seedling. However, to this time their impact and role in plant is not fully understood. Eudes et al. (1997) demonstrated that plant toxicity of several trichothecene was very different to their toxicity in animals. Then concomitantly Eudes et al., (2000), Pavlová and Šrobárová (2001) proved the relationship between resistance of cvs and toxins There is also a question, how the structure of plant organs, respectively the resistance mechanism of the host plant will be revealed when mixture of the main mycotoxins are accumulated in infected seed and may be transmitted to the seedling.

MATERIALS AND METHODS

Preparation of culture filtrate. The isolate of *F.culmorum* was cultured in potato-glucose extract (200 g of potatoes, 10 g glucose per 1 litter of extract) in Erlenmeyer flasks for 3 weeks. The cultures were then autoclaved at 120 ^{0}C, pressure 1, 8 MPa, for 20 min and filtered. The filtrates were autoclaved again at the same temperature and pressure.

Set-up of experiment. From the wheat cultivars of preliminary tests we selected the cvs. Hana–resistant, Barbara-susceptible one. Seeds of the cultivars were disinfected in a 0, 6 % solution of NaOCl for 1 min, rinsed several times in distilled water, and then kept in double distilled water for 36 h to swell. Two layers of filter paper moistened by sterilised distilled water were put into glass jars (sterilised by hot air), and 25 seeds of each cultivar were placed on it. The vessels were covered with Petri dishes and placed in a growth chamber with a temperature of 20-25 ^{0}C and a 12 h light period. After 4 days, at which the coleoptiles and three roots had developed, we added 10 ml of the culture filtrate of *F. culmorum* and mixture of its mycotoxins (SIGMA): deoxynivalenol (DON), T-2 toxin and diacetoxyscirpenol (DAS), each in concentration 30µg/ml^{-1}, into the vessels. After ten days of cultivation phytotoxicity was evaluated according to biomass production and ultrastructure of root and leaves cells. To the control plants 10 ml of distilled water was added.

Ultrastructural evaluation. For electron microscopy, segments from central part of 3rd leaves and 5mm long root apices were fixed with 3% glutaraldehyde and 1% OsO_4, dehydrated in ethanol and embedded in Spur's medium. Ultrathin sections were stained with uranyl acetate and Pb-citrate and investigated with the EM Tesla BS 500.

Statistical analysis. All statistical calculations were performed by computer software STATGRAPHICS Version 2, 1, Statistical Graphics Corporation.

RESULTS AND DISCUSSION

***Biomass*.** Treatment with the culture filtrate resulted in an increase of biomass production, especially of roots (length, fresh and dry matter) of seedlings of the cv-s Hana (resistant), in the susceptible cv. Barbara there was generally a decrease of root biomass. The culture filtrate stimulated the growth of leaves of this cultivar, data with statistic importance of the observed parameters are shown in Tab. 1 and 2.and Fig. 1.). The growth inhibitive effect of both the culture filtrate and the toxins was more pronounced in roots than in leaves.

Ultrastructure. The incubation of roots of susceptible cv. Barbara with toxins related in a variety of morphological alterations, vesiculation and loss of ribosomes is extremely. Cells of mesophyll had a disturb structure of chloroplasts, while in resistant one only disturbed thylakoids arrangement (Fig.2) as usually under stress condition (iamporová and TrgiHová 1999). In the cytoplasm of root the cisternae and long tubular elements of endoplasmatic reticulum (ER) disintegrate into short ER tubules, fragmentation, dilatation and vesiculation of cisternae is extremely developed.

This alteration is accompanied by loss of ribosomes from the surface of the cisternae.The large dark central vacuoles and many small vacuolar compartments with membrane-bound electrolucent are present in the cytoplasm (Fig.3).The formation of vacuoles can be important for creating a compartment for protein storage.

Ultrastructural modifications were seen mainly in the main structures participating in protein metabolism.In the root cells of susceptible cv. the chromatin clamping and margination in the nucleus is a characteristic feature. Disaggregating of cytoplasmic organelles or fragmentation of the nucleus was observed (Fig. 4). The highly condensed chromatin masses do not allow differentiation of the nucleolus or part of it. Clear identification of ribosome is restricted to some areas of the outer nuclear membrane. For the resistant cv (Hana) to the pathogen, toxins didn't destroyed cells thoroughly. After ten days of incubation, about 80 % of root cell show cellular alteration. In both cultivars the inhibitive effect was higher of the culture filtrate then the toxins .The efficacy was more pronounced in roots than in leaves. Root cells after treatment by crude metabolites were destroyed to the higher degree than by toxins: compartmetation (*"like structural apoptosis"*) of nucleus was observed.

Structure of tissue observed under transmission electron microscope (TEM) proved the same response of cvs as to the pathogen.

The cv. Hana, which is resistant both to the pathogen and to the accumulation of zearalenone (Šrobárová and Bachan 1994; Pavlová and Šrobárová 1997), or to single DON (Šrobárová and Pavlová 2001) it has the same response to mixture of trichothecene. While susceptible cv had the cell thoroughly destroyed, the resistant one has just some membrane disoorded;.

The used fusariotoxins are trichothecene, the most effective specific inhibitors of protein synthesis known and free amino acids f.e.free proline are accumulated in tissue Bandurska et al.1994). These changes are accompanied with structural modifications or destruction mainly of the cellular organelles and compartments which are involved in transnasional protein processing as well as in its transport. In this connection we suppose a possible mechanism of resistance in this level for the pathogen and its toxins: A high level of free proline in tissues can suppres (Jacobelis and Botalicco 1981) the leakage of electrolytes from cells caused by a pathogen and its toxins (Wang and Miller 1988). The accumulation of free proline is also stimulated by abscisic acid which is produced in wheat seedlings infected by *F. culmorum* (Michniewicz et al., 1990). This mechanism can stabilise the consequences of the attack on the host and renew the function of membranes (Bandurska et al., 1994). From this point of view, susceptibility or resistance, better sensitivity is characterized by the speed of this protective mechanism.

ACKNOWLEDGEMENTS

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Table 1. Analysis of variance for the production criteria of cvs treated by secondary metabolites.

variable	root length	leaf growth	root number	leaf number	root fwght	leaf fwght	root dwght	leaf dwght
cultivar	$21,97^{++}$	$121,25^{++}$	$1,39^{-}$	$6,30^{-}$	$3,77^{-}$	$7,78^{+}$	$13,97^{++}$	$41,31^{++}$
treatment	$5,43^{-}$	$8,46^{+}$	$15,12^{+}$	$4,53^{-}$	$3,5^{-}$	$4,50^{-}$	$14,04^{++}$	$6,70^{-}$
sum of squares	38,67	37,59	7	0,52	0,47	1,36	0,01	0,03
Standard error	2,4	0,51	0,93	0,1	0,107	0,192	0,001	0,002

$^{+}P \leq 0, 05$ $^{++}P \leq 0, 01$

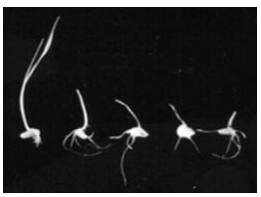

Figure 1. Different level of growth inhibition of wheat seedlings treated by metabolites of *F. culmorum*, compare to control (left), then to the right direction: two seedlings by crude metabolite, and last ones by fusariotoxins.

Table 2. Correlation coefficients between mean values of all production criteria in winter wheat cultivars treated with secondary metabolites.

		total	Hana/	control/	control/
		biomass	Barbara	filtrate	toxins
length	roots	$0,89^{++}$	$0,87^{+}$	$0,89^{++}$	$0,94^{++}$
	leaves	$0,84^{++}$	$0,92^{+}$	$0,94^{++}$	$0,89^{++}$
number	roots	$0,97^{++}$	$0,99^{+}$	$0,44^{-}$	$0,98^{+}$
	leaves	$0,54^{-}$	$0,89^{++}$	$0,91^{+}$	$0,98^{+}$
fresh	roots	$0,93^{+}$	$0,96^{++}$	$0,94^{+}$	$0,99^{+}$
weight	leaves	$0,88^{++}$	$0,87^{+}$	$0,90^{++}$	$0,99^{+}$
dry	roots	$0,95^{+}$	$0,73^{+}$	$0,50^{-}$	$0,89^{++}$
weight	leaves	$0,90^{++}$	$0,96^{++}$	$0,97^{++}$	$0,61^{-}$

$^{+}P \leq 0,05$ $^{++}P \leq 0,01$

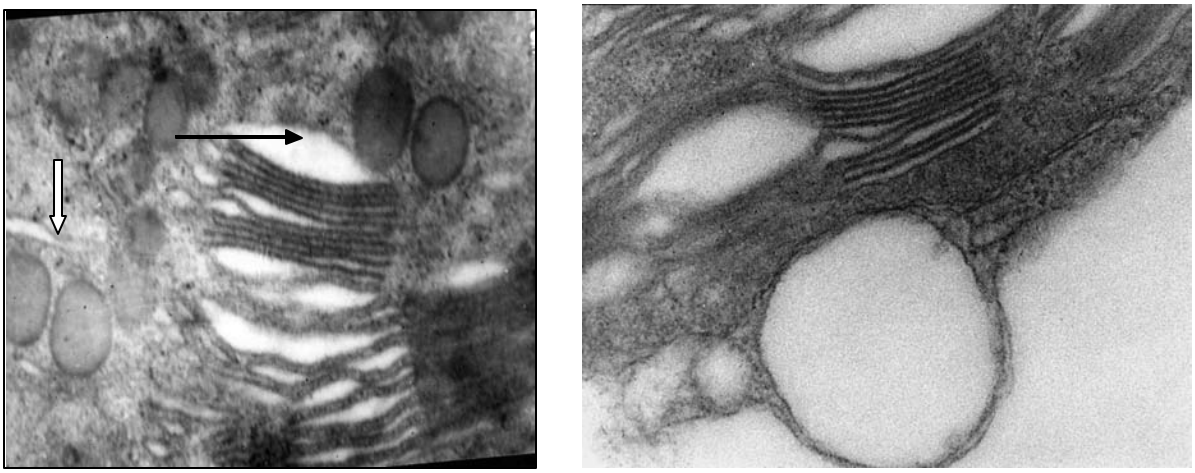

Figure 2. The ultrastructure of mesophyll cells: right) vacuolated chloroplast, white stroma (arrow) of susceptible cv.Barbara and left) resistant cv.Hana.

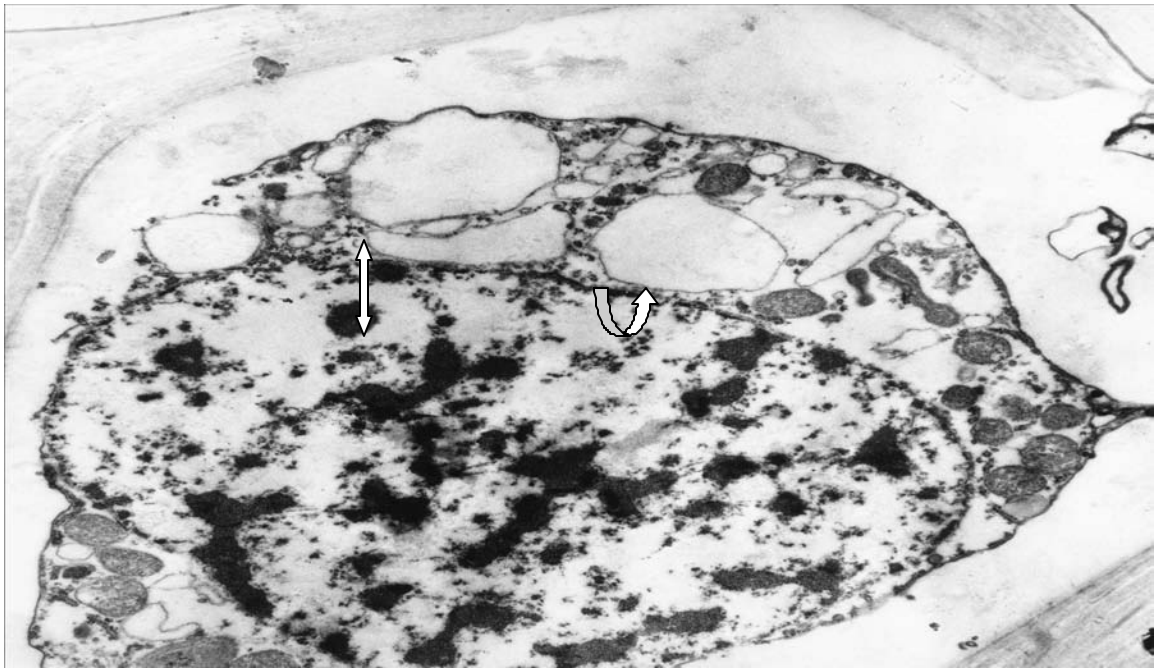

Figure 3. The root cell of resistant cv.: plasmolysis of cell, dark central vacuole and electron transparent vacuoles in cytoplasm.

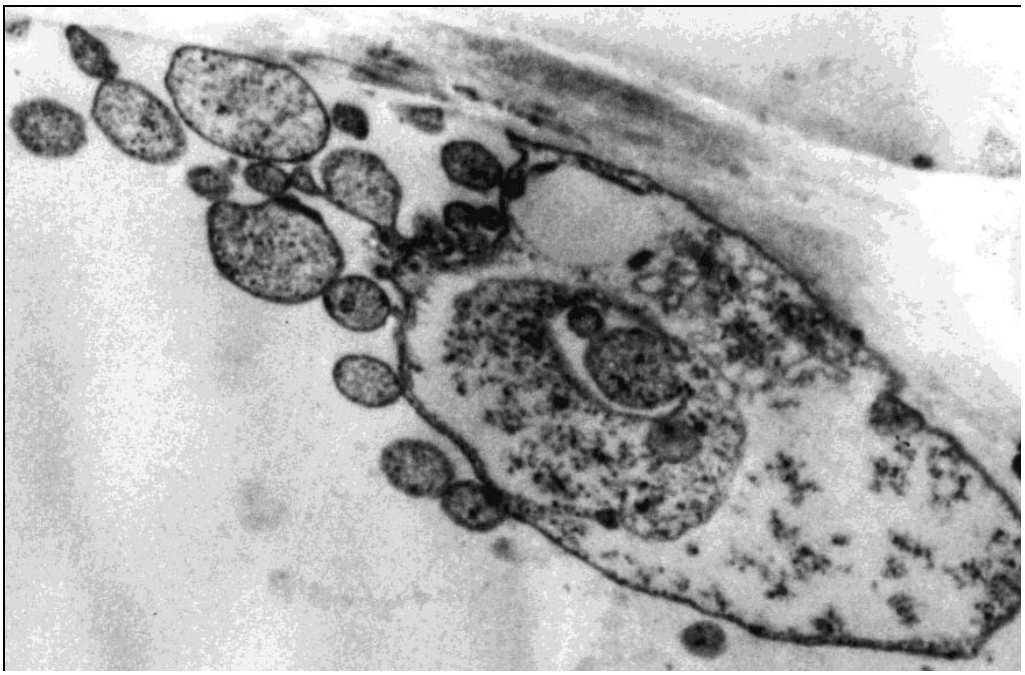

Figure 4. Root cell of susceptible wheat cv.Barbara treated by metabolites: rest of cell cytoplasm and fragmentation of the nucleus (arrows) is observed.

A QTL ON *TRITICUM DICOCCOIDES* CHROMOSOME 6B ASSOCIATED WITH RESISTANCE TO FHB

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is a serious disease problem on durum wheat (*Triticum turgidum* L. var. *durum*) in the USA. To date, the resistance to FHB available in hexaploid wheat (*T. aestivum* L.) sources has not successfully been transferred to tetraploid durum. In the 1980's, USDA geneticist L.R. Joppa produced a set of disomic chromosome substitution lines in the background of "Langdon" durum using the tetraploid wild emmer (*T. turgidum* L. var. *dicoccoides*) line FA-15-3 (= "Israel A"). Each LDN-DIC line had a different pair of chromosomes from wild emmer substituted for the corresponding Langdon durum chromosomes (Joppa and Williams. 1988. Genome 30:222-228). In 2002, Stack et al. (Crop Sci. 42:642-647) reported that several of the LDN-DIC substitution lines showed resistance to FHB. One of these, the LDN(DIC-3A), has been studied in some detail (Otto et al. 2002. Plant Mol. Biol. 48:625-632). In the trials of Stack et al., the LDN(DIC-6B) line also showed significantly reduced FHB. The 6B line is of interest because the 6B chromosome is the site of a gene for high grain protein content which has been extensively studied and mapped (Joppa et al. 1997. Crop Sci. 37:1586-1589). We evaluated a population of 85 recombinant inbred chromosome lines (RICLs) derived from LDN x LDN(DIC-6B) for reaction to FHB. Markers along the map of 6B, previously constructed by Du and Hart (1998. Theor. Appl. Genet. 96:645-653) and Olmos et al. (2003. Theor. Appl. Genet. 107:1243-1251), were surveyed for associations with FHB resistance. Simple linear regression and composite interval regression analysis mapping indicated the presence of a quantitative trait locus (QTL) on the short arm of 6B. This QTL accounted for about 20 percent of the phenotypic variation for resistance to FHB. It will be beneficial to combine this QTL with others to increase the levels of FHB resistance in durum cultivars. It remains to be determined whether this resistance locus is comparable to the one identified on 6BS in hexaploid wheat (Anderson et al. 2001. Theor.Appl.Genet.102:1164-1168).

ASSESSING TYPE 1 RESISTANCE IN WHEAT TO FUSARIUM HEAD BLIGHT IN HIGH DISEASE PRESSURE SCREENING NURSERIES

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ABSTRACT

Phenotypic expression of resistance in wheat to Fusarium head blight (FHB) is of three principal types: a) reduced initial infection (A.K.A. "type 1" resistance); b) reduced lesion expansion beyond primary infections (A.K.A. "type 2" resistance); c) reduced kernel damage beyond that accounted for by (a) or (b). Expression of strong type 2 resistance, found in some Chinese wheats, has largely been the basis for recent breeding efforts in spring wheat in North America. Attempts to develop commercial wheats with strong type 1 resistance have been less successful. Resistance to primary infection is often estimated by disease incidence. Inoculated, irrigated nurseries are widely used in breeding wheat for resistance to FHB, but these often give very high levels of disease incidence, good for limiting escapes and comparing FHB severity, but such high disease makes comparisons for incidence difficult. For example, in several recent years the FHB incidence across all locations in the uniform regional spring wheat FHB nursery has been above 80%, and has often exceeded 90%. A practical consequence of such high disease pressure is that identifying and incorporating strong type 1 resistance into cultivars has lagged behind the use of type 2 resistance. We propose that counting of individual primary infections ("hits") per infected spike, will allow better estimation of type 1 resistance in high disease pressure nurseries. The relationship between the number of hits per spike and the incidence of infected spikes is the same one that P.H. Gregory worked out some 60 years ago when he applied the "multiple infection transformation" to plant disease. Hits can be counted when symptomatic spikelets appear after the latent period is past but before vegetative spread within the head has proceeded. In spring wheat, hits can be easily identified at ten days postanthesis even in very susceptible lines. Between 1998 and 2003 we sampled 583 plots of spring wheat in the NDSU spring wheat breeding nursery at Prosper, ND. Inoculation in this nursery is by *G. zeae* ascospores produced in perithecia on colonized grain spread on the ground. In this nursery many wheat heads show symptoms of multiple hits. By counting hits we have identified lines with reduced incidence of FHB, independent of FHB severity. Acknowledgement: The authors thank NDSU spring wheat and durum breeders for use of this nursery. (This poster was presented at the 2004 Annual meeting of the American Phytopathological Society in Anaheim, CA. The abstract appeared in Phytopathology 94:6(suppl.):S98.)

ASSESSMENT OF DISEASE SPREAD IN BARLEY SPIKES AFTER SINGLE FLORET INOCULATION WITH *FUSARIUM GRAMINEARUM*

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OBJECTIVE

To assess disease spread in barley spikes after single floret inoculation with *Fusarium graminearum*.

INTRODUCTION

In wheat, Schroeder and Christensen (1963) described two types of resistance to Fusarium head blight (FHB): "type I" resistance operates against initial pathogen infection, and "type II" resistance operates against spread of the pathogen in the spike after an initial infection site is established. In practice, "type I" resistance is usually measured by spray-inoculating spikes with a suspension of *Fusarium graminearum* macroconidia or ascospores and then assessing the number of infected spikelets. In contrast, "type II" resistance is usually measured by inoculating a single floret within the center of a spike and then assessing the spread of infection from that initial site (Schroeder and Christensen 1963). Resistance to spread in wheat is very important, and much effort has been expended to identify accessions with this type of resistance. Sumai 3 is a wheat accession that possesses a high level of resistance to spread and has been used extensively in breeding for resistance to FHB (Bai and Shaner 2003; Mesterhazy 2003). Sources of resistance to initial infection are also being sought in wheat.

Based on studies conducted in wheat, several researchers began screening barley for similar types of FHB resistance. In the ICARDA/CIMMYT barley improvement program in Mexico, barley accessions are screened for resistance to initial infection and to spread in the field. Resistance to initial infection is assessed after spraying spikes with a conidial suspension of *F. graminearum*. Resistance to spread is assessed after placing a cotton ball soaked in a suspension of *F. graminearum* macroconidia within a central floret of spikes. Inoculated spikes are then covered with glassine bags to prevent allo-infection and evaluated for disease spread about 30 days later (Vivar et al. 1997). These screening tests are conducted at the experiment station in Toluca where rain falls nearly every day during the incubation period and evening temperatures are cool. From this screening program, several cultivars (e.g. Shyri and Atahualpa) and lines (e.g. DH-52, a line derived from the Gobernadora x CMB643 population) were reported to carry resistance to spread (Vivar et al. 1997). Lines with different levels of resistance to initial infection and spread were also described. For example, DH-98, also derived from the Gobernadora x CMB643 population, exhibited a high level of resistance to initial infection and a low level of resistance to spread.

In another study, Capettini (1999) assessed resistance to spread in barley in the greenhouse by inoculating plants with the single floret technique and then incubating them at 100% RH for 72 hours. Significant differences were observed for the amount of spread in the spike (this included mostly lateral spread from the central to lateral spikelets and not vertical spread) among six-rowed cultivars, suggesting that genetic variability exists for this type of resistance. However, the overall mean number of kernels infected by spread after inoculation was only 7.3 (12% of kernels in a spike); thus, disease spread was not extensive. Capettini (1999) stated that his results should be interpreted with caution due to the extreme level of variability observed in the experiments.

Numerous observations have been made on thousands of barley accessions growing in FHB nurseries established in the Upper Midwest region of the United

States. From these observations, it appears that vertical spread of FHB in barley spikes is extremely rare, if it occurs at all (B. Steffenson, unpublished). In the cases where vertically adjacent spikelets were found infected, one could not rule out the possibility of simultaneous infection of adjacent kernels by propagules of *F. graminearum* or mycelial infections. It is certain, however, that FHB spread in barley does not occur to anywhere near the same degree as it does in susceptible wheat. Lateral disease spread in barley is fairly common and occurs among the three spikelets at a rachis node in six-rowed types (Steffenson 2003). The issue of whether barley possesses identifiably distinct resistance mechanisms (i.e. resistance to initial infection and to spread) against *F. graminearum* has important implications in resistance screening and breeding. In this study, we investigated whether barley exhibits variation for resistance to spread in the spike as was reported in wheat.

MATERIALS AND METHODS

To obtain rigorous control over the inoculation procedure and incubation conditions, all experiments were conducted in a controlled growth room and greenhouse. Six barley accessions, known to vary widely for their reaction to FHB in the field, were evaluated for resistance to spread in the spike. Chevron and CIho 4196 are resistant six-rowed and two-rowed cultivars, respectively. PI 383933 is six-rowed and one of the most susceptible barley accessions known (B. Steffenson, unpublished). It has a very dense spike, an attribute likely to favor disease spread in the spike due to the close vertical proximity of nodes. In the field, PI 383933 often exhibits FHB severities approaching 100%. ICB 111809 is one of the most susceptible two-rowed accessions we have observed in evaluations of barley germplasm over the past eight years. DH-52 and DH-98 are double haploid lines from the Gobernadora x CMB643 population (Vivar et al. 1997). DH-52 was reported to possess a high level of resistance to spread and a low level of resistance to initial infection. In contrast, DH-98 was reported to possess a high level of resistance to initial infection and a low level of resistance to spread. Two wheat accessions (Roblin and Sumai 3) were included in the experiment to assess the validity and consistency of the inoculation method. Roblin is a highly susceptible wheat cultivar. Disease spread after single floret inoculation is often extensive in Roblin. In contrast, Sumai 3 possesses a high level of resistance to spread.

Assessments for resistance to spread were made according to standard methods. A single centrally located floret within each spike was inoculated using a Hamilton PB600-1 repeating dispenser with 500ml luer glass syringe (model 750). Inoculum (10 ml of a 40,000 conidia/ml solution) was placed into the floret without wounding it. For the infection period, plants were placed in mist chambers where ultrasonic humidifiers operated continuously for 40 minutes to establish a layer of free moisture on the spikes. Thereafter, the humidifiers were set to come on for 10 minutes every 60 minutes so as to maintain a saturated environment. During the 40-hour infection period in the dark, the temperature was maintained at 18-24°C. After the infection period, the chamber doors were opened to facilitate the slow drying of plant surfaces. Then, plants were then placed in a greenhouse at 18-23°C. In preliminary experiments, we varied the length of the infection period from 48-72 hours. We found that inoculated plants incubated for 48 hours or more in the mist chambers usually had mycelium growing out from the inoculated floret. The mycelial growth was often extensive and completely covered other adjacent spikelets. Thus, a misting period of 40 hours was selected because it resulted in a high rate of successful infection of the inoculated floret, but reduced significantly the incidence of mycelial growth. In this way, we could favor vertical disease spread occurring via the vascular system—not by external mycelial growth across adjacent spikelets. To further verify whether mycelial infections occurred, we took detailed notes on the presence of mycelial growing from inoculated spikelets immediately after the 40-hour infection period.

RESULTS AND DISCUSSION

The single floret inoculations were highly successful as over 95% infection was achieved in all accessions and in all experiments. Vertical spread occurred in every inoculated spike of Roblin, thereby confirming

the validity and consistency of our methods. In Roblin, vertical spread was extensive often reaching 100% (mean=66%). In contrast, vertical spread in Sumai 3 was less common occurring in only 10% of inoculated spikes. FHB severity resulting from vertical spread in Sumai 3 ranged from 0 to 25% with a mean of 11%. Our results with these wheat accessions are consistent with many previous reports by other investigators. In start contrast to the susceptible wheat cultivar Roblin, vertical spread from inoculated florets in barley was extremely rare. Just two of 25 inoculated spikes (8%) of the highly susceptible accession PI 383933 showed any vertical spread. Vertical spread in PI 383933 was minimal and occurred only in the adjacent spikelets above the inoculated floret. We cannot rule out completely the possibility that these two cases of vertical spread were due to mycelial infection. Mycelial growth can be sparse and difficult to detect on florets. Moreover, it can disappear completely after only a few hours of drying conditions. DH-52 exhibited vertical spread to the adjacent spikelet in two of 20 inoculated spikes (10%), but in these cases mycelial growth was detected on the infected spikelets. DH-98 was reported to have a very low level of resistance to spread by Vivar et al. (1997), but no spread whatsoever was observed in our study. The same result was also observed in the resistant controls of Chevron and CIho 4196 and the susceptible two-rowed control of ICB 111809. Our results clearly indicate that vertical spread (via vascular tissue) in barley is very rare, if it occurs at all. This conclusion is in agreement with previously unpublished research by R. Dill-Macky (personal communication), who conducted similar single floret inoculations with various barley accessions. Our results are also corroborated by numerous anecdotal observations from the field where most spikes show vertically discontinuous spikelet infection. In surveys of naturally infected six-rowed spikes from the field, no visible FHB infections were observed to extend across vertically adjacent nodes within the rachis, again suggesting the lack of spread via the vascular tissue (S. Lewandowski and W. Bushnell). In cases where vertically adjacent spikelets are found infected, one must consider the possibility that they occurred due to mycelial infection or simultaneous (or near-simultaneous) infection episodes. We conducted all of our experiments under the carefully controlled conditions of the growth room and greenhouse to eliminate these factors as much as possible.

Based on the results presented here and of numerous observations in the field, it is apparent that Midwestern barley germplasm possesses a very high level of resistance to spread in the spike. Therefore, screening nurseries for assessing this type of resistance (e.g. by the ICARDA/CIMMYT program in Mexico) may not be appropriate for Midwestern germplasm under the northern Great Plains environment. Instead, emphasis should (and is being) be placed on identifying and breeding for the highest levels of resistance to initial infection (i.e. "type I" resistance sensu wheat). This type of resistance is of paramount importance because a relatively low incidence of infected spikelets within a field can contain sufficient quantities of deoxynivalenol to render the crop unsuitable for malting. Discounts due to mycotoxin contamination in barley have been economically disastrous for growers in the Midwest region.

In contrast to the lack of vertical spread reported above, lateral spread from spikelet to spikelet within a node of six-rowed spikes is common in barley (Steffenson 2003). From many large-scale germplasm evaluation studies, six-rowed accessions were generally found to exhibit higher FHB severities than two-rowed accessions (reviewed in Steffenson 2003). The higher severities observed in six-rowed accessions may largely be due to the frequency of lateral infection spread—something that does not occur in two-rowed types because they possess only one spikelet per node. This aspect, as it relates to overall disease severity between the two row types, should be investigated further.

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DIGITAL DISEASE IMAGES FOR ESTIMATING FUSARIUM HEAD BLIGHT SEVERITY IN BARLEY

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OBJECTIVE

To develop digital disease images for assessing the severity of Fusarium head blight (FHB) in two-rowed and six-rowed barley.

INTRODUCTION

Accuracy, precision, reproducibility, and efficiency are important aspects for disease assessment of plants (Campbell and Madden 1990). Rating scales (i.e. descriptive categories of different disease levels) and disease diagrams (i.e. illustrations of different disease levels) are often developed to facilitate disease assessment in various pathosystems. Several different rating scales have been used to assess FHB severity in barley. Takeda and Heta (1989) used a five class scale for assessing the percentage of infected spikelets on barley accessions in their resistance screening efforts where 0=0% severity, 2=1-5% severity, 4=6-20% severity, 6=21-40% severity, and 8=41-100% severity. A five class scale is also routinely used for assessing FHB severity in barley by several research groups in the United States. The severity intervals of this scale are 1=0-20%, 2=21-40%, 3=41-60%, 4=61-80%, and 5=81-100%. The five class scales work well for selecting accessions that possess FHB resistance in general screening tests of barley germplasm, but may not provide adequate resolution for differentiating among accessions with more narrow severity differences. Another method for assessing the level of FHB infection is to actually count the number of infected spikelets (Prom et al. 1996). This number is then divided by the total number of spikelets in the spike to derive a FHB severity. The "spikelet count" method provides a measure of the true or actual FHB severity and also allows for direct comparisons to be made of the resistance level between two-rowed and six-rowed barley accessions because the severity is standardized based on the total number of spikelets in the spike. However, the spikelet count method is extremely laborious and time-consuming to use (Steffenson 2003). Disease diagrams have been developed for a number of diseases and have proven useful for estimating disease severities (James 1971; Madden and Campbell 1990). Comprehensive disease diagrams or images have not, however, been developed for FHB of barley to our knowledge. Thus, the objective of this study was to develop a comprehensive set of digital disease images for estimating FHB severity in both six-rowed and two-rowed barley.

MATERIALS AND METHODS

Digital color images were taken of FHB-infected barley spikes collected from the field using a Nikon D-100 digital camera and Nikkor 105mm lens. Archetypal healthy and diseased spikelets were selected and digitally cut from the spike images. The healthy and diseased spikelets were then used as "building blocks" to create idealized two-rowed and six-rowed spikes exhibiting various disease severities. All image manipulations were done using Adobe Photoshop.

RESULTS AND DISCUSSION

Fourteen standard digital spike images exhibiting disease severities from 1-100% were developed for both two-rowed (Figure 1) and six-rowed barley (Figure 2). To increase rater accuracy under low disease situations, four low severity classes of 1%, 2%, 3%, and 5% were included in the disease image scale. Thereafter, severity classes ranging from 10-100% were established in intervals of 10%. The represented patterns of diseased spikelets in each image were based on numerous observations of naturally infected spikes from the field. Standard spikelet numbers of 26 and 60 were chosen for two-rowed and six-rowed spikes,

respectively, because they represent the average spikelet number in midwestern cultivars. Correction factors for spikes having greater or fewer spikelets can be applied if needed.

For ease of use in the field, the images were reproduced at 100% the size of actual spikes and arranged sequentially from lowest to highest severity on a standard letter size paper with the two-rowed images on one side and the six-rowed images on the opposite side. Unlike disease diagrams, which are often line drawing representations of the disease (see James 1971), our scale consists of actual color images of FHB-infected spikelets assembled into a composite spike. The realistic nature of the images should facilitate disease severity assessments, especially by novice raters.

In the barley-FHB pathosystem, variation for disease severity can be extremely high. Moreover, it is sometimes important to resolve small differences among treatments for FHB severity—a goal best achieved using the spikelet count method. The standard error for disease severity assessments can be reduced adding more replications or environments; however, this will result in additional costs for labor and time. Disease images can help to reduce these extra costs, but should not sacrifice accuracy and efficiency. The accuracy, reproducibility, and efficiency of using these disease images for assessing FHB severity will be tested in 2005 and subsequent years. The accuracy of using the disease images will be made in comparison with the spikelet count method, which is considered the true or actual FHB severity. Reproducibility also will be tested with several experienced and novice raters. Efficiency is an important consideration in disease assessment; thus, we will consider the quantity and quality of data obtained within a given unit of time. We are soliciting cooperators to assist in validating the utility of these disease images in different types of studies including disease resistance evaluations of germplasm, fungicide efficacy trials, and various epidemiological investigations. If you are interested participating in this project, please contact us.

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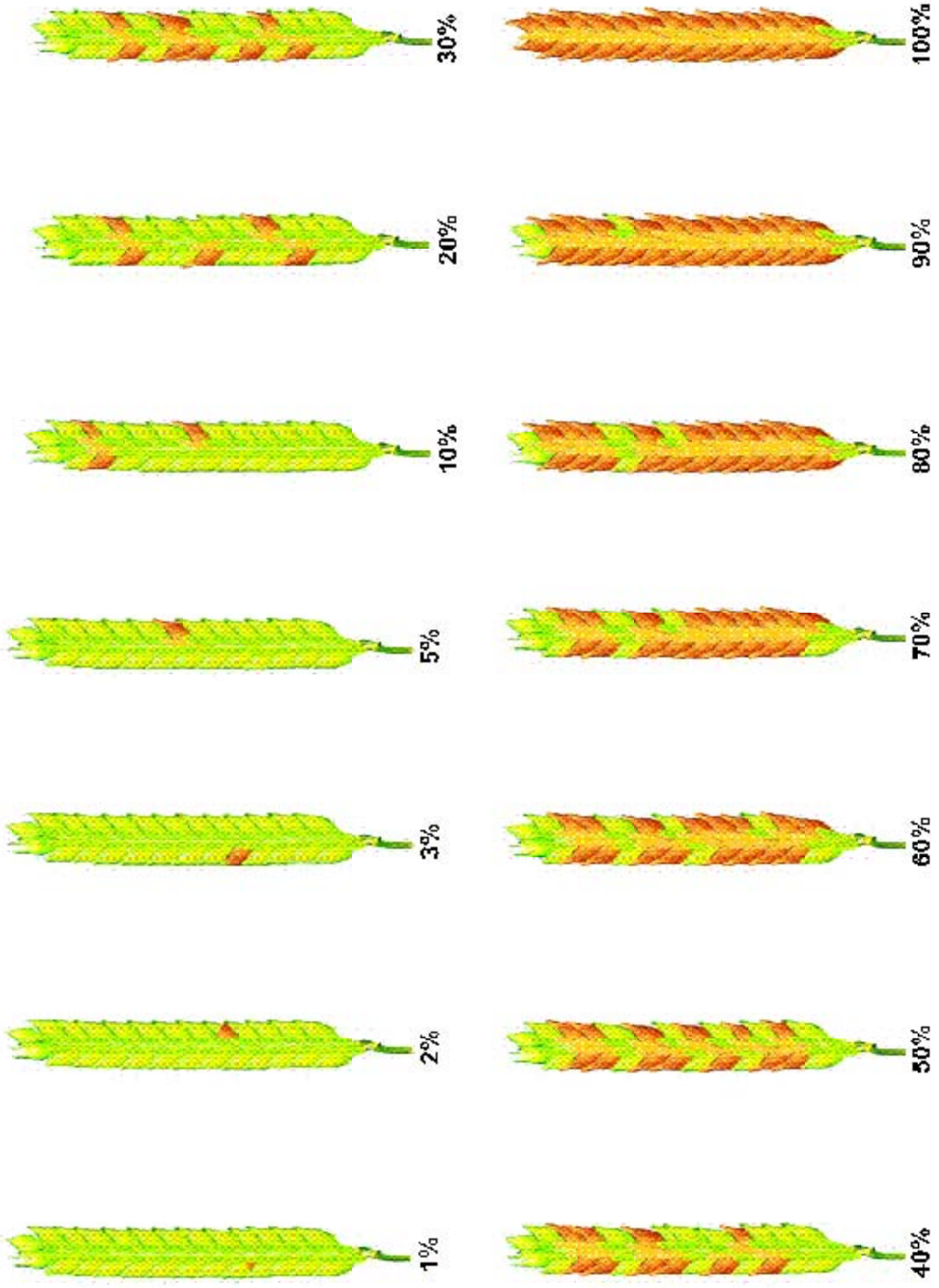

Figure 1. Digital disease images for assessing Fusarium head blight severity in two-rowed barley.

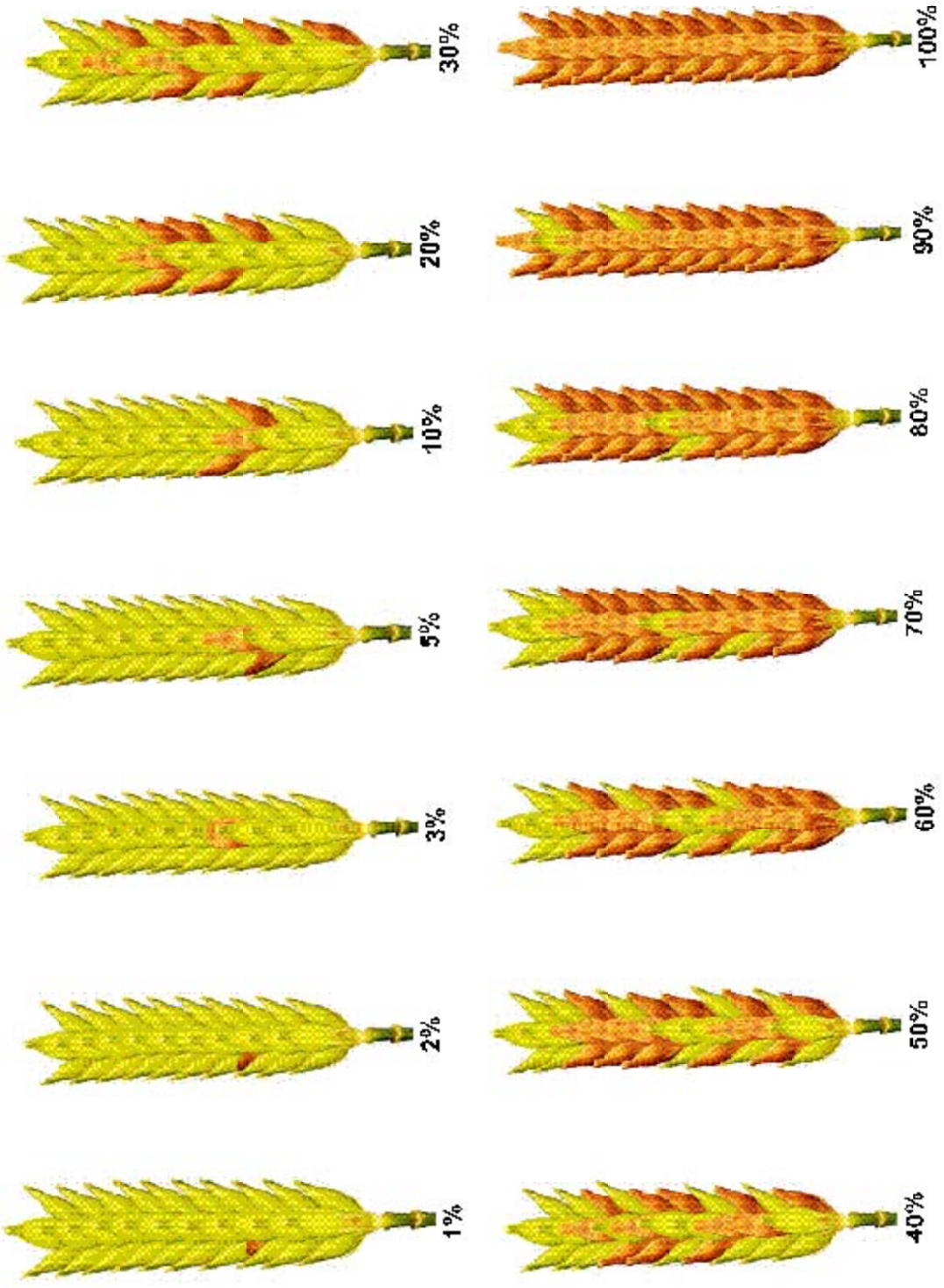

Figure 2. Digital disease images for assessing Fusarium head blight severity in six-rowed barley.

IDENTIFICATION AND CHARACTERIZATION OF EXPRESSED GENES INVOLVED IN FUSARIUM HEAD BLIGHT RESISTANCE OF WHEAT

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ABSTRACT

Although several chromosomal regions with quantitative effects on Fusarium head blight (FHB) resistance have been identified, the actual function of the resistance genes is still unknown. In this project we aim to identify expressed genes involved in the resistance reaction of wheat against FHB and to contribute to the functional clarification of the resistance reaction.

Near isogenic lines (NILs) differing in the major FHB resistance QTL, *Qfhs.ndsu-3BS* and *Qfhs.ifa-5A*, were developed. The spring wheat lines CM-82036 (FHB resistant) and Remus (FHB susceptible) were crossed, and NILs were developed by repeated backcrossing with the susceptible Remus. In each cycle plants were selected for the next backcross generation which possessed *Qfhs.ndsu-3BS* and *Qfhs.ifa-5A* in heterozygous condition. Selection was done by genotyping with SSR markers flanking *Qfhs.ndsu-3BS* (GWM389, GWM533, GWM493) and *Qfhs.ifa-5A* (GWM129, GWM156, GWM293). In the BC_5F_2 generation homozygous lines for all QTL classes, class 1 with both resistance QTL (*Qfhs.ndsu-3BS* and *Qfhs.ifa-5A*), class 2 and 3 with one resistance QTL (either *Qfhs.ndsu-3BS* or *Qfhs.ifa-5A*) and class 4 with the susceptible alleles at both QTL positions were selected.

In fall 2004 these NILs were challenged by inoculating with *Fusarium graminearum*. At anthesis either a *Fusarium graminearum* suspension or water were pipetted between the palea and lemma of the two basal florets of four central spikelets per spike. Inoculations were carried out in the greenhouse under controlled conditions. After inoculation the heads were kept at 80 % humidity to ensure infectious conditions. 4h, 24h, 48h, 72h and 96h after inoculation spikelets were harvested, separated into compartments and shock frozen in liquid nitrogen. RNA extraction of these samples is in progress. Initially, we will use the cDNA-AFLP method, a gel-based transcript profiling system, to analyze differential gene expression between the NILs at several time points after Fusarium inoculation. Differentially expressed transcripts will be recovered, sequenced and further characterized.

ACKNOWLEDGMENTS

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QTL MAPPING AND VALIDATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN THE SPRING WHEAT CULTIVAR FRONTANA

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ABSTRACT

The Brazilian spring wheat cultivar Frontana is a widely used Fusarium head blight (FHB) resistance source. Molecular mapping of a Frontana/Remus doubled haploid population led to the identification of several FHB resistance QTLs. Effects on chromosomes 3A and 5A showed consistent association with FHB severity over three years and accounted for 16% and 9% of the phenotypic variation, respectively. The study indicated that FHB resistance of Frontana primarily inhibits fungal penetration, but has minor effect on fungal spread after infection.

To validate the two major FHB resistance QTL of Frontana on chromosomes 3A and 5A a population of 110 F_7 recombinant inbred lines from a cross of Frontana/Inia66 (FHB susceptible) was evaluated for FHB resistance. In 2004 the population was sown in three replications in the field and spray inoculated at anthesis. The severity and incidence of the disease were assessed by visual scoring. The population was genotyped with 320 SSR markers. Preliminary results for QTL mapping revealed no association of the 3A and 5A genomic regions with FHB resistance in the Frontana/Inia66 population. The most prominent QTL in the Frontana/Inia66 population for FHB resistance mapped to chromosome 6D, explaining 17% of the phenotypic variation in 2004.

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SCREENING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN AN EPIDEMIC YEAR

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ABSTRACT

Kentucky's 2004 wheat crop was hit with a severe FHB epidemic. Ideal temperatures and abundant rainfall combined with mist irrigation led to ideal conditions for FHB development in our nursery. Extreme disease pressure prevented accurate assessment of breeding lines. Severity and incidence were recorded in the 2004 scab nursery at 21 days after anthesis (DAA). Symptoms were slow to develop but after June 1 symptoms appeared and developed very rapidly. The data collected at 21 days after anthesis had almost no predictive value; at 28 DAA, many lines rated moderately resistant at 21 DAA had been obliterated by FHB. Given these circumstances we were interested to learn what, if any, value could be assigned to the data we collected. Because the spike symptoms from the FHB nursery were not informative, we hoped that Fusarium damaged kernels (FDK) and DON would be reasonable indicators of resistance. Unfortunately, the frequency of tombstone kernels was so high that genotypic differences were masked, and there was often insufficient seed to submit for DON analysis. We also point-inoculated bagged heads outside of the irrigated nursery in 2004. The point inoculation study was evaluated at two locations (Lexington and Woodford County, KY), which were not irrigated. Approximately 120 heads per location of Clark were point inoculated at anthesis. Water was injected into 120 heads per location as the control. Both sets were bagged with glassine bags and stapled closed for 48 hours before removing. Data were collected at 7-day intervals after inoculation, except for Day 21 at Woodford County. At this location, the plants had senesced, masking visual symptoms of FHB. The plants injected with water were only read once. The results collected in 2004 field injections demonstrated that bagged heads could be a useful tool. The most representative data collected in 2004 was from non-irrigated plots. Seed damage was more variable and susceptibility levels were more prominent without irrigation, so we were able to infer genotypic differences. Results from point-inoculated bagged heads were promising. This technique allowed disease severity to be read and analyzed from an off-site station without irrigation. This could be useful in testing advanced breeding lines in different locations in Kentucky. In 2005, testing will involve using a small air sprayer to deliver spores instead of point inoculations.

RELATIONSHIP BETWEEN SEVERITY OF FUSARIUM HEAD BLIGHT, AMOUNT OF PATHOGEN DNA AND DON CONTENT IN WHEAT AND BARLEY GRAIN

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OBJECTIVES

To examine relationship between accumulation of mycotoxin DON, pathogen DNA content and disease severity traits in winter wheat cultivars after infection with *Fusarium culmorum* in field conditions and fungicide treatment.

INTRODUCTION

Fusarium head blight (FHB) predominantly caused in the examined conditions by *Fusarium graminearum* (Schwabe) and *Fusarium culmorum* (W.G. Smith) Sacc. belongs to the most damaging diseases of cereal crops. Mycotoxin contamination of human food and animal feed became a more important feature than the direct yield losses that often occur irregularly. Both species were reported to produce deoxynivalenol (DON), but also ability to produce other DON – derivatives and zearalenone has been recognized in some isolates of both species (Mirocha *et al.*, 1994; Sýkorová et al., 2003). Different studies revealed significant relations between DON content and characters measuring severity of FHB infection (Miedaner et al., 2001; Šíp et al., 2002, Šíp et al., 2004), but relationship between disease symptoms and DON content is not yet well understood. The analyses on molecular level, able to determine the quantity of pathogen DNA, are expected to contribute significantly to better identification of resistance level and understanding factors that influence FHB, which is important for eliminating the risk of mycotoxin contamination of grains and foodstuffs (Nicholson *et al.*, 2003).

MATERIALS AND METHODS

Plant material: Response to artificial infection with *Fusarium culmorum* and fungicide treatment was studied in nine winter wheat cultivars: Bona, Šárka (early), Nela, Sepstra (medium early), Petrus, Siria, Arina and Ebi (late in heading) and eight spring barley cultivars: Jersey, Scarlett, Olbram, Akcent, Tolar, Kompakt (cultivars registered in the Czech Republic), CI 4196 (resistant Chinese landrace) and Chevron (resistant Swiss landrace), with varying level of resistance to FHB and accumulation of DON.

Description of field experiments: Results are based on three year (2001-2003) experiments with wheat and two year (2002-2003) experiments with barley at the location Prague – Ruzyne. Each cultivar was grown on 2.5 m^2 plots in three replicates of four variants: 1/ Infection variant (I), 2/ Infection variant treated with fungicide (IF) 3/ Control uninfected variant (C) and 4/ uninfected variant treated with fungicide (CF). Highly pathogenic isolate (B) of *Fusarium culmorum* (Šíp et al. 2002) was used for inoculation. The spore mixture was sprayed directly onto the plot in two terms: at full flowering (>50% of flowering spikes) and one week later. Fungal infection was promoted by mist irrigation of plots. In 2001 the fungicide Caramba (Metconazole) and in the years 2002 and 2003 Horizon 250 EW (Tebuconazole) were applied in IF and CF variants. Inoculation with *Fusarium* conidia suspension followed in IF variant after 24 hours, when positive occurrence of fungicide in plant tissue was assured.

Examined FHB resistance traits: Head blight symptoms (VSS) of wheat were evaluated usually 28 days after inoculation on a scale of 0 to 9, where 0 = 0 %, and 9= 100% of the spikelets with FHB symp-

toms. *Fusarium* damaged kernels were calculated as percentage by total seed number (%FDK). From seed samples (of randomly selected 50 spikes) the traits GNS (number of grains per spike) TGW (thousand grain weight), and GWS (grain weight per spike) were determined in all variants. Tolerance to FHB was expressed both in wheat and barley as percent reduction (R) from control variant C in these traits. In barley, the analysis of extent of infection penetration into grain consisted in determination of percentage of *Fusarium* colonies and percentage of non-germinating seeds from samples that contained 100 randomly selected seeds. ***The content of DON*** was determined by ELISA on RIDASCREEN- FAST DON kits from R- Biopharm GmbH, Darmstadt, Germany (Šíp et al., 2002). ***DNA content of the pathogen in analyzed seed samples*** was estimated by transformed C_T (threshold cycle) values obtained in 2002 and 2003 from real time quantitative PCR analysis (Šíp et al., 2004).

Statistical analyses: The UNISTAT 5.0 package (UNISTAT Ltd., London W9 3DY, UK) was used for statistical analyses of the data.

RESULTS AND DISCUSSION

Relationship between DON content and other examined traits. It comes from Tables 1 and 2 that the associations between the traits were highly influenced by experimental years and fungicide treatment. Table 1 for wheat shows prevalence of significant relations between traits, but different traits were evidently needed to explain FHB effects. FDK (% of *Fusarium* damaged kernels), which was possible to determine only in wheat, appeared to be the best predictor of DON content and examined disease severity traits. All traits were interrelated on a genotypic base (G-I: correlations between cultivar means over environments in infection variant) and, therefore, it can be expected that high resistance to DON accumulation in a cultivar will be accompanied with resistance to other resistance components (Mesterházy et al. 1999).

Other trait relations were either insignificant or significant at P=0.1. As indicated by correlation coefficients G (IF), cultivars differently responded to fungicide treatment in single traits. After application of fungicide a low DON content in a genotype was significantly related only to a low percentage of FDK.

In barley, correlation studies (Table 2) support in general the conclusions of Jones and Mirocha (1999) who found that DON concentration could not be effectively estimated by yield traits, visual index or discolorations of grain. Due to often unclear FHB symptoms (Šíp et al., 2004), there was in this crop restricted choice of characters. Therefore, it is highly stressed the importance of determination of pathogen DNA content to explain cultivar differences in response to FHB. DON content was closely related (r=0.92) only to pathogen DNA content (CT values).

Exploitation of real time PCR assays for quantification of FHB causal agents. It is firstly to note that present results in molecular field are based on two year studies in wheat and one year studies in barley. It is planned to complete the analysis of this experimental series.

Figures 1 and 2 clearly demonstrate different pictures in wheat and barley. While in barley (Fig. 2) DON content in a genotype was closely related pathogen DNA content and fungicide effect on reduction of DON was closely related to reduction of DNA content, in wheat it was found that DON production need not be proportionate to quantity of pathogen DNA in grain. Fig. 1 gives evidence of high differences in relations between accumulation of DON and pathogen DNA content between years 2002 and 2003. Similar average DON content in both years (2002: 26.6 mg/kg; 2003: 26.3 mg/kg) was reached at highly different DNA content (CT: 2002- 0.175; 2003- 0.072). Efficacy of fungicide treatment for DON content was highly different in both years (2002: 29.1%; 2003: 70%), but percent reduction of DNA content was in these years similar (2002: 23.4%; 2003: 23.3%).

Mesterházy et al. (2003) reported that efficacy of fungicides in controlling FHB in wheat was highly variable and often unsatisfactory. Also in these experiments data can be supplemented showing high cultivar by year by fungicide treatment interaction effects. At sufficient moisture content, particularly differences in sums

of average day temperatures in period of disease development played an important role. Fungicide treatment was little effective in relatively colder conditions that promoted slow, long lasting disease development (2001 with high DON content) or conditions that caused high disease incidence (2002 with high pathogen quantity and yield reduction). Percent reductions of DON content due to fungicide treatment were in years 2001-3 markedly different for early (38%, 56%, 47%) and late (5%, 15%, 89%) genotype groups inoculated in different terms. Differential control of the disease (choice of appropriate fungicide, its dosage and timing, repeated applications) based on prognosis of disease development in particular year (region) for certain cultivar types and respectful of other factors (pathogen populations aggressiveness, preceding crops, etc.) appears to be highly desirable. It is expected that real time PCR analysis that would enable to quantify the relative amounts of causal agent could significantly contribute to increase of effectiveness of disease control both by genetic means and chemicals.

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Table 1. Phenotypic (2001, 2002 and 2003) and genotypic (G) coefficients of correlation between six traits in variants I and IF at wheat.

Combination	2001		2002		2003		G (I)	G (IF)
of traits	I	IF	I	IF	I	IF		
CT/DON			0.62 ***	0.80 ***	0.41 *	0.58 ***	0.69 *	0.67 *
CT/FDK			0.61 ***	0.41 *	0.65 ***	0.23	0.76 **	0.52
CT/VSS			0.67 ***	0.23	0.25	0.44 *	0.86 **	0.69 *
CT/TGWR			0.45 **	0.25	0.16	0.67 ***	0.73 *	0.14
CT/GWSR			0.41 *	0.10	0.58 ***	0.23	0.67 *	0.26
DON/FDK	0.41 *	0.87 ***	0.83 ***	0.41 *	0.56 **	0.75 ***	0.88 ***	0.87 **
DON/VSS	0.45 *	0.48 **	0.74 ***	0.19	0.23	0.72 ***	0.70 *	0.36
DON/TGWR	0.19	0.71 ***	0.69 ***	0.49 **	0.34 *	0.59 ***	0.84 **	0.58
DON/GWSR	0.34	0.79 ***	0.61 ***	0.28	0.29	0.54 **	0.77 **	0.55
FDK/VSS	0.64 ***	0.44	0.78 ***	0.03	0.39 *	0.63 ***	0.86 **	0.51
FDK/TGWR	0.48 **	0.82 ***	0.70 ***	0.38 *	0.53 **	0.11	0.92 ***	0.45
FDK/GWSR	0.43 *	0.88 ***	0.76 ***	-0.11	0.56 **	0.61 ***	0.85 **	0.49
VSS/TGWR	0.64 ***	0.70 ***	0.73 ***	0.51 **	0.62 ***	0.43 *	0.85 **	0.25
VSS/GWSR	0.54 **	0.44	0.77 ***	0.52 **	0.80 ***	0.86 ***	0.87 **	0.27
TGWR/GWSR	0.75 ***	0.89 ***	0.71 ***	0.61 ***	0.42 *	0.26	0.91 ***	0.49

Table 2. Phenotypic coefficients of correlation between examined traits in 2002 (below diagonal) and 2003 (above diagonal).

	DON	FUC	NGS	GNSR	TGWR	GWSR
C_T	0.92 ***	0.32	0.25	0.32	0.48 **	0.42 *
DON	---	0.38 *	0.29	0.33	0.50 **	0.43 *
FUC	0.50 **	---	0.68 ***	-0.34	-0.26	-0.33
NGS	0.04	0.82 ***	---	-0.27	-0.32	-0.16
GNSR	-0.07	-0.30	-0.12	---	0.81 ***	0.95 ***
TGWR	-0.07	0.00	-0.02	0.33	---	0.95 ***
GWSR	-0.15	-0.22	-0.14	0.84 ***	0.78 ***	---

Explanation of trait symbols used in Tables 1 and 2:

CT = CT Fus transformed, DON= DON content, FDK= percentage of Fusarium damaged kernels, VSS= visual scoring of symptoms on 0.9 scale, FUC= percentage of Fusarium colonies, NGS=percentage of non-germinating seeds, GNSR= reduction of grain number per spike, TGWR= reduction of thousand grain weight, GWSR= reduction of grain weight per spike

*** P<0.001, **P<0.01, *P<0.05

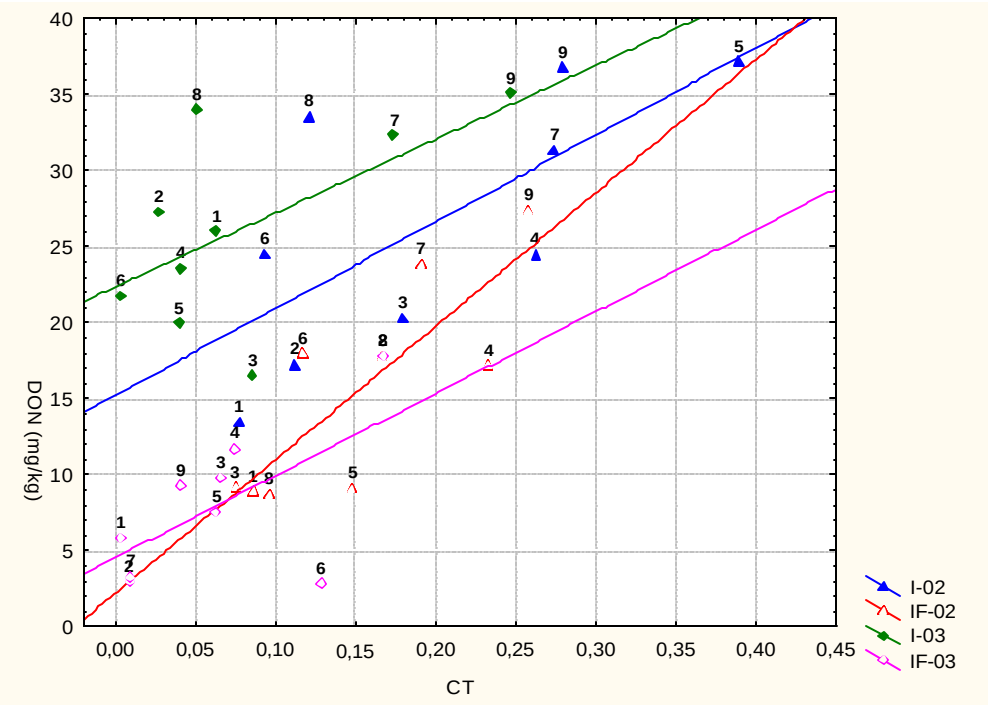

Figure 1. Relation between pathogen DNA content (CT) and content of DON for 9 wheat cultivars (1-Arina,2-Petrus,3-Nela,4-Bona,5-Saskia,6-Ebi,7-Sepstra,8-Šárka,9-Siria) in 2 years (2002, 2003) after infection (I) and fungicide treatment (IF).

INCORPORATION OF FUSARIUM HEAD BLIGHT RESISTANCE INTO EUROPEAN WINTER WHEAT BREEDING PROGRAMMES

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ABSTRACT

The European Union of 25 States is climatically very diverse and the importance of *Fusarium* pathogens ranges from almost negligible in some countries to extremely high in others. The risk of an epidemic ranges from about one year in twenty in the United Kingdom to almost every year in Hungary. The areas at highest risk are those with a continental climate, a high proportion of maize in the rotation and a tradition of direct drilling and minimal cultivation. An increase in cultivation of maize and winter wheat, together with narrower crop rotations, changed cultivation practices and climate change (global warming) has increased the area under threat in the last 15 years. Surveys have shown a relative increase in the proportion of *F. graminearum* and decrease in *F. culmorum* over the same period .Even traditionally low-risk areas such as Denmark and Sweden are beginning to take the threat seriously. As yet there has been no dramatic change in the situation in Europe comparable to that which occurred in the US and Canada in the early 1990's.

The European Union has acted to set limits for the primary toxins, DON and ZEA. At present the level is to be set at 1250µg/kg DON for uncleaned bread wheat and 1750µg/kg DON for durum wheat. Germany has already introduced stricter national limits in 2004. The DON level has been set at 500µg/kg for roughly cleaned bread wheat. The problems of standardised ELISA testing and lot sampling have not yet been adequately solved in Europe.

Most cereal breeding in the European Union is in the hands of private breeders. The former state breeding programmes of central and eastern Europe are also at various stages of privatisation. Bought seed rates for winter wheat in the European Union are around 50 % and vary from around 8 % in Poland to 85 % in Denmark and Sweden. Variety testing for *Fusarium* resistance is a national responsibility and there has been no attempt at harmonisation between the member states. It is very difficult to obtain direct comparisons of varieties between different countries.

Most wheat crops in the old EU 15 member states receive at least one fungicide spray – on average two. The lower economic level of the 10 new EU member states has historically meant a lower use of fungicides, but this is rapidly changing with economic progress. High yield potential under fungicide treated regimes remains the highest priority in wheat breeding programmes. The breeder can only go so far in incorporating *Fusarium* resistance that the yield is not (significantly) reduced. Many highly resistant varieties in the last few years have not been taken up because the yield potential has not been able to match that of more susceptible varieties .A combination of variety choice, fungicide treatment and cultivation practice has to be used to reduce the danger of infection to a minimum. At present the contact fungicides Folicur and Caramba offer the best protection.

Ratings for variety *Fusarium* resistance are included in the national descriptive lists of the United Kingdom, France, Germany and the Netherlands. Official testing procedures vary widely between countries. Where specific nurseries have been used, these have generally involved inoculation with spore suspensions followed by a combined frequency/intensity score. Germany has now moved on from this to using nurseries with maize stubbles as the only infection source. So far only ratings for visual infection have been used. Only France has considered introducing direct measurements of DON for variety ratings.

Breeders use a range of testing procedures for selection and screening. Spore suspension sprays have been used widely, either in specific nurseries, or in yield plot cross ways, or on the edge 0,5 m of yield plots. The use of maize-stubble nurseries is becoming popular in Germany. Breeding for *Fusarium* resistance has been given a new impulse on a European level by the introduction of the toxin limits and further specifically in Germany by the refusal, since 2001, of the Regional Agricultural Chambers to test susceptible varieties in post-registration trials.

In Germany and other continental regions, breeders have been able to use morphological components - plant height; lax, hanging head type - as a basis for resistance breeding in the past. In maritime areas, with high lodging pressure, tall varieties are not an option. In these areas other forms of resistance need to be found.

Flowering biology may offer an opportunity to produce short strawed varieties with acceptable *Fusarium* resistance. Reports from the US and Canada indicate that the open-flowering character may play a role in primary infection. It is also known that non-extruded or partially-extruded anthers offer a primary entry point for *Fusarium* spores to the grain site. Although it is too early to say how useful these characters could be on a wider scale, the use of anther extrusion data has already proved of value in our own programme in preselection of earlier generation material in years with low *Fusarium* pressure. It must also be clear that flowering biology is only having an effect on primary infection.

There are noticeable differences in West-European germplasm to Type II - spreading in the ear - resistance, though these are at a much lower level in comparison to Sumai 3. The need to incorporate specific Type II resistances depends on the regional *Fusarium* pressure. Countries such as Rumania and Hungary, with high *Fusarium* pressure, are certainly more advanced than in the West. Most breeders in Germany and Austria are incorporating Sumai 3, Rumanian and Hungarian sources of resistance. The development and use of markers for selection in this respect is very important. Without the use of specific markers in early generations the Type II resistance is almost certain to be lost during field selection.

Although markers for Type II resistance are now well described, their use is only just leaving the research project stage in West-European breeding programmes. Markers are generally only accepted by breeders where field screening is not effective.

While bio-technology and the use of markers to support breeding work are widely accepted, the use of green gene-technology in Europe is not. It is unlikely that any gene-tech solution to the Fusarium problem would be acceptable in the foreseeable future. The current costs for registration and also restrictions on the freedom to operate with gene-technologies make it unlikely that private breeders will be looking to this route.

In summary, changes in climate and agricultural practice are increasing the risk of *Fusarium* epidemics in Europe, but as yet there have been no catastrophic epidemics as seen in the US and Canada. It is to be hoped that private-breeding supported by an effective system of royalty income in co-operation with basic research from Institutes and Universities can avoid such a catastrophe on the “Old-Continent”.

CORRELATIONS AMONG COMPONENTS OF FUSARIUM HEAD BLIGHT IN WHEAT, BARLEY AND OAT

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ABSTRACT

Fusarium head blight (FHB) damages cereal crops by reducing yields and quality, and by contaminating the grain with deoxynivalenol (DON) or other mycotoxins. Researchers need to quantify levels of FHB damage to identify and compare resistant sources, and to assess the outcomes of breeding programs striving to develop adapted cultivars with enhanced resistance profiles. Industry must quantify disease damage (primarily mycotoxin levels) to ensure that both raw and end-products are suitable and safe for human or animal consumption. During the past eight years, we have looked at the responses of wheat, barley and oat genotypes to FHB, to inform producers and others, of any differential levels of resistance (or susceptibility) in currently registered cultivars. Such 'cultivar selection' can be a strategic tool for integrated disease management. By quantifying the levels of FHB using various measurable components, we have also been able to assess how these are correlated within each crop. This latter information is necessary to clarify which of the disease components can provide the key information desired. Wheat, barley and oat genotypes were tested in replicated field trials in southern Manitoba from 1997 to 2004, under natural conditions or supplemented by the addition of *Fusarium graminearum*-infested corn kernel inoculum. Components of FHB measured included disease severity (FHB-Index), and in harvested plots, the level of fusarium damaged kernels (FDK) (>scabby= kernels), level and identity of *Fusarium* spp., and grain contamination by DON. Correlations, relative to final DON levels, were strongest (mainly between 0.80 and 0.90) between DON and *F. graminearum* for all three crop species; DON and total *Fusarium* spp. also were highly correlated. In wheat, DON was highly correlated to both FHB-I and FDK. In barley, these correlations were less strong and results varied among tests (year, location); weak or non-significant correlations sometimes occurred. In oat, DON and FHB-I were not correlated, and DON and FDK either not or only weakly so. As such, in the absence of DON data (or the means to assess these) various other components of FHB could provide critical information, depending on the crop species. Ultimately, determining actual DON levels (and/or those of other mycotoxins) provides the best assurance, and comfort, that putatively FHB-affected grain is suitable for its designated use(s). This is particularly true if harvesting of mature crops is delayed due to wet conditions, and *Fusarium* and DON continue to accumulate (as occurred in 1993 in the eastern Canadian prairies and the upper American mid-west, and likely occurred in 2004 in Manitoba), without attendant changes to the visible components of FHB.

IDENTIFICATION OF BARLEY (*HORDEUM VULGARE* L.) ACCESSIONS WITH LOW DEOXYNIVALENOL ACCUMULATION

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ABSTRACT

Although sources of genetic resistance to Fusarium head blight (FHB) are available for barley (*H. vulgare* L.), all show some degree of deoxynivalenol (DON) accumulation when disease pressure is high. Exceptionally low limits for acceptable DON levels have been set by the various end-use barley industries, therefore new sources of resistance are desired. An initiative was undertaken to identify new sources of FHB resistance that might carry novel genes that could be pyramided with those currently being used by barley breeders. To avoid duplication of effort, a database (provided by B. J. Steffenson) was used to eliminate accessions that had already been screened for FHB resistance in the United States. In contrast to American efforts which have focused more on 6-row barley, all barley types (2-row, 6-row, covered, hulless) were included in the current study. Since 2001, over 1,500 barley accessions from Plant Gene Resources of Canada, Saskatoon, SK have been evaluated for low DON accumulation. Accessions were grown in the FHB nursery at Brandon, MB, where short rows of the material were artificially inoculated with 3 isolates of *Fusarium graminearum* using the grain spawn method. Disease symptoms were rated visually on a 0-5 scale, with resistant lines harvested for DON content analysis at Ottawa using the ELISA technique. Accessions with low DON content were reevaluated in the FHB nursery in each subsequent year. Among the most promising accessions were: Azul (CN 35781; 6-row, covered); Doneckij 6 (CN 32593; 2-row, covered); CN 5317 (2-row, covered); CN 60364 (2-row, hulless); CN 89138 (2-row, hulless); CN 91465 (2-row, covered); and CN 91521 (2-row, covered). These accessions originating from various countries, including Russian Federation, Georgia, Ethiopia and China, may be useful as parental germplasm in development of varieties with improved FHB resistance.

FUSARIUM HEAD BLIGHT REACTIONS AND ACCUMULATION OF DEOXYNIVALENOL (DON) AND EFFECT ON QUALITY OF WHEAT

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ABSTRACT

Sixty-one commercial cultivars and germplasm lines of wheat were inoculated at middle of the anthesis with spore suspension of *Fusarium graminearum* and *Fusarium culmorum*. Wheat spikes were inoculated using a syringe with conidia of *Fusarium graminearum* and *Fusarium culmorum*. Head blight incidence, thousand kernel weight, visually scabby kernels and Deoxynivalenol content was determined from harvested samples during 2002 and 2004. Samples were analyzed using high performance liquid chromatography (HPLC). Lines BVD 6, BVD 7 and cultivars Osmangazi, Acar and Martar were the best resistance sources and had small amounts of DON during 2002. Deoxynivalenol was detected range 0.01 to 22.34 µg/g. A total of 28 of commercial cultivars and lines (%70) contained DON concentration excess of the 2.0µg/g that is U.S., FDA toxin limit for human consumptions. 32 commercial cultivars and lines also (%80) contained DON concentration excess of the 1.0µg/g.

Samples of four Chinese commercial cultivars and 16 germ plasm lines analyzed in 2004 to determine the effects on FHB. 16 lines were highly resistant to crown rot(*F. pseudograminearum* and *F.culmorum*) and common root-rot(*Bipolaris sorokiniana*) diseases They were from Turkish germ plasm lines of wheat.

Additional key words: wheat, deoxynivalenol, *Fusarium graminearum*, *Fusarium culmorum*, cultivars, lines.

CIMMYT'S FHB FIELD RESEARCH APPROACH, EVIDENCE FOR HOST-BY-LOCATION INTERACTION AND POTENTIALLY NEW GENETIC DIVERSITY IN RESISTANCE IN WHEAT

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INTRODUCTION

In the mid-1980s, the International Maize and Wheat Improvement Center (CIMMYT) recognized the seriousness of the threat posed by Fusarium head blight (FHB) and made the search for resistance/tolerance a high priority. Since then, the realization of the importance of this disease has grown for a number of reasons. Firstly, following increased global interaction, it became apparent that FHB was one of the major wheat diseases in China, but that unique FHB resistance had already been identified in Chinese varieties. Secondly, zero- or minimum tillage practices were more widely being adopted, allowing intensification of cropping and a faster turnover of rotations. Crop residues left on the soil surface allowed the *Fusarium* fungus to survive, ready to spread to the next wheat crop. In addition, the increase in maize rotation acreage in several parts of the world provided an alternate host for the fungus. Finally, FHB gained further notoriety as it was associated with the presence of mycotoxins in the grain that are hazardous to humans and certain animals. Soon, several strong research groups began investigating the FHB disease and today a vigorous global community of FHB scientists maintains active ties and regular communication. When the USA and Canada experienced major epidemics in the past decade, CIMMYT shared germplasm with them under the umbrella of the United States Wheat and Barley Scab Initiative.

MATERIALS AND METHODS

Field Activities in Mexico

In our field research station in Toluca, Mexico (2640 masl; >800 mm rainfall per crop cycle), we plant just over 1 ha for FHB screening of bread wheat, durum wheat, triticale and barley, enhancing humidity with sprinklers. Between six and ten spikes per entry are hand-inoculated with *Fusarium graminearum* and the plants evaluated for the five well-known Types of resistance (infection, spread, toxin level, yield loss, and grain appearance). About 10,000 spring habit types are evaluated annually and winter habit types on an *ad hoc* basis, including global introductions (e.g., from Argentina, Austria, Brazil, Bulgaria, Canada, Chile, China, France, Germany, Japan, Hungary, Romania, South Africa, Turkey, UK, Uruguay, and USA).

International Testing

Resistant spring habit entries are made available globally through the international Scab Resistance Screening Nursery (SRSN). A Winter Wheat Scab Resistance Screening Nursery (WWSRSN) is being distributed on an *ad hoc* basis. Data on these nurseries has been returned by: Argentina, Brazil, Canada, China, France, Germany, Guatemala, India, Iran, Korea, Pakistan, Paraguay, Peru, Poland, Tanzania, Ukraine, and Uruguay. The most resistant (Type II) lines over years are presented in Table 1.

Genetic Diversity for Resistance

We have shown that high levels of resistance can be achieved with only a few genes, and that the genes in the Chinese variety Ning7840 and the Brazilian variety Frontana are different (Singh et al., 1995; van Ginkel et al., 1996). Recently, we determined that among the 500 very diverse FHB resistant bread wheat lines, less than 10% carry the 3BS locus from Sumai#3 using two flanking molecular markers (*Xgwm493* and *Xgwm533*) (see Figure 1; unpublished). This may indicate that a great amount of novel resistance could be present in these materials. Our current breeding

strategy is to combine diverse resistance mechanisms and diverse genes (Singh and van Ginkel, 1997).

Host-By-Location Interaction: An Issue of Great Concern

Some host-by-location interaction was observed when the same wheat genotypes were exposed to FHB in different countries (see Figure 2). This may be driven by differences in the *Fusarium* species present, environmental effects on the defense mechanisms, true virulence, interactions among these factors or other causes.

The work by Kerry O'Donnell and colleagues (2004) identifies FHB as a pathogen with numerous subspecies and clades. At the local level, simple screening will adequately identify locally effective resistance. But a problem arises the moment one aims to extrapolate from one local situation to a more global one. Past international testing has identified some resistance sources that do have wide effectiveness over space and time. For an international center like CIMMYT, these unresolved interactions issues are top priority. But also national breeding programs targeting diverse environments need to consider the potential implications. Additional research should center on the effect on resistance of the presence of distinct species/clades, and differential variation in pathogenicity, aggressiveness and possibly even the expression of actual virulence. We need to know what we are really fighting against, in order to develop an effective resistance strategy expected to provide long-term protection.

CONCLUSIONS

The three main goals before us working in the area of genetics of resistance are:

1. To better understand the host-by-location interactions and implications for resistance.

2. To broaden the genetic base of resistance by studying various genetic resources.

3. To expand the emphasis from plant to the end-product, the grain and its quality including above all the issue of mycotoxins.

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Table 1. Top Type II resistant wheat entries in global testing of Scab Resistance Screening Nursery (equal or better than Sumai#3 and Frontana) (van Ginkel et al., 2003).

1st SRSN	2nd SRSN	3rd SRSN	4th SRSN	5th/6th SRSN	7th SRSN
Shanghai #3	Ng82149/Kauz	Wuhan #3	Ng8675/Ng8645	Sha5/Weaver	Catbird
CMH78A.544	Ng8201/Kauz	China #7	Mayoor	Catbird	Gondo
Fan #1	Sha#3/Kauz	Ning7840	Ng8675/Cbrd	Chum18//Jup/Bjy	Shanghai
Ning7840		Shanghai #3	Lu 95	Gondo	Ng8675/Cbrd
Yangmai #6		F3.71/Trm//3383.20			Sha3/Cbrd
		Suzhoe #6			
		Ng82149			

Xgwm533 *Xgwm493*

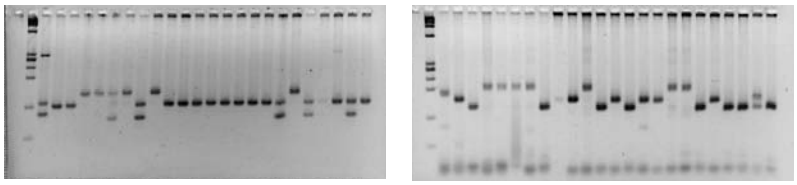

Figure 1. 500 wheat entries were compared to 8 check varieties using four markers. Examples shown.

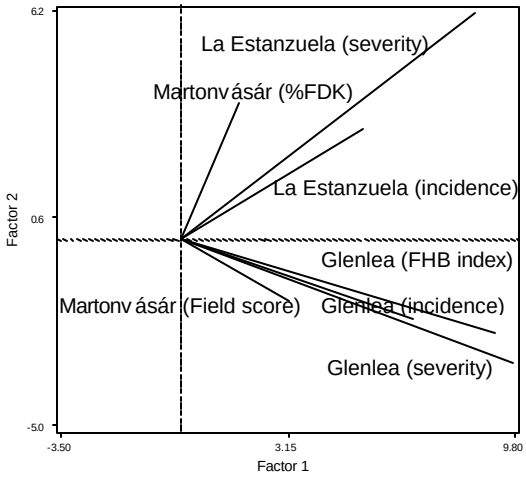

Figure 2. Bi-plot analysis (SREG model) of wheat genotype response to FHB in the 6th SRSN, indicating some genotype-by-location interaction (van Ginkel et al., 2004).

GLOBAL PROGRESS IN IDENTIFYING AND DEPLOYING FHB RESISTANCE GENES

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ABSTRACT

No single strain of wheat, barley, or related species completely withstands Fusarium head blight (FHB), a disease that is making increasing inroads on health and harvests worldwide. In fact, it is not clear if immunity to FHB exists in the cereals. In addition, the genetic constitution and chromosomal location of FHB resistance genes are not fully known, although current research suggests that several quantitative trait loci (QTL) or minor genes control resistance. It is encouraging that several DNA markers linked to FHB resistance QTLs have been identified and evaluated since the 1st International Symposium on FHB in 2000 (Suzhou and Nanjing, 2000). Progress also continues to be made in developing more resistant or tolerant varieties; however, the level of resistance to FHB in these varieties to date is considered by many not adequate for the long-term sustainability, especially on a global a basis and under conditions optimal for the pathogen. The resistance in the best lines often breaks down under particular conditions such as high-inoculum pressure or high-humidity conditions. Our presentation will provide updated information on the global progress in identifying FHB resistance genes in previously untapped sources and deploying them in wheat and barley. Finally, we will present our ideas on the need and options for further international collaboration to combat FHB as a global problem.

IMPROVEMENT OF FUSARIUM HEAD BLIGHT RESISTANCE IN THREE WINTER WHEAT POPULATIONS USING A RECURRENT SELECTION SCHEME

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INTRODUCTION

Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe) causes significant losses in the SRW wheat crop in Kentucky and in small grain crops worldwide. FHB epidemics result in significant yield losses, and the toxin deoxynivalenol (DON) can cause serious problems with grain quality and food safety. Genetic variation in FHB resistance is present in wheat (Buerstmayer et al., 1996, Bai et al., 2001, Mesterhazy et al., 1999). The amount of genetic variation among and within segregating populations and the generation in which selection is practiced is very important for optimizing selection progress. Breeding FHB-resistant wheat cultivars is a goal of the University of Kentucky breeding program. With this objective in mind two different selection schemes were conducted for three winter wheat populations.

MATERIALS AND METHODS

Three $F_{2:5}$ populations of 40 lines each were evaluated during 2004 at two locations (Lexington and Princeton, KY). The populations were developed from the following crosses:

Population1: Ning7840/2691//2684/3/Elkhart
Population2:Purdue5/Foster//Foster
Population3: Ning7840/2691//2684/3/25R57

In 2003 two selection schemes were conducted and are described below.

Within-family selection: Five spikes with low severity and five unselected spikes were harvested from each plot in 2003. Approximately 10 seeds of the forty $F_{4:5}$ families were planted in three replications at Lexington and two at Princeton in 2004. The experimental design was a split-plot design with the lines as the main plot and the selection treatment (selected vs. unselected) as the subplot.

Among-family selection: The 8 $F_{2:4}$ families in each population in 2003 with the lowest FHB index were planted as $F_{2:5}$ seed in a randomized complete block design in 4 row plots with three replications at Lexington and two at Princeton.

Field inoculation: The field inoculation protocol was modeled after the method of Fauzi and Paulitz (1994) with some modification. *F. graminearum* colonized corn was spread in wheat plots prior to heading (GS 7). Plots were mist irrigated daily beginning just prior to heading for five minutes every ten minutes between 6 and 8 AM and between 10 PM and 12 AM.

Field disease evaluations: Disease evaluations were initiated when scab symptoms were detected on several of the susceptible cultivars, approximately 3 weeks post anthesis. Those lines that flowered first were read first. Average head severity was determined by counting the number of infected spikelets divided by the total number of spikelets per head on 10 infected heads per plot. Also the percentage of Fusarium damaged kernels (FDK) was estimated and a DON test was done on grain samples.

Heritability Estimates: Analysis of variance was computed for FHB severity in each experiment. Broad sense and narrow sense heritability estimates were calculated on an entry-mean basis from variance components.

Actual Selection Gain: Realized heritabilites were calculated based on the 2003-04 data following the formula: $h_r = R/S$

For each population, the selection differential (S) was calculated as the difference in the mean head blight severity of the original $F_{2:4}$ populations (x_0) and that of the selected sample of resistant plants. The response to selection (R) was calculated as the difference between the mean head blight severity of the unselected plants in 2004 (x_0) and the mean head blight severity of the progeny of the selected plants (x_1).

RESULTS

At Lexington (Table1), one cycle of recurrent selection for FHB resistance reduced the percentage of diseased spikelets from 50.8 to 40.3% in Population 1, from 38.9 to 29.5% in Population 2 and from 41.6 to 39.3% in Population 3. Similar results were found by Jiang et al., (1994) who obtained an average reduction of diseased spikelets of 9 % after two cycles of recurrent selection in three populations.

At the second location, Princeton (Table 2), one cycle of recurrent selection for FHB resistance reduced the percentage of diseased spikelets from 32.2 to 27.8% in Population 1, from 34.7 to 27.1% in population 2 and from 39.4 to 37.1% in population 3. The selection response was higher at Lexington than at Princeton. If we compare the realized heritabilities calculated in 2004 with the BSH estimated from the components of variance in 2003 (Verges V, unpublished, 2004), in two of the three populations the h^2_r was higher than the h^2_{bs}. This means that for these families the obtained increase in resistance was higher than the expected from the estimates of variance components in the $F_{2:4}$ generation.

One cycle of among-family selection for low FHB index reduced the mean severity in some families compared to the population mean (Table 4). FDK and DON also showed significant progress. The top families in Population 2 showed the highest progress in selection: 6 of 8 families showed lower mean severity and FDK than the population mean, and the 8 families showed lower DON than the mean DON concentration. Population 1 and 3 also showed progress with one cycle of selection.

DISCUSION

One cycle of recurrent selection was successful in the three populations, especially in Population 1 and 2, where mean severity had a reduction of 10 %. The success of recurrent selection was not so evident in Princeton. As the selection was conducted in Lexington, we suggest that the selection environment could have influenced these results; if this trend is confirmed it might be a disadvantage when selecting in early generations.

One cycle of among family selection for low FHB index showed that good progress could be achieved through selecting the top families on each population. Not only was mean severity reduced on the selected families; but many top families had a lower FDK and DON concentration than the population mean.

In 2003, these three populations were genotyped for the presence of the Sumai 3 resistance alleles (Verges et al., 2003). Only population 2 had the resistance alleles, which might explain its superior performance.Many of these families have been planted in 2004 in preliminary trails of the University of Kentucky breeding program.

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Table 1. Mean FHB severity (%) of the original three $F_{2:4}$ winter wheat families in 2003 (μ_0), the proportion (P) of selected plants out of the $F_{2:4}$ families, the selection differential S, the mean FHB severity (%) of the progeny produced by the selected plants based on $F_{4:5}$ line means (μ_1) , the mean FHB severity (%) of the progeny produced by the non selected plants based on $F_{2:5}$ line means (μ_0), the mean selection response R, the realized heritability h_r^2, Lexington, KY,2004.

	2003			2004			
	μ_0	P	S	μ_1	μ_0	R	h^2_r
Population 1	29.5	2.5	-14.4	40.2	50.8	-10.6	0.73
Population 2	30.1	2.5	-15.1	29.5	38.9	-9.4	0.62
Population 3	31.2	2.5	-16.2	39.3	41.6	-2.3	0.14

Table 2. Mean FHB severity (%) of the original three $F_{2:4}$ winter wheat families in 2003 (μ_0), the proportion (P) of selected plants out of the $F_{2:4}$ families, the selection differential S, the mean FHB severity (%) of the progeny produced by the selected plants based on $F_{4:5}$ line means (μ_1) , the mean FHB severity (%) of the progeny produced by the non selected plants based on $F_{2:5}$ line means (μ_0), the mean selection response R, the realized heritability h_r^2, Princeton, KY, 2004.

	2003			2004			
	μ_0	P	S	μ_1	μ_0	R	h^2_r
Population 1	29.5	2.5	-14.4	27.8	32.2	-4.4	0.30
Population 2	30.1	2.5	-15.1	27.1	34.7	-7.6	0.62
Population 3	31.2	2.5	-16.2	37.1	39.4	-2.3	0.14

Table 3. Effect of one cycles of among-family selection for low FHB index on severity of infection, Fusarium damaged kernels (FDK) and DON concentration in three winter wheat populations, Lexington, KY. 2004.

	Population 1			Population 2			Population 3		
Entry	Severity (%)	FDK (%)	DON (ppm)	Severity (%)	FDK (%)	DON (ppm)	Severity (%)	FDK (%)	DON (ppm)
C_0	50.8	65.8	13.2	38.9	42.6	18.8	41.6	57.2	13.6
C_1-1	57.4	38.4	15.2	29.3	28.1	5.8	36.2	53.3	7.8
C_1-2	61.8	55.2	9.3	31.6	37.5	16.5	32.8	38.6	9.4
C_1-3	54.0	75.0	11.7	38.3	33.1	10.9	39.4	75.4	15.8
C_1-4	44.9	51.1	11.1	40.4	17.1	7.5	44.9	61.1	9.2
C_1-5	52.8	80.0	13.4	36.6	32.8	12.1	33.5	61.4	10.6
C_1-6	54.4	40.2	11.6	36.3	69.2	10.9	33.0	25.8	12.4
C_1-7	41.0	33.5	12.4	36.4	50.4	12.2	30.0	42.6	16.5
C_1-8	37.7	43.5	16.2	22.5	47.9	10.2	42.0	75.2	11.5
LSD (0.05)	9.2	10.1	4.6	8.0	9.1	3.4	7.8	8.5	4.3

GENETIC CORRELATIONS OF KERNEL WEIGHT AND TEST WEIGHT WITH FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL ACCUMULATION IN BARLEY

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ABSTRACT

Various traits of barley can influence the level of Fusarium head blight (FHB) infection and deoxynivalenol (DON) accumulation in barley. The objective of this study was to determine if deoxynivalenol and FHB incidence are correlated with kernel weight and test-weight of a doubled haploid (DH) progeny of a two- and six-row cross of Leger/CI9831. Doubled haploid lines were equally divided between two-row spikes (*Vrs1.t*) and six-row (*vrs1.a*) ones. These lines were tested in natural conditions at Charlottetown (Prince Edward Island) and Ottawa (Ontario). In 1993, they were also tested in FHB nursery in 2001 and 2002 at Ottawa. Kernel weight and test-weight were measured in each environment. Kernel weight was found to be genetically correlated with FHB incidence and DON content. Barley lines with better resistance to FHB had larger kernel weight even in natural conditions compared to susceptible ones. Test-weight was more variable across environments and is less genetically associated with FHB resistance compared to kernel weight. It appears that kernel weight is a better selection criterion compared to test-weight for FHB resistance in barley.

QUANTITATIVE ASSAY OF THE EXPRESSION OF GENE *G12* IN SCAB RESISTANT AND SUSCEPTIBLE WHEAT VARIETIES

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ABSTRACT

Wheat gene *G12* isolated from 'Sumai 3' was previously found to be up-regulated by *Fusarium graminearum* infection using mRNA Difference Display technology. The aim of this study was using Real-Time PCR to quantitatively assay its expression patterns in both scab resistant and susceptible wheat varieties in response to *F. graminearum* infection. Spikes of scab-resistant 'Sumai 3', 'Tokai 66' and 'Abura' and scab-susceptible 'Wheaton' and 'Y-1193' were inoculated in a growth chamber by injecting *F. graminearum* microconidia suspension into the first flowering spikelets. Water was used in mock inoculations as a negative control. mRNA samples were collected in 24 and 32 hours after inoculation and used for Real-Time PCR assays with *G12* primers, the Applied Biosystem SYBR Green DNA Core Reagent Kit and a Cepheid's SmartCycler II. Generally, threshold cycles (Ct) appeared at the 20th to the 36rd cycles (Melt Deriv 76—77) for all the samples. Differential expression of gene *G12* between scab-resistant and scab-susceptible entries was observed in both 24 and 32 hours before and after inoculation. Our data suggested that *F. graminearum* infection down-regulated the expression of gene *G12* in both the resistant and susceptible entries in 24 hours after infection. However, in 32 hours after inoculation, the expression in susceptible entries seemed rebounding back over the negative controls, while the expression in the resistant entries remaining down-regulated.

PHENOTYPIC *VS.* MARKER-BASED SELECTION TO FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT

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ABSTRACT

Phenotypic selection is a current method to improve Fusarium head blight (FHB) resistance in wheat breeding. Markers for several QTL have been published that may be useful for the development of FHB resistant cultivars in the future. The aim of this study is to compare the realized selection gain from phenotypic and marker-based selection for FHB resistance in spring wheat. Resistance sources were taken from two recent mapping populations. Derivatives of the resistant cultivars CM82036 (Sumai3/Thornbird) and Frontana were crossed with the two susceptible German spring wheat cultivars Munk and Nandu to establish the source population in the year 2000. Phenotypic evaluation and selection of the experimental lines was performed in spray-inoculated field experiments. Within a selection program starting with evaluation of 1,075 $F_{1:2}$ lines, the 300 best genotypes were selected for low FHB severity at two locations in 2001. In the following year, these genotypes were re-tested at four locations and the 20 best lines were selected and recombined in a factorial design. The resulting 1,100 F_1 plants were selfed and tested as $F_{1:2}$ lines in 2003 at two locations. Thereof, 135 $F_{1:3}$ genotypes were selected and re-tested in 2004. Marker-based selection was done for QTL on chromosomes 3B and 5A (CM82036) and 3A (Frontana) with SSR markers in three subsequent selfing generations. In the original mapping populations these QTL accounted for 32, 23, and 16% of phenotypic variation, respectively. Finally, 30 $F_{3:4}$ lines were selected that harbored the three QTL for the respective resistance alleles homozygously and selfed. All plants per line were bulked at harvest to produce enough seed for testing. The resulting $F_{3:5}$ bulks were tested along with the phenotypically selected $F_{1:3}$ bulks at four locations in 2004. Mean disease severity (% infected spikelets per plot) of the unselected source population was 22.7%. Phenotypically selected progeny had a mean FHB severity of 9%, marker-selected genotypes of 12.4%. Phenotypic selection, however, took twice as long as marker-based selection, but has the advantage of selecting towards several traits simultaneously. In conclusion, use of molecular markers for FHB resistance is valuable for the breeder, especially when the time frame is narrow. A combination of both selection methods seems to be most favorable: (1) marker-based selection to include genotypes with the relevant QTL and (2) phenotypic evaluation in the field to exploit the full selection gain and to consider additional agronomic traits, like flowering date, plant height, and other disease resistances.

EVALUATION OF MOLECULAR MARKERS ASSOCIATED WITH FHB RESISTANCE QTL IN SOFT RED WINTER WHEAT BREEDING LINES

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ABSTRACT

Fusarium head blight (FHB), also called head scab, infection results in significant economic losses to wheat and barley producers and the wheat and barley industries. Economic losses are associated with reduced yield and quality as well as mycotoxin (DON) accumulation in infected kernels. Significant research effort has focused on the identification of microsatellites (SSRs) associated with resistance quantitative trait loci (QTL), for use in marker-assisted selection (MAS) within wheat and barley breeding programs. MAS has proven to be an effective way of predicting host resistance, which is the most economical and effective way of controlling FHB in wheat. Information comparing the FHB resistance QTL haplotype data of adapted parental breeding lines would be useful in implementing a MAS program. In this study we compared the haplotype of 41 breeding lines from the University of Illinois soft red winter wheat breeding program (all with moderate to high resistance to FHB) with known sources of FHB resistance that had previously been characterized with SSR alleles linked to FHB resistance QTL. The SSRs evaluated were associated with the following seven chromosome regions: 2DL, 3A, 3BS (distal to the centromere), 3BSc (proximal to the centromere), 4B, 5AS, and 6BS. Of the 41 lines evaluated, 9 lines had Ning7840, a Sumai 3 derived source of FHB resistance, in the pedigree. For the remaining 32 lines the FHB resistance was derived from so-called "native" sources of resistance present in the soft red winter wheat gene pool. Our objective was to determine if the QTL conferring FHB resistance in the University of Illinois breeding program lines was associated with known resistance sources, Frontana, Maringa, Sumai 3, Wangshuibai and Wuhan1. Based on marker analysis the 9 breeding lines with Ning7840 in the pedigree appear to carry some SSR alleles similar to those in Sumai 3, but the lines vary in the number of SSR alleles that are the same as Sumai 3, and none of the lines appear to carry all of the Sumai 3 QTL. Based on the 46 SSRs evaluated, the 32 breeding lines that do not have a Sumai 3 derived source of resistance in the pedigree carry some SSR alleles that are similar to SSR alleles associated with known FHB resistance QTL, but in most cases the SSR alleles are different from those found in well-known FHB resistance varieties. Thus, there is extensive deviation of the Illinois breeding lines from the SSR alleles in known FHB resistance sources. Several possible explanations include: some of the breeding lines may not be as FHB resistant as Sumai 3; the breeding lines may not carry the QTL studied; the linkage phase between the SSR markers and the FHB resistance QTL may not be the same as in the FHB resistance sources; or the breeding lines may derive FHB resistance from other QTL not included in this study.

NEW STS MARKERS ASSOCIATED WITH FHB RESISTANCE IN WHEAT DEVELOPED WITH AN EXTENSION-AFLP METHOD

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ABSTRACT

The QTL for Fusarium head blight (FHB) resistance on wheat chromosome 3BS has often been reported. Recent studies have revealed another FHB resistance QTL on wheat chromosome 2DS. In two doubled haploid mapping populations derived from Sumai #3 (R)×Gamenya (S) and Sumai #3 (R)×Nobeokabouzu-komugi (VR), QTLs for Type I resistance (to initial penetration) and Type II resistance (to fungal spread within plant tissues) were detected on chromosome 2DS by using AFLP and SSR markers, but further studies of QTLs on chromosome 2DS in different populations were needed. We developed an "extension-AFLP" method to efficiently convert AFLP markers into STS markers in wheat. When an AFLP marker of interest was detected with an *Eco*RI+3/*Mse*I+4-selective primer combination, the PCR product was used as a template for additional selective amplification with four primer pairs in which one additional selective base (A, C, G, or T) was added to the 3'-end of one of the two primers. The extended primer pair that produced the targeted band was further extended by adding each of the four selective nucleotide bases in the next round of selective amplification. Extension selective amplification was performed until the targeted bands became clear enough for subsequent cloning and sequencing. In our previous study (Genome 47: 660-665, 2004), we successfully used the extension-AFLP method to convert two AFLP markers located on chromosome 3BS into STS markers. In this study, we further converted a dominant AFLP marker (AGT/CAAC396) located on chromosome 2DS into STS marker. Using the extension-AFLP method, we obtained DNA sequence of the AFLP band and then designed STS primer based on the obtained sequence. However, when the STS primer (STS396) was applied to amplification of total genomic DNA of the parents of the mapping populations, it did not show polymorphism as its original AFLP markers did. When the PCR products were subjected to digestion with five restriction enzymes (*Hae*III, *Msp* I, *Hin*f I, *Rsa* I, and *Hha* I), STS396 showed polymorphism upon digestion with *Msp* I, *Hin*f I, *Rsa* I, and *Hha* I. Mapping result showed that the STS marker co-segregate with its corresponding AFLP marker. These results again suggest that the extension-AFLP method is an efficient approach for converting AFLPs into STS markers. The resulting STS markers might be useful to improve FHB resistance in wheat.

COMPARISON OF INOCULUM SOURCES ON DEVELOPMENT OF FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL CONTENT IN WHEAT IN A DISEASE NURSERY

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ABSTRACT

The influence of inoculum source (conidial suspension, infested barley/corn grains, and infested wheat debris) and inoculation frequency on development of Fusarium head blight (FHB) and deoxynivalenol (DON) content was examined using three spring wheat genotypes in a disease nursery in Ottawa, Ontario from 2001-2003. The development of FHB was monitored by visually estimating disease severity on a 0-9 scale six times during each growing season. Severity of FHB over time was summarized as area under the disease progress curve (AUDPC). Symptoms of FHB were also rated as critical-time disease severity (DS), percentage of infected spikelets (IS), and FHB index at the soft dough stage. Percentage of *fusarium*-damaged kernels (FDK) and DON content in the wheat grains were assessed after harvesting. The disease parameters for the inoculation treatments with conidial suspension sprayed two and three times and infested grains twice were significantly greater than the uninoculated control. Treatment with infested grains spread three times had significantly greater AUDPC, DS, FHB index, FDK, and DON, but did not differ in IS from the control. Treatments inoculated with debris two or three times were not significantly different from the uninoculated control in all parameters except for FDK, in which, 2-applications of debris gave significantly higher percentage of FDK. Regardless of the inoculum source, two inoculations produced as much FHB, FDK, and DON as three inoculations. Correlations among the assessment parameters were significant ($P < 0.05$) for all inoculum sources in 2001 and 2002 and for infested grains only in 2003. Under high disease pressure in 2003, correlation between DS and IS was not significant when inoculated with debris, nor between FDK and other parameters following conidial suspension or debris as the inoculum source. Greater correlations among the assessment parameters using infested grains than conidial suspension and debris suggest that infested grains may be a more effective source of inoculum for FHB nurseries.

IDENTIFYING NEW QTLS FOR WHEAT RESISTANCE TO FUSARIUM HEAD BLIGHT USING SSR AND TRAP MARKERS

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ABSRACT

Fusarium Head Blight (FHB) is the most destructive disease in wheat. One of the most effective strategies for controlling this disease is to grow FHB resistant cultivars. The major resistance QTLs from Sumai 3 have been used extensively in breeding programs worldwide. Identification new sources of resistance will enhance genetic diversity of FHB resistance sources in breeding programs. Chokwang, a Korean wheat cultivar, contained different QTL from these in Sumai 3 and its derivatives. To identify the new QTL and closely linked markers, 678 simple sequence repeats (SSRs) and 275 target region amplified polymorphism (TRAP) markers were analyzed in a population of recombinant inbred lines (RILs) derived from the cross Chokwang/Clark. Three new QTLs were identified. Two of them tentatively were located on chromosome 5DL (Qfhb.usda-5DL-1 and Qfhb.usda-5DL-2). Two SSR markers (XBarc239 and Xcfd3) linked to the two QTL were identified, which explained 30.1% and 26.1% of phenotypic variance respectively. Another QTL Qfhb.usda-7BL was identified by SSR Xbarc1096 and tentatively mapped on 7BL, which explained 19.1% of phenotypic variance. The QTL on 3BS was also detected by SSR Xgwm533 in this population, which explained 24.1% of phenotypic variance. In addition, Qfhb.usda-5DL-1, a major QTL with the largest effect, was physically mapped to bin of 5DL-0.60-0.74 using Chinese Spring deletion lines. These results suggested that Chokwang contains new QTL for FHB resistance that are different from these in Sumai 3. Pyramiding resistance QTL from Sumai 3 and Chokwang may enhance FHB resistance in wheat cultivars.

EVALUATION OF EAST ASIAN BARLEY CULTIVARS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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OBJECTIVES

The objective of this study is to identify barley (*Hordeum vulgare* L.) introductions with better Fusarium head blight (FHB) resistance by screening East Asian cultivars for FHB severity and deoxynivalenol (DON) level.

INTRODUCTION

Fusarium head blight, incited principally by *Fusarium graminearum* Schwabe, in recent years has been the most destructive disease of barley in North America (McMullen et al., 1997; Steffenson, 1998). The major loss in barley from FHB is a reduction in grain quality caused by the presence of the mycotoxin DON. Barley that has more than 0.5 ¼g/g of DON is not acceptable to many malting and brewing companies and is often not purchased or is heavily discounted.

Success of developing barley cultivars with FHB resistance has been limited due to low levels of FHB resistance and associations with undesirable agronomic traits (Rudd et al., 2001). Unlike Sumai 3 in wheat (*Triticum aestivum* L.), no single barley cultivar or accession is being used widely as a source of FHB resistance. The resistance sources currently used in barley, CIho 4196 (two-rowed) and Chevron (six-rowed), are associated with undesirable traits such late heading date and tall plants (de la Pena et al., 1999; Urrea et al., 2002).

For decades, development of FHB-resistant barley cultivars has been a major goal in parts of East Asia. Most sources of resistance currently used in barley and wheat breeding programs in North America came from this region. Most East Asian cultivars are fall-sown and head under short-day conditions. The objective of this study is to identify introductions with better FHB resistance by screening East Asian cultivars for FHB severity and DON level.

MATERIALS AND METHODS

The experiments were conducted at Zhejiang University in Hangzhou, China in 2002 and in 2003, and at Langdon and Osnabrock, North Dakota (ND) in 2003. Forty-eight cultivars from China and Japan and checks were tested using randomized complete block design with three replicates. Nurseries were inoculated by spreading maize (*Zea mays* L.) and barley kernels colonized by local isolates of *F. graminearum*. The FHB severity was measured as the percentage of infected kernels divided by total number of kernels on 10 spikes. Deoxynivalenol was tested on harvested grains from China 2002, China 2003, and Langdon ND 2003 with gas chromatography method (ASBC, 1999) in the barley malting and brewing lab at North Dakota State University. In addition, data on heading date as days from January 1 until 50% of the spikes were half emerged from the boot and plant height as cm from the ground to the tip of the spike excluding the awns were collected.

RESULTS AND DISCUSSION

The evaluation of barley cultivars for FHB resistance produced variable results because many East Asian introductions are highly photoperiod sensitive. Variations in heading date and plant height were observed over locations. The late heading date of CIho 4196, the resistant check (Urrea et al., 2002), made evaluation of its FHB response difficult.

Fusarium head blight severity - The average FHB severity of the 48 cultivars across four environments ranged from 11.0 to 42.6% with an average of 27.4%. The interactions between cultivars and environments were significant; thus, data from individual nursery sites are presented. In Hangzhou, China 2002 and Osnabrock, ND 2003, Shenmai 3 (a cultivar released by the Shanghai Academy of Agricultural Sciences and selected from a Gobernadora/Humai 10 cross made by the ICARDA/CIMMYT barley breeding program in Mexico), Zhaori 19 (from Japan), and Guan 78-01 were significantly lower in severity than CIho 4196 (Table 1). Compared to Conlon, a Midwest two-rowed malting barley cultivar, the FHB severities of Shenmai 3, Zhaori 19, and Guan 78-01 were significantly lower in Hangzhou, China 2002 and 2003, and Osnabrock, ND 2003. The severity of CIho 4196 was significantly lower than those of Conlon in Hangzhou, China in 2002 and 2003, and those of Lacey, a Midwest six-rowed malting barley cultivar, in all four environments. Asahi 5 and Zaoshu 3 from Japan and Supi 1 and ZAU 7 from China are examples of cultivars with moderate FHB resistance (Table 1).

Deoxynivalenol accumulation - The DON concentrations of the 48 barley cultivars across three environments ranged from 7.2 to 48.4 µg/g. The average DON level was 18.9 µg/g. Since the error variances in three environments were not homogeneous, the results from individual environment analyses are presented. The DON levels of Shenmai 3, Zhaori 19, and Guan 78-01 were significantly lower than those of CIho 4196 in Hangzhou, China in 2002 and Langdon, ND in 2003 (Table 2). Compared to Conlon, the DON levels for these three cultivars were not significantly lower. The differences in DON level between CIho 4196 and Lacey were not consistent across the three environments.

Heading date and plant height - In the Langdon and Osnabrock trials, Shenmai 3 had heading dates close to those of Conlon and Lacey and the plants were shorter than those of Conlon and Lacey. Zhaori 19 was similar in plant height to Conlon, but headed about four days earlier (Table 3). Guan 78-01 was close to Conlon in plant height, but almost one week later than Conlon (Tables 3 and 4). CIho 4196 was later and taller than the other cultivars tested in China and ND. Shenmai 3 apparently lacks the *Eam1* gene of Zhaori 19 for earliness under long days and the *eam6* gene of CIho 4196 for lateness under long days. Shenmai 3 likely has the *Eam5* and *eam9* genes for earliness under short days (Franckowiak et al., 2003).

Correlations - Within an environment, the simple linear correlations between FHB severity and DON were significant in Hangzhou, China in 2002 and 2003, but not significant in Langdon, ND in 2003 (Table 5). Among environments, the correlation for FHB severity was significant between Hangzhou, China in 2003 and Langdon, ND in 2003. For DON concentration, the correlations between Hangzhou, China in 2002 and Langdon, ND in 2003 and between Hangzhou, China in 2003 and Langdon ND in 2003 were significant. This indicated the effects of environments on FHB severity and DON level were not consistent or highly repeatable for the 48 barley cultivars tested.

CONCLUSIONS

Shenmai 3, Zhaori 19, and Guan 78-01 had significant lower values for both FHB severity and DON level in two out of four and two out of three environments, respectively. These East Asian cultivars may have better FHB resistance than CIho 4196. CIho 4196 is tall and late in China and ND, Zhaori 19 is very early in ND, and Guan 78-01 is late and naked. Shenmai 3 appears better adapted to ND in terms of heading date and plant height; therefore, Shenmai 3 may be a better source of FHB resistance than accessions currently used in barley improvement programs.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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Table 1. Fusarium head blight severities for selected barley cultivars grown in China and North Dakota (ND).

Cultivar	Origin	Spike type	Hangzhou China 2002	Hangzhou China 2003	Langdon ND 2003	Osnabrock ND 2003
			----------% infected kernels----------			
Shenmai 3	China	2	9.0	1.9	10.7	22.3
Zhaori 19	Japan	2	9.9	5.0	23.8	21.3
Guan 78-01	China	2	6.2	7.3	17.5	27.7
Asahi 5	Japan	2	10.2	8.8	24.6	34.7
Zaoshu 3	Japan	2	16.3	24.1	25.4	31.7
Supi 1	China	2	23.0	15.7	23.1	27.7
ZAU 7	China	2	14.1	21.9	42.0	36.3
CIho 4196	China	2	19.6	0.9	17.3	39.0
Conlon	ND	2	43.3	24.5	17.0	39.0
Lacey	Minn	6	55.9	17.8	28.9	50.3
$LSD_{0.05}$			---------- 9.4 ----------			

Table 2. Deoxynivalenol (DON) levels in grain samples from selected barley cultivars grown in China and North Dakota (ND).

Cultivar	Origin	Spike type	Hangzhou China 2002	Hangzhou China 2003	Langdon ND 2003
			----------µg/g----------		
Shenmai 3	China	2	0.2	12.5	9.1
Zhaori 19	Japan	2	0.9	25.2	4.3
Guan 78-01	China	2	0.1	31.4	5.9
Asahi 5	Japan	2	0.7	49.2	10.1
Zaoshu 3	Japan	2	0.6	22.4	17.2
Supi 1	China	2	2.1	76.5	29.4
ZAU 7	China	2	1.3	47.8	12.8
CIho 4196	China	2	15.6	11.6	20.6
Conlon	ND	2	5.7	25.4	3.3
Lacey	Minn	6	4.1	56.5	23.3
$LSD_{0.05}$			7.6	17.1	10.8

FINE MAPPING OF WHEAT QTL FOR RESISTANCE TO FHB AND DON IN CHINESE LANDRACE WANGSHUIBAI

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, can significantly reduce both grain yield and quality. Growing FHB resistant varieties is an effective means to reduce losses caused by the disease. However, currently used FHB resistance sources are mainly Sumai 3 and its derivatives. Utilization of FHB resistance sources different from Sumai 3 may enrich the genetic pool of FHB resistance sources. Wangshuibai is a FHB resistant Chinese landrace unrelated to Sumai 3. To map QTL for Type II FHB resistance and for a low level of deoxynivalenol (DON), a mycotoxin produced by the pathogen, 139 F_6 derived recombinant inbred lines (RILs) was developed from a cross Wangshuibai/Wheaton. Totally 1259 simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers were analyzed in this population, and seven QTL for Type II resistance and four for low DON accumulation were detected. Two QTL for Type II resistance located on 3BS were detected which explained 37.5% and 9.4% of the phenotypic variation, respectively. Five additional QTL for Type II resistance on chromosomes 3A, 3D, 2A, 6B and 1B explained 7.4% to 11.9% of the phenotypic variation. The major QTL on 3BS also explained 12% phenotypic variation for low DON accumulation. Three additional QTL for low DON accumulation explained 7.2% to 8.9% of phenotypic variation. New QTL for FHB resistance identified in Wangshuibai have potential to be used in developing cultivars with enhanced FHB resistance by pyramiding FHB resistance QTL from different sources.

IDENTIFICATION OF NEW FHB RESISTANCE SOURCES FROM ASIAN WHEAT GERMPLASM

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OBJECTIVES

To identify wheat germplasm that expresses a high level of Type II resistance and accumulates low DON content in harvested grain.

INTRODUCTION

Fusarium head blight or scab is an economically important disease of cereal crops worldwide. Severe infection can dramatically reduce grain yield and quality. In addition, infected grain is often contaminated with deoxynivalenol (DON), which has become a major concern for animal production and human health (Bai et al. 2004). FHB resistant cultivars have been identified from different countries. However, only the Chinese cultivar 'Sumai 3' and its derivatives show consistent Type II resistance across different environments and have been extensively used as the major source of resistance in breeding programs (Bai and Shaner 2004). Therefore, new sources of resistance are needed to prevent the potential breakdown of resistance from Sumai 3 and diversify FHB resistance sources in breeding programs.

MATERIALS AND METHODS

One hundred and ten wheat accessions were evaluated for Type II FHB resistance and DON contents. Among them, 79 originated from China, 23 from Japan, one from Korea and seven from the USA and other countries (Table 2). The disease evaluation was conducted in the greenhouse of Kansas State University, Manhattan, KS in 2003 as described by Bai et al. (2001). In brief, the Type II FHB resistance was evaluated by injecting 1000 conidia spores of *F. graminearum* into a central floret of a spike at anthesis. The inoculum was a Kansas field isolate (GZ 3639). The experiment was repeated twice with three replications (pots). Four to six plants in each pot, depending on uniformity of flowering time, were inoculated. The inoculated plants were incubated in a moist chamber for three days at 25 ºC to initiate infection. Infected and total spikelets in a spike were counted at 21 days after inoculation and proportion of scabbed spikelets (PSS) in a spike was calculated as final disease severity. The seeds from inoculated spikes were evaluated for DON content by using combination of gas chromatography/mass spectrometry (GC/MS, Mirocha et al, 1998) and DON content was expressed as mg/kg. Statistical analysis was conducted by using SAS software package (SAS Institute, Inc., Cary, NC)

RESULTS AND DISCUSSION

All inoculated wheat accessions showed FHB symptoms after single floret inoculation. Differences in PSS and DON content were significant among accessions. The mean PSS of FHB over two experiments ranged from 7% (F60096) to 100% (ChanjiBaiDongMai, CheJianZi and LingShuiSanYuehHwang) and showed continuous variation across the accessions evaluated. All accessions could be classified into four categories based on their average PSS: resistant (0-30%), moderate resistant (31-50%), moderate susceptible (51-70%) and susceptible lines (71-100%). About 60% of the accessions were resistant or moderately resistant to FHB (Table 1). Only 20% of accessions were highly susceptible. The DON contents in the harvested grain of inoculated spikes ranged from 0.4 mg/kg

(Fu5114) to 188.9 mg/kg (ChanjiBaiDongMai) (Table 2). Thirty-five resistant accessions (PSS < 30%) mainly originated from China and Japan, 15 of them had a DON content less than 2 mg/kg. Most of these resistant accessions do not relate to Sumai 3 in their pedigree, suggesting that they may carry genes (QTL) for FHB resistance and low DON content different from those in Sumai 3.

The wheat accessions with a low DON content usually had a low PSS value (Table 1 and Table 2). Significant positive correlations were observed between PSS and DON contents (Fig. 2) in both experiments. The correlation for spring experiment ($r = 0.73$, $p < 0.0001$) was higher than that of fall experiment ($r = 0.32$, $p = 0.002$), this may be due to removal of several highly susceptible accessions in the fall experiment. Thirty-five resistant wheat accessions with less than 20% PSS had an average low DON of 3.19 mg/kg. Among them, two Chinese landraces (Huangcandou and Baisanyuehuang) and two Japanese landrace (Minamikyushu 69 and AsoZairai) had similar Type II resistance and DON content as that in Ning 7840 (Table 1). These landraces may have potential to be new FHB resistance sources for molecular mapping and breeding.

In an average, moderately resistant and moderately susceptible accessions had a higher DON content than that for resistant accessions, but with some exceptions (Table 2). Among these accessions with less than 2 mg/kg DON, seven were moderately resistant and three are moderately susceptible (Table 1). Moderately susceptible Chinese landrace 'HongMongBai' had 70% PSS, but showed a low DON content (0.83 mg/kg); while, another Chinese landrace 'MeiQianWu' showed low infected spikelets (19%), but accumulated a relatively higher level of DON in the harvested grain (9.52 mg/kg, Table 2). Inconsistent relationship between PSS and DON content may be due to genetic difference among the accessions, but non-genetic factor may also affect DON measurement in harvested grains (Bai and Shaner 2004). These observations suggested that selection for Type II resistance based on the visual FHB symptoms on the infected spikes could be anticipated to get wheat genotypes with a low DON content. However, a high level of DON accumulation in harvested grains may not always be expected for those accessions with moderate Type II resistance.

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Table 1. Number of lines, minimum, maximum and mean deoxynivalenol (DON) content in mg/kg for wheat accession with different levels of FHB resistance based on proportion of scabbed spikelets (PSS).

FHB Rating	**R***	**MR**	**MS**	**S**
Minimum DON	0.39	0.71	0.83	2.60
Maximum DON	10.50	28.13	41.18	188.89
Mean DON	3.19	5.81	12.00	22.81
Number of lines	35 (31.8%)	30 (27.3%)	23 (20.9%)	22 (20%)

*****R stands for resistant with PSS ranging from 0 to 30%; MR stands for moderately resistant with PSS ranging from 31% to 50%; MS stands for moderately susceptible with PSS ranging from 51% to 70%; S stands for susceptible with PSS ranging from 71% to 100%.

Table 2. Fusarium head blight ratings and deoxynivalenol (DON) levels for selected wheat lines from different origins based on 2003 experiments.

Name	**Country**	**Source**[¶]	**Average PSS**[†] **(%)**	**Average DON**[†] **(ppm)**
F60096	China	JAAS	7.0	3.16
Fu5114	China	JAAS	7.4	0.39
Minamikyushu 69	Japan	PI382152	7.5	2.37
Su49	China	JAAS	8.2	2.74
Linghaimao Yang Mo	China	PI435124	8.2	6.98
WangShuiBai	China	JAAS	8.8	0.68
Asozairaiii	Japan	JIRCAS	9.3	1.97
Huangcandou	China	JAAS	11.0	1.10
Baisanyuehuang	China	JAAS	11.7	1.30
Ning 7840	China	JAAS	13.1	0.61
Taiwan Wheat	China	JAAS	13.3	0.92
Asozairai(Yuuboukapp u)	Japan	JIRCAS	13.3	1.63
Huang Fang Zhu	China	JAAS	13.7	2.10
HaiYanZhong	China	JAAS	14.1	3.23
Nyubai	Japan	PI382154	14.7	1.68
Huoshaomai	China	JAAS	17.5	2.58
MeiQianWu	China	PI525071	19.2	9.52
Fumai3	China	JAAS	19.6	5.49
Yangmai1	China	JAAS	19.8	1.76
CaiZiHuang	China	JAAS	20.6	9.21
Ernie	USA	PI592001	21.6	2.34
Shirasaya No1	Japan	PI197129	22.9	4.17
WZHHS	China	JAAS	23.5	3.14
Huoshaobairimai	China	JAAS	23.9	1.59
Yangmai 5	China	JAAS	24.0	6.54
Sobakomugi 1C	Japan	JIRCAS	24.8	10.5

Table 2. cont's

Name	Country	Source	Average PSS	Average DON (ppm)
Sumai 3	China	JAAS	25.1	3.04
Shoukomugi II	Japan	JIRCAS	25.4	1.70
Qiaomaixiaomai	Japan	JIRCAS	25.7	1.03
Tokai66	Brazil	PI382161	27.4	2.60
Nobeokabozu	Japan	PI382153	27.7	1.50
Freedom	USA	PI592002	29.1	2.04
Itoukomugi	Japan	JIRCAS	29.5	0.93
Qiangshuihuang	China	PI502931	29.6	8.01
Asotomea	Japan	JIRCAS	30.1	3.83
Chokwang	Korea	Purdue	30.8	3.10
Nobeokabouzukomugi	Japan	JIRCAS	31.4	1.96
Nyuubai	Japan	JIRCAS	31.5	1.60
FSW	China	JAAS	31.8	1.36
Yangmai 158	China	JAAS	31.8	0.71
Hongjianzi	China	JAAS	31.9	3.01
Sobakomugi 1B	Japan	JIRCAS	32.6	3.81
Kagoshima	Japan	JIRCAS	33.0	1.04
Can Lao Mai	China	JAAS	33.9	9.80
ShuiLiZhan	China	PI502930	35.0	5.68
HuiShanYangMai	China	PI462154	35.0	4.68
XingHuaBaiYuHua	China	PI462150	36.9	21.12
MuTanChiang	China	CItr9018(PI70675)	37.8	-[§]
YouBaoMai	China	PI524980	38.1	28.13
Sotome	Japan	JIRCAS	38.3	2.63
Yangmai 4	China	JAAS	39.1	5.77
YangLaZi	China	PI502932	39.3	5.96
Shinchunaga	Japan	PI197128	39.7	1.66
JiangDongMen	China	PI462135	42.2	11.15
Aburakomugi	Japan	JIRCAS	42.3	3.24
Abura	Brazil	PI382140	42.4	8.50
Wannin 2	China	JAAS	45.1	3.69
Sapporoharukomugijugo	Japan	PI81791	45.5	2.17
Xueliqing	China	JAAS	46.1	7.40
Kikuchi	Japan	JIRCAS	46.4	7.62
Sanshukomugi	Japan	PI197130	48.0	5.94
DaHuangPi	China	PI502939	48.7	5.52
LiangGuangTou	China	PI435109	49.0	0.92
Fusuihuang	China	JAAS	50.0	6.63
SanChaHo	China	CItr9017(PI70674)	51.0	15.36
YouZiMai	China	PI435110	51.1	13.30
Funo	China	JAAS	51.2	5.22

Table 2. cont's

Name	Country	Source	Average PSS	Average DON (ppm)
Clark	USA	PI 512337	53.5	30.47
Dafanliuzhu	China	JAAS	53.6	5.22
Dahongpao	China	JAAS	53.6	20.16
ChuShanBao	China	PI524973	54.6	5.19
ShanhaiCaiZiHuang	China	PI462140	56.0	3.27
Zhen 7495	China	JAAS	56.1	8.25
HongHuaWu	China	PI502949	56.5	10.46
KuangTuErShiaoMai	China	CItr7158 (PI57347)	58.1	1.97
Chile	Chili	JIRCAS	58.2	12.9
FangTouHongMang	China	PI502938	61.4	12.50
Shironankin	Japan	JIRCAS	61.5	5.79
Zalraiyuubou	Japan	JIRCAS	61.7	11.95
Heshangmai	China	JAAS	63.4	1.36
PaiMaiTze	China	PI64285 (8349)	65.8	8.45
HungGuangTou	China	PI447389	66.1	41.18
NTDHP	China	JAAS	66.4	6.61
Jingzhou 1	China	JAAS	68.2	11.09
Taiwan Shiaomai	China	CItr7171(PI57360)	68.7	-
Avrora (Abpopa)	Russia	JAAS	69.5	32.48
HongMongBai	China	PI518598	69.9	0.83
FangTouBaiMang	China	PI502935	70.9	2.95
Chinese Spring	China	JAAS	73.0	3.25
YuLinBai	China	PI591997	74.2	31.15
HongMangMai	China	PI525072	74.6	-
SanYueHuang	China	PI518834	77.8	10.53
ChangShuTongZhuTou	China	PI452263	81.2	13.27
MeiXiuHuang	China	PI525070	81.6	3.90
Nanda 2419	China	JAAS	82.6	4.35
JuRongHuoShanTian	China	PI462138	83.3	9.42
DaBaiPao	China	PI525074	83.7	9.58
YaZuiZi	China	PI524987	84.7	15.48
HongTouZi	China	PI502946	87.0	10.11
PaHuaiMai	China	PI430506	88.5	14.86
SanBaiMai	China	PI524979	89.1	25.00
Sanyuehuang	China	JAAS	90.8	17.85
TaFangShen	China	CItr9009	91.3	26.87
ChingChowWhite	China	Cltr5086	93.4	2.60
BaiMang	China	PI502943	96.0	4.63
BaiChaoYu	China	PI502947	99.1	14.55
CheJianZi	China	PI524983	100	11.63
LingShuiSanYuehHwang	China	PI445867	100	16.07
ChanjiBaiDongMai	China	PI445868	100	188.89

[†]DON is the average value in mg/kg; PSS is the proportion of scabbed spikelets in a spike
[¶]JAAS - Jiangsu Academy of Agricultural Science, P.R. China; JIRCAS - Japan International Research Center for Agricultural Sciences
[§] Data missing

GRAIN SHATTERING AND ITS RELATIONSHIP WITH FUSARIUM HEAD BLIGHT IN SPRING WHEAT

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OBJECTIVES

This study aims to investigate the genetics of grain shattering and its relationship with Fusarium head blight (FHB) resistance in spring wheat.

INTRODUCTION

Grain shattering can cause substantial loss in spring wheat (Ball and French, 1985; Harrington and Waywell, 1950; Vogel, 1938). Therefore, resistance to shattering is an important trait to consider when developing new adapted spring wheat cultivars. Much progress has been achieved in this area and most modern cultivars possessed good resistance to shattering (Kadkol et al., 1989). Recently, the grain shattering problem has resurfaced with the introduction of FHB resistant germplasm, Sumai3, which is susceptible to shattering (Rudd et al., 2001). Preliminary observations indicate that FHB-resistant derivatives of Sumai3 have a tendency to shatter more. There are a number of studies addressing the genetics of shattering during 1930's to 1950's (Lewicki, 1929; Hughes, 1940; Porter, 1959). Depending on the genetic materials used, a single gene, two genes, or multi-gene model for shattering resistance was proposed. Grain shattering inheritance, however, is still not clear. Therefore, studying the genetics of shattering for Sumai3 and the relationship between FHB resistance and shattering susceptibility, which has not been done previously, is warranted. This information is crucial for breeders in order to develop new wheat cultivars with FHB and shattering resistance.

MATERIALS AND METHODS

Plant materials- A population of 107 F_5-derived recombinant inbred lines (RILs), developed by NDSU HRSW breeding program using single seed descent method, was used in this study. The population was derived from the "Sumai3/Stoa" cross. Sumai3, a chinese genotype used as the main source of resistance to FHB disease by many breeding programs is susceptible to shattering. Stoa, a hard red spring wheat cultivar released by NDSU, is resistant to shattering and susceptible to FHB.

Methods- In 2004, the 107 RILs of the population and their parents were grown at Casselton and Prosper, ND. At Casselton, each RIL was sown in a two-row plot, 2.4 m long and 17 cm apart. The lines were arranged in a randomized complete block design with two replicates. The experiment was sprayed with Folicur fungicide to minimize the confounding of FHB on shattering results, as described by Hofman et al. (2000). Shattering was determined by two methods. The first method consisted of assigning visual score on a 1 to 5 scale with 1 being the most resistant and 5 the most susceptible. The shattering score were taken about 20 days after Feekes stage 11.4 when susceptible genotypes showed a lot of shattering. The second method consisted of randomly sampling 20 spikes per plot three weeks after Feekes stage 11.4 and counting the shattered grain number to determine the shattering percentage averages. At Prosper, the experiment was planted in a hill-plot in the FHB nursery. Grain spawn inoculation method, as described by Stack and Frohberg (1997), was used to inoculate the RILs and their parents by FHB. Three weeks af-

ter inoculation, 20 to 30 spikes per RIL were scored for FHB severity on a 0-100% scale (Stack and McMullen,1995).

RESULTS AND DISCUSSIONS

Genetic analysis of grain shattering- Data for grain shattering are presented in Table 1. The parental cultivar Sumai3 was significantly higher for both shattering percentage and visual score than the parent cultivar Stoa. There was a wide range of shattering reaction among the 107 RILs. The correlation between shattering percentages and visual scores was highly significant (Table 3). The shattering percentage for the population showed a skewed continuous distribution (Figure 1). Among the RILs, 27 lines had less shattering percentage than the resistant parent and only one line had more shattering percentage than the susceptible parent. This suggests that shattering resistance was dominant and major genes may control shattering resistance in this cross. Based on the mean shattering percentage values of resistant, susceptible parents, and the standard deviation of the population, the RILs were grouped into three classes i.e. resistant (R), moderate (M), and susceptible (S). The segregation of the population fitted the 4: 3: 1 ratio for R: M: S. This result suggests that three major genes may control shattering resistance in the resistant parent Stoa. The environmental effect and/or minor genes however, may be involved resulting in the continuous distribution.

Correlation between grain shattering and FHB- The FHB severity scores showed that there were significant differences between the RILs (Table 1). The FHB severity ranged from 2.45-81.35%. The shattering resistant parent Stoa showed high FHB severity (57.38%) while shattering susceptible parent Sumai3 had low FHB severity (6.25%). Correlation coefficients between grain shattering and FHB severity of all RILs with their parents were also calculated (Table 3). A significantly negative correlation between the two traits was obtained. This indicates that FHB resistant lines are more susceptible to shattering. However, the relationship was not high, although, it was significant. This is due, probably to the environmental conditions prevalent in the field and to some agronomic traits of the genotypes such as plant height, kernel weight, glume pair angle, and lodging. In another study we have conducted in 2003 and 2004 (unpublished data), these traits were shown to affect significantly grain shattering in several HRSW genotypes. Therefore, further investigation of agronomic traits and shattering reaction at different environments are needed in order to explain this relationship. Based on the preliminary data obtained from this study, we can conclude that the genes controlling these two traits are not closely linked. Hence, developing new varieties with resistance to both shattering and FHB with Sumai3 as FHB resistant parent is possible.

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Table 1. Means and ranges of shattering percentage, visual score, and FHB severity of RILs and their parental genotypes, Casselton, ND, 2004.

	Shatter percentage (%)	**Visual score (1 - 5)**	**FHB severity (%)**
Sumai3	93.8	5	6.25
Stoa	7.0	2	57.38
Population mean	36.61	3.04	19.66
Population range	0 - 97.22	1 - 5	2.45 - 81.38
C.V. (%)	29.10	14.93	55.37
Standard Deviation (SD)	10.66	0.45	10.88
LSD (0.05)	21.32	0.90	21.76

Table 2. Frequency distribution and probability (X^2) of segregation ratio of RILs for shattering percentage, Casselton, ND, 2004.

	Line No.	**X^2 (4R: 3M: 1S)**	**P**
R (Resistant parental line mean $\pm$ SD)	44		
M (Intermediate level between R and S)	49	3.68	0.16
S (Susceptible parental line mean $\pm$ SD)	14		

Table 3. Correlation coefficients between shattering percentage, visual score for shattering, and FHB severity of the RILs and parental lines, Casselton, 2004.

	Visual score for shattering	**FHB severity**
Shattering percentage	0.95***	-0.30**
Visual score for shattering		-0.29**

: Significant at P<0.01. *: Significant at P<0.001.

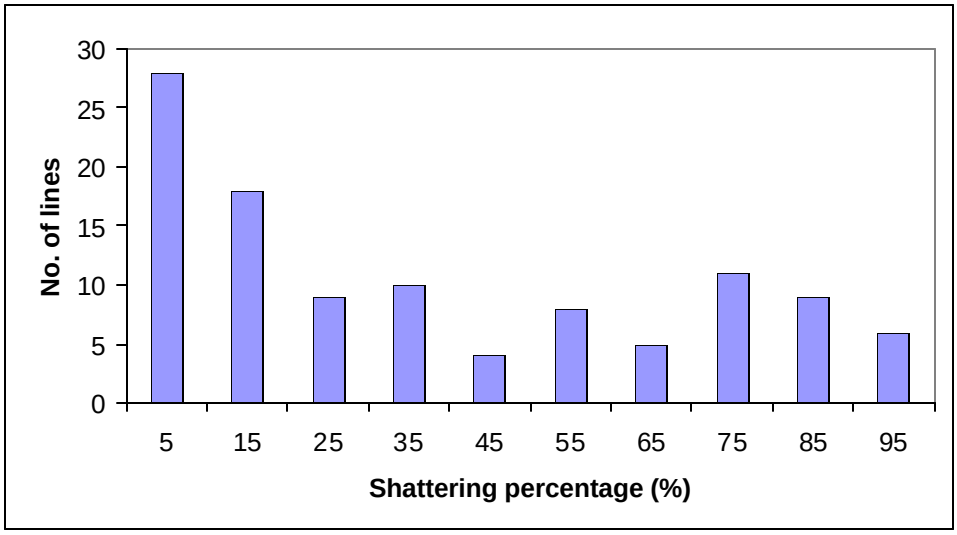

Figure 1. Frequencies distribution of RILs for shattering percentages, Casselton, ND, 2004.

SCREENING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT GERMPLASM

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ABSTRACT

The use of resistant cultivars in wheat will be one of the major components in managing Fusarium Head Blight (FHB or scab). Systematic screening of spring wheat germplasm in the USDA National Small Grain Collection was initiated in 1998. Diverse sources of resistance have been identified in the spring wheat germplasm (Zhang and Jin, 2003). Continued efforts are being made to identify additional sources of resistance, and to characterize and introgress resistance into adapted germplasm. This report summarizes the research approaches and progress in the 2004 spring wheat germplasm screening project. In 2004 materials at different stages of FHB screening were planted in multiple and inter-related nurseries (field and greenhouse) following Zhang et al. (2000) with some modification. The newly introduced materials were planted in non-replicated single-row plots and evaluated for FHB reaction in the Preliminary Screening Nursery (PSN). Materials selected from 2003 FHB nursery were planted in the Elite Germplasm Nurseries (EGN). The EGN entries were planted in single row plots with two replicates/location. The EGN materials were blocked based on year of selection and maturity groups within year of selection. The primary FHB screening nursery was in St. Paul, MN. Materials entering third year EGN were also planted in a FHB screening nursery in Crookston, MN. The St. Paul nursery was inoculated with a macroconidial suspension twice starting at anthesis. The Crookston nursery was inoculated with *Fusarium*-colonized corn kernels. The nurseries were mist irrigated until disease assessment. In 2004, a total of 384 accessions of spring wheat, originated primarily from Russia, eastern Europe, and Heilongjiang and Sichuan provinces of China were planted in the PSN nursery. The EGN consisted of 254 entries, with 105 accessions selected from the 2001 PSN, 64 accession selected from the 2002 PSN, 85 accessions from the 2003 PSN. Thirty accessions were selected as putative sources of resistance from the PSN. Severe lodging occurred in the St. Paul nursery and it was not harvested, thus the usefulness of the data from the 2004 St. Paul field nursery will be limited. The Crookston nursery was hand-harvested. Disease index and VSK of the third year screening materials will be presented. Field selections were screened in the greenhouse by single floret inoculation. Similar to our previous findings, many of the lines exhibiting moderate resistance in the field were highly susceptible to point-inoculation in the greenhouse.

ACKNOWLEDGEMENT AND DISCLAIMER

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QUANTITATIVE TRAIT LOCI OF FUSARIUM HEAD BLIGHT RESISTANCE IN TWO WHEAT POPULATIONS

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OBJECTIVES

To characterize QTL for FHB resistance in Wangshuibai in two genetic populations and identify markers closely linked to FHB resistance for marker-assisted selection.

INTRODUCTION

In China, Fusarium head blight (FHB) was first reported in the late 1930's (Dai, 1941) and is becoming more frequent, severe and widespread (Chen et al., 2000). Breeding wheat cultivars resistant to FHB is the preferable approach to minimize FHB damage. However, progress in breeding FHB resistant cultivars has been hindered by the lack of effective resistance sources and by the complex nature of wheat resistance. Furthermore, selection based on visual symptoms may not be effective because of confounding environment effects such as temperature and humidity at flowering on expression of resistance genes.

Resistance genes from Sumai 3 and related lines such as Ning 7840 have been well characterized through molecular mapping (Bai et al., 1999; Waldron et al., 1999; Zhou et al., 2002). The quantitative trait locus (QTL) on chromosome 3B of Sumai 3 demonstrated a major effect on FHB resistance and has been validated in several other populations (Buerstmayr et al., 2002; Anderson et al., 2001). Exploration of new genetic sources other than Sumai 3 is still necessary to enhance genetic diversity of FHB resistance. Wangshuibai is a Chinese landrace and repeated evaluations of FHB type II resistance under multi-environments in China have shown that FHB resistance in Wangshuibai is more stable than that of Sumai3 (Lu et al., 2001). Thus, exploration of FHB resistance in Wangshuibai may provide breeders with alternative resistance genes for the improvement of FHB resistance in wheat. The objectives of this study were to characterize QTL for FHB resistance in Wangshuibai with molecular markers and identify markers closely linked to FHB resistance for marker-assisted selection.

MATERIALS AND METHODS

Plant materials - By single-seed-descent from the cross Wangshuibai/Alondra's' and Wangshuibai/Annong8455, 104 and 118 F7-derived RILs was developed, respectively.

Evaluation of FHB resistance - Wangshuibai/Alondra's' RILs were evaluated for type II FHB resistance with single floret inoculation method (Lu et al., 2000) in Nanjing and Wuhan in 2000. Percentage of scabbed spikelets was calculated as FHB severity at the 21st day after inoculation. The same materials were also evaluated in a greenhouse at Oklahoma State University, USA, in 2001 and 2002. Wangshuibai/Annong8455 RILs were evaluated for FHB resistance in Nanjing in 2003.

Molecular Marker Analysis - AFLP analysis was conducted according to Bai et al. (1999). Wheat SSR primers were synthesized according to the sequences described by Röder et al. (1998) and the United States Wheat and Barley Scab Initiative (website: www.scabusa.org).

Linkage analysis - An integrated SSR-AFLP map was constructed with JoinMap® 3.0 Program (van Ooijen & Voorips, 2001). Interval mapping analysis was carried out for further QTL analysis at LOD threshold of 2.5 with cofactor selection using MapQTL 4.0 (van Ooijen et al., 2002).

RESULTS AND DISCUSSION

FHB resistance - The correlation of FHB severity of RILs among experiments was not as high as we expected although appropriate moisture was provided for fungal initial infection. However, the frequency distributions of FHB severity for the population were similar among different years and locations.

Chromosomal location of QTL for FHB *resistance* -Interval mapping based on the data from USA2001 and USA2002 of Wangshuibai/Alondra's' RILs revealed one major QTL is on chromosome 3B located between Xbarc147 and Xgwm493 and explained 13.7% and 23.8% of the phenotypic variation, respectively (Table 1). The other QTL on chromosome 3BS was mapped between Xgwm285 and XEtcgMctc11 with LOD values of 1.69 and 3.43, respectively. Interval mapping based on the data from WH2000 also revealed a putative QTL for FHB resistance on chromosome 1BS (Table 1).

Interval mapping of Wangshuibai/Annong 8455 RILs indicated the major QTL was also mapped on chromosome 3B with LOD value of 5.57 explaining 21.9% of the phenotypic variation. Another QTL was mapped on chromosome 2A with LOD value of 2.5. It was located between Xgwm425-Xgwm372 and was contributed by Annong8455. The other two QTLs with minor effect in Wangshuibai/Alondra population were not detected. Comparison of QTLs in these two populations indicated that Wangshuibai possessed one major resistance QTL on chromosome 3BS in spite of the different genetic interval location. SSR markers Xbarc147 and Xgwm533.1 tightly associated with this QTL could be used for marker-assisted selection. QTLs with minor effect were different in these two populations because of the susceptible parents.

The major QTL in Sumai 3 was placed in the map interval Xgwm533.1 to Xgwm493 and was designated Qfhs.ndsu-3BS (Anderson et al., 2001). Shen et al. (2003) found that a major QTL in Ning 894037, another Chinese FHB resistance resource, was also in the same genomic region on 3BS. Results from the present study indicated that FHB resistance QTL with significant effect from Wangshuibai was located on chromosome 3BS flanked by markers Xbarc147 and Xgwm493. It is possible that a resistance gene cluster exists on chromosome 3B with different alleles at linked loci. It is also possible that these 3BS QTL from different sources are indeed the same QTL. The difference in QTL effects reported in the different resistant varieties and mapping studies may be due to the size of the mapping populations, the accuracy and methods of the resistance evaluations used in the different studies and the interaction of the 3BS QTL with different genetic backgrounds. Further research is needed to clarify the relationship between FHB-resistance QTL on 3BS mapped in different FHB resistant sources.

In this study, major FHB resistance QTL in the Chinese landrace Wangshuibai was identified, which would provide more FHB resistance resources to improve wheat resistance to FHB by using gene pyramiding. Marker-assisted selection (MAS) for FHB resistance genotypes can be performed with some of the closely linked markers, especially breeder-friendly SSR markers. Furthermore, markers associated with agronomic traits such as plant height and heading date can also be identified in the AFLP-SSR integrated map and can be used for MAS to break undesired association between FHB resistance and other agronomic traits.

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Table 1. QTL for FHB resistance by interval mapping (W: Wangshuibai, Al: Alondra's', An: Annong8455).

Population	Map interval	Location	Origin	Data set	LOD	$R^2 \times 100$
Wangshuibai/ Alondra's'	Xbarc147-Xgwm493	3B	W	USA2001	2.82	13.7
				USA2002	4.58	23.8
	Xgwm285-XEtcg.Mctc-11	3B	W	USA2001	1.69	7.1
				USA2002	3.43	15.7
	XEtcg.Magc-7-XEaccg.Mctc-7	1B	Al	WH2000	2.71	15.6
Wangshuibai/ Annong8455	Xgwm389-Xgwm533.1	3B	W	NJ2003	5.57	21.9
	Xgwm425-Xgwm372	2A	An	NJ2003	2.80	11.0

IDENTIFICATION OF PROTEINS INDUCED BY FUSARIUM HEAD BLIGHT INFECTION IN THE SPIKES OF HEXAPLOID WHEAT (*TRITICUM AESTIVUM*)

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ABSTRACT

Fusarium head blight (FHB), also called head scab, is a very destructive disease of wheat and barley. It causes economic losses due to reduction both in yield and quality. Although FHB resistance has been well documented and resistant cultivars have been developed to manage FHB, there is a limited understanding of the molecular mechanisms involved in plant resistance against the infection and spread of *Fusarium graminearum*. In the current study, two-dimensional displays of proteins extracted from spikelets infected with *F. graminearum* were compared with those from spikelets inoculated with sterile H_2O. Fifteen protein spots were detected which were induced (qualitatively different) or up-regulated (quantitatively increased) following *F. graminearum* infection of spikelets of Ning7840, a resistant cultivar. These proteins were further identified by LC-MS/MS analysis. Proteins with antioxidant function such as superoxide dismutase (SOD), dehydroascorbate reductase (DHAR), and glutathione *S*-transferases (GSTs) were up-regulated or induced 5 days after inoculation with *F. graminearum*, indicating an oxidative burst of H_2O_2 inside the tissues infected by FHB. An ascorbate-glutathione cycle is likely involved in reduction of H_2O_2. DHAR and TaGSTF5 (a glutathione *S*-transferase) responses to FHB infection differed between resistant and susceptible cultivars. A 14-3-3 protein homolog was also identified in FHB-infected spikelets. The function of 14-3-3 protein homologs during the interaction between plants and other pathogens indicates that they might be involved in the wheat defense response to FHB, and may be related to initial infection and mycotoxin accumulation in wheat grains. A PR-2 protein (b-1, 3 glucanase) was up-regulated in the FHB-infected spikes, which is in accord with a previous study using a cDNA approach.

GENETIC ENGINEERING

Chairperson: Kay Walker-Simmons

WHEAT TRANSFORMATION: A NEEDED TOOL FOR WHEAT GENETICS AND GERMPLASM IMPROVEMENT

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ABSTRACT

Fusarium head blight (FHB) is a major disease of wheat in the north central and eastern United States that affects both grain yield and end-use quality. It is generally agreed that the most cost effective way of preventing this disease would be through the release of FHB resistant cultivars. A major limitation to developing FHB resistant cultivars is the limited availability of resistant germplasm. Our project has attempted to increase genetic resources expressing FHB resistance through wheat transformation. Though microprojectile bombardment is commonly used, wheat transformation using *Agrobacterium tumefaciens* is preferred because a higher probability of simple, low copy integration events and ease in generating 'marker-free' transgenic lines by simultaneous delivery of two T-DNA elements. Currently we are using two strategies for increasing FHB resistance. The first strategy relies upon anti-fungal protein expression, such as bovine lactoferrin along with its derivative bovine lactoferricin, and the synthetic lytic peptide D4E1. The second strategy is to develop lines expressing inhibitors of programmed cell death (anti-apoptotic genes; Bcl-xL, Op-IAP, Sf-IAP, and ced9). FHB and its toxin, deoxynivalenol, have been suggested to regulate programmed cell death during pathogen infection. By transforming wheat with inhibitors of programmed cell death we hope to not only increase tolerance to FHB in wheat, but inhibit necrotrophic pathogen infection in general. The expression of inhibitors of programmed cell death may result in other agronomically beneficial traits as well. Preliminary results suggest improved tolerance to high levels of salinity and cold stress, as transgenic plants demonstrate reduced levels of DNA fragmentation and increased hardiness, respectively. We have also demonstrated the ability of deoxynivalenol to induce increased levels of programmed cell death via TUNEL staining of treated leaf sections. Finally, while our interest lays in the augmentation of FHB resistance present in wheat and its relatives, the importance of wheat transformation as a key genomics tools is clear. Transformation technology provides the ability to insert beneficial genes as well as silence existing genes in order to elucidate host-pathogen interactions during necrotrophic infection. For example, a newly discovered extremely efficient gene silencing system called DRIGS (direct repeat induced post transcriptional gene silencing) is currently being tested in wheat.

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MAPPING FHB RESISTANCE QTL IN A BARLEY POPULATION DERIVED FROM AN ATAHUALPA X M81 CROSS

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ABSTRACT

Previous barley mapping studies, using four sources of resistance (Chevron, Frederickson, Gobernadora and Zhedar), report four major *Fusarium* head blight (FHB) quantitative trait loci (QTLs): one on chromosome 2 near the centromere, one on chromosome 2 near the *Vrs1* locus, one on chromosome 2 near the southern telomere and one on chromosome 6. The first objective of this study was to identify QTL for resistance to FHB in an Atahualpa x M81 (AxM81) population in hopes that they will complement those previously identified. Atahualpa was chosen because it is genetically dissimilar to other sources of resistance in barley. The second objective was to investigate the effect of major spike morphology traits on the detection of FHB QTLs. To accomplish this, we created a selected subset of the AxM81 population that was fixed for two single gene traits that have a phenotypic association with FHB. The *nud1* locus, located on chromosome 1, determines covered v. hulless kernels. The *Vrs1* locus, located on chromosome 2, determines two-rowed v. six-rowed spike type. The random subset contains 102 individuals segregating at both the *nud1* and *Vrs1* loci. The selected subset contains 67 individuals fixed at both the *nud1* (for covered kernels) and *Vrs1* (for six-rowed spike type) loci. Phenotypic data for FHB severity was collected from four environments; deoxynivalenol (DON) accumulation data was collected from one environment. A simple sequence repeat (SSR) linkage map was constructed using JoinMap 3.0. The map currently covers approximately 60% of the barley genome. Composite interval mapping QTL analysis with PlabQTL 3.0 has located a major FHB QTL using the random population on chromosome 2 associated with the *Vrs1* locus at Crookston 2003 (LOD=13.9; R^2=46.9%), China 2003 (LOD=19.6; R^2=59.1%) and China 2004 (LOD=9.6; R^2=35.5%). A single DON QTL located in the random population was coincident with the major *Vrs1* QTL at Crookston 2002 (LOD=3.7; R^2=15.4%). One FHB QTL, identified in a single environment, was located in the random population on chromosome 2 associated with *GBM1062* (approximately 15 cM distal to *Vrs1*) at Crookston 2002 (LOD=3.3; R^2=14.1%). Three FHB QTLs, identified in single environments, were located using the selected population; two are located on chromosome 1 associated with *HvCMA* at China 2004 (LOD=3.2; R^2=20.2%), and *Bmag0321* at Crookston 2003 (LOD=3.1; R^2=19.5%), and one is located on chromosome 2 associated with *Bmac0093* at Crookston 2003 (LOD=4.0; R^2=23.9%). One DON QTL was located in the selected population on chromosome 1 associated with *Bmag0321* at Crookston 2002 (LOD=3.7; R^2=22.9%). Preliminary results indicate several QTL for FHB resistance were identified using the selected subsets that were not identified using the random subset.

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ROLE OF DIOXYGENASES IN FUNGAL SPORULATION

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ABSTRACT

Oxylipins comprise a family of structurally related oxygenated long chain fatty acid-derived molecules that exhibit crucial biological activities as signals of intra- and inter-cellular communication in mammals, plants and fungi. Oxylipin production is ubiquitous among pathogenic and saprophytic fungi and appears to play a role in life cycle control particularly in sexual and asexual development. For instance, in various members of Mucorales, immunofluorescence microscopy showed that 3-OH oxylipins are associated with asexual reproductive structures (e.g. sporangium, columella and aggregating sporangiospores), and in the yeast *Dipodascopsis uninucleata* with the sexual reproductive phase of the life cycle (e.g. gametangia, asci and matrix of released aggregating ascospores). We have recently identified three fatty acid oxygenases (PpoA, PpoB and PpoC) in the model fungus *Aspergillus nidulans*. Deletion of the encoding genes were correlated with changes in the asexual to sexual spore development, alterations in mycotoxin biosynthesis and decreased virulence as measured by spore reduction on host seed. Phylogenetic analyses showed that *ppo* genes are present in both saprophytic and pathogenic Ascomycetes and Basidiomycetes, suggesting a conserved role for Ppo enzymes in the life cycle of fungi. We have identified four putative Ppo proteins in *Fusarium graminearum* and will describe strategies in inactivating these genes.

SATURATION MAPPING OF THE FHB RESISTANCE QTL *QFHS-NDSU-3A* IN TETRAPLOID WHEAT

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ABSTRACT

Fusarium head blight (FHB), a destructive disease of wheat, has posed a significant threat to wheat production, processing, and consumption. Sources of effective resistance to FHB have not been found in durum wheat (*Triticum turgidum* var. *durum* L., 2n=4x=28, AABB). A major FHB resistance quantitative trait locus (QTL) *Qfhs.ndsu-3AS* was identified from a wild tetraploid wheat accession (*T. dococcoides* L., 2n=4x=28, AABB) and mapped within a 29.3 cM interval on chromosome 3A. A mapping population of 83 recombinant inbred chromosome lines (RICLs) derived from a cross between the *T. turgidum* var. *durum* cv. Langdon (LDN)-*T. dicoccoides* substitution line 3A and LDN has been used for saturation mapping of this QTL region in the present study. To date, we have assigned 30 new molecular markers to the QTL region, which extended the map distance from 155.2 cM to 248.4 cM. These markers, including SSR, STS, TRAP (target region amplification polymorphism), SSCP (single-strand conformation polymorphism), and CAPS (cleaved amplified polymorphic sequence), were generated from the ESTs mapped within the deletion bin 3AS-4 where the microsatellite marker closely linked with the peak of the QTL, *Xgwm2*, was assigned. We have identified new markers flanking the QTL and placed the QTL within a 9.4 cM chromosomal interval that is over three times smaller than the previous interval (29.3 cM). Thermal asymmetric interlaced PCR (TAIL-PCR) has been employed to extend DNA sequences surrounding the loci of interest. Single or low-copy TAIL-PCR products have been used to screen BAC libraries of LDN and *T. tauschii* (2n=2x=14, DD) and generate more markers to saturate this QTL region. A large F_2 population (over 1,000 individuals) was developed from a cross between LDN and a RICL with a smaller *T. dicoccoides* chromosomal fragment containing *Qfhs.ndsu-3AS*. This population has been used to generate more recombinants for fine mapping of the QTL region. F_3 offspring of the heterozygous recombinant F_2 individuals were produced to generate homozygous recombinants for FHB evaluation. Comparative mapping suggested that the FHB resistance QTL *Qfhs.ndsu-3AS* and *Qfhs.ndsu-3BS* localized on the short arm of chromosome 3A and 3B respectively, are not homoeoloci.

TISSUE SPECIFIC EXPRESSION OF A CHITINASE GENE FROM *TRICHODERMA ATROVIRIDE* CONFERS *FUSARIUM* RESISTANCE TO GM-BARLEY

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ABSTRACT

Since many *Fusarium*-mycotoxins are heat stable, these compounds cannot be removed through the chain of food processing, and once present in the grains at harvest, they will also be present in the final product. The reduction of the original infection of *Fusarium* will thus be the only way to reduce the amount of *Fusarium*-produced mycotoxins in the final food products. Cereal genes conferring resistance to *Fusarium* infection have not yet been identified, but in some wheat cultivars, resistance loci have been mapped. *Trichoderma* genes encoding chitindegrading enzymes have been introduced into several plant species and have been shown to increase the plants' resistance against fungal pathogens. At the Norwegian Crop Research Institute we have produced GM-barley where a fungal endochitinase gene, *ech42* from *T. atroviride* regulated by the barley promoter *Ltp2*, has been inserted resulting in increased resistance towards *Fusarium* infection of the seeds. The advantage of the *Ltp*2 promoter is that it permits a gene to be expressed only in the aleurone layer of developing seeds, corresponding to the point of time when *Fusarium* infects the spikes of barley. One of the resulting transformed plant lines, PL9, seemed to be especially promising. The copy number was estimated by the real-time PCR method to be low. Study on the inheritance of the transgenes in T_1 progeny revealed a 3:1 segregation. The expression of the chitinase gene, *ech42,* was studied in the T_1 generation using quantitative real-time RT-PCR assay. Some T_1 progenies showed very high *ech42* expression while others had either very low or no detectable expression. After inoculation with *Fusarium culmorum,* all *ech42* containing T_1 progenies coming from PL9 showed high resistance. The amount of *F. culmorum* present after point inoculation of the spikes was quantified by real-time PCR analysis. Extremely low amounts or no *F. culmorum* could be detected in seeds located at the same spike close to the point inoculated grains compared to the huge amounts found in wild type control plants. Further studies will be performed on plants from the T_2 generation currently grown in the greenhouse.

A TRUNCATED FORM OF RIBOSOMAL PROTEIN L3 ELIMINATES RIBOSOME DEPURINATION AND CELL DEATH CAUSED BY POKEWEED ANTIVIRAL PROTEIN AND CONFERS RESISTANCE TO TRICHOTHECENE MYCOTOXINS

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ABSTRACT

The contamination of important agricultural products, such as wheat, barley or maize with the trichothecene mycotoxin, deoxynivalenol (DON) due to infection with *Fusarium graminearum* or *Fusarium culmorum* is a worldwide problem. Trichothecenes inhibit translation by targeting ribosomal protein L3. We have previously shown that pokeweed antiviral protein (PAP), a single chain ribosome inactivating protein, depurinates ribosomes by binding to L3. Co-expression of a truncated form of yeast L3 (L3D), which contains only the first 100 amino acids, together with wild type PAP in transgenic tobacco plants led to a dramatic increase in PAP mRNA and protein expression. Unlike plants expressing PAP alone, transgenic plants expressing wild type PAP and yeast L3D were phenotypically normal. Ribosomes from these plants were not depurinated, even though high levels of PAP was associated with ribosomes. Expression of the endogenous tobacco ribosomal protein L3 was upregulated in transgenic lines containing L3D and PAP. Transgenic lines that showed high level of PAP and L3 protein expression were resistant to the *Fusarium* mycotoxins, DON and 4,15-diacetoxyscirpenol (DAS). These results demonstrate that co-expression of yeast L3D and PAP eliminates ribosome depurination, mRNA destabilization and cell death caused by PAP and increases endogenous L3 expression. High levels of PAP and L3 expressed in these plants confer resistance to trichothecene mycotoxins.

OVEREXPRESSION OF ANTIFUNGAL PROTEINS INCREASES THE RESISTANCE OF WHEAT TO FUSARIUM HEAD BLIGHT

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ABSTRACT

We are developing and testing transgenic wheat for resistance to Fusarium Head Blight (FHB). Anti-fungal proteins (AFPs) such as ß-1,3-glucanases, thionins, chitinases, thaumatin-like proteins (tlps) and ribosome-inactivating proteins (RIPs) are thought to inhibit fungal growth via different mechanisms. Chitinases and ß-1,3-glucanases degrade fungal cell walls, tlps and thionins degrade fungal cell membranes and RIPs inhibit fungal protein synthesis. Transgenic wheat lines over-expressing these AFPs in the cultivar Bobwhite were generated using micro-projectile bombardment. In transgenic lines carrying a ß-1,3-glucanase, an α-purothionin and a tlp-1, our previous results showed statistically significant reductions in scab severity compared to the nontransgenic Bobwhite controls in the greenhouse. These lines were evaluated in field trials in the summer of 2004 and five lines exhibited statistically significant reductions in scab severity compared to nontransgenic Bobwhite controls. We also developed seventeen and eight lines carrying a barley chitinase and barley RIP, respectively. In addition, we developed four, eleven and six lines expressing a combination of chitinase/RIP, chitinase/tlp-1 and RIP/tlp-1, respectively. These combinations each employ two of the three different mechanisms of fungal growth inhibition. We screened these lines for FHB resistance in the greenhouse three to four times. Eight chitinase, one RIP, three chitinase/tlp-1, one chitinase/RIP and three RIP/tlp-1 lines consistently show enhanced resistance towards FHB when compared to Bobwhite, the untransformed control. Western blot analyses of these lines are discussed.

EXPRESSION OF ARABIDOPSIS NPR1 IN WHEAT CONFERS RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Plant productivity and quality is severely limited by Fusarium Head Blight (FHB) or scab, which has re-emerged as a devastating disease of wheat and barley. Breeding has been at the forefront in developing wheat with improved FHB resistance. Biotechnology provides an alternative approach for augmenting resistance to FHB. The *NPR1* gene is a key regulator of basal and inducible defense responses in Arabidopsis to several pathogens. Moreover, over expression of NPR1 in Arabidopsis and rice was found to confer resistance to biotrophic pathogens. However, the impact of NPR1 on resistance to necrotrophs remains to be determined. We provide evidence that expression of NPR1 confers resistance to FHB in wheat. The Arabidopsis NPR1 gene was expressed in wheat plants from the ubiquitously expressed maize *Ubi1* promoter. A strong type II resistance to FHB was observed in two independently derived *Ubi1::AtNPR1* transgenic lines. FHB resistance was inherited as a dominant trait; significant correlation was observed between the FHB resistance phenotype and the expression of the *Ubi1*::*AtNPR1* transgene. Expression of the transgene and FHB resistance was stably maintained in the T_2 and T_3 generations. Comparisons of grain yield between a transgenic line and the non-transgenic control plant revealed no detrimental effects of the *Ubi1::AtNPR1* transgene expression on grain yield in healthy green house grown plants. These two promising lines are being readied for field studies on yield, the durability of FHB resistance and broad-spectrum resistance to other pathogens.

Previously, we had cloned a partial cDNA for a wheat homolog (*WhNPR1*) of the Arabidopsis and rice *NPR1* genes from a rust-infected Lr21 wheat cDNA library. The predicted WhNPR1 protein exhibits 80% similarity to the Rice NPR1 protein. We have used RACE to clone the 5‘end of the *WhNPR1* gene. Using this RACE product, we are currently reconstructing the full-length *WhNPR1* cDNA. Since NPR1 function in plant defense requires its interaction with other proteins, we hypothesize that increased expression of WhNPR1 will be more effective than the Arabidopsis NPR1 in conferring durable resistance to FHB in wheat. To test the hypothesis, we will generate transgenic plants that overexpress WhNPR1.

MICROARRAY ANALYSIS OF BARLEY INFECTED WITH *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a serious problem for barley and wheat cultivation. The objectives of this study were to identify barley defense response mechanisms operating against *F. graminearum*. The Barley1 Affymetrix GeneChip provides a means for evaluating the differential transcript accumulation in barley from large sets of genes under defined conditions. We used the Barley1 GeneChip to study the differential transcript accumulation from barley genes in spikes challenged with *F. graminierum* and mock inoculation water controls. We also examined *F. graminearum* infection structures and deoxynivalenol (DON) accumulation. Four replicate experiments were conducted at five different time points, 24h, 48h, 72h, 96h and 144h. Two classes of genes were identified namely, quantitatively expressing and qualitatively expressing genes. Genes exhibiting quantitative differences in transcript accumulation were defined as those transcripts that accumulated in the *Fusarium*-treated spikes at a statistically significant higher level than the water controls. A total of 186 such genes were found. Genes exhibiting qualitative differences in transcript accumulation are those that are exclusively found in the water controls or the *Fusarium* treated samples. A total of 389 such genes were found. Twenty genes were randomly selected and validated on the northern blots, indicating the GeneChip data are robust. Based on the defined patterns of differential transcript accumulation, histology and DON accumulation, we classified the disease progression into four broad classifications: preinfection (24h), early (48h), middle (72h and 96h) and late phase (144h). Functional classification of the identified genes was done based on annotation, number and mean expression values and attempts were made to tag the biological significance to these groups. One major pathway showing significant induction during *Fusarium* infection was found to be tryptophan metabolism. Our results show the power of the Barley1 GeneChip to identify patterns and pathways of genes expression during *F. graminearum* infection.

HIGH RESOLUTION MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE AND HEADING DATE QTL ON CHROMOSOME 2H OF BARLEY

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OBJECTIVES

To identify precise locations of Fusarium head blight resistance and heading date QTL on chromosome 2H and determine if the association between the two traits is due to linkage or pleiotropy.

INTRODUCTION

A major QTL for Fusarium head blight (FHB) resistance, discovered in the Chevron x M69 mapping population was located in a 45 centimorgan (cM) genomic region of chromosome 2 (2H) (de la Pena et al., 1999). The resistant allele at this QTL was also associated with late heading. A follow-up validation study comparing the Chevron x M69 mapping population with two other Chevron derived populations confirmed the coincidence of HD and FHB QTL in this region (Canci et al., 2004). Subsequently, a marker-assisted selection (MAS) study using markers carrying the Chevron allele at the chromosome 2H QTL region resulted in a 43% reduction in FHB severity and a 2-day increase in heading date (HD) (Gustus et al., 2001). In a more recent study, six backcross (BC3) near-isogenic lines carrying the chevron alleles for markers in the chromosome 2 FHB QTL region were found to reduce FHB by 44% in St. Paul and 41% in Crookston (Nduulu et al., 2002). This same QTL region also increased HD by six days; thus creating uncertainty as to whether the association is due to linkage or pleiotropy.

In this current study, we report the construction of a fine map for the chromosome 2H target QTL region using an F2 population derived from a cross between a BC5 line carrying the Chevron alleles in the QTL region and the recurrent parent M69. Because these backcross-derived F2 lines are isogenic for the entire genome and only segregate at the target QTL region, evaluating the recombinant lines allows us to more precisely estimate QTL positions for FHB and HD.

MATERIALS AND METHODS

Development of the parental near isogenic line (pNIL): To develop the parental NIL, a progeny from the 101 F4:7 mapping population (de La Pena et al., 1999) was crossed with an elite line M69. Subsequently, a marker-assisted backcrossing scheme using M69 as the recurrent parent was used to advance lines to the BC4F2 generation. A BC4F2 line carrying the FHB-resistance Chevron alleles at the target QTL region was selected as the pNIL.

Development of recombinant NILs (rNILs): We derived an F2 population of 532 plants from a cross between the pNIL and M69. The F2s were screened with SSR markers *Bmag0140* and *Ebmac0521* flanking the target QTL region and 40 rNILs were identified that had a recombination event between the flanking markers. These 40 putative recombinants were further screened with 11 additional SSR markers previously mapped at the *Bmag0140-EBmac0521* interval. Using marker data from the entire F2 population, a linkage map for the target QTL region was created using the GMendel 3.0 program (Holloway and Knapp, 1994). The 40 rNILs were further advanced to the F2:4 generation and used for field testing.

Field evaluations of rNILs: The rNILs and the parental lines Chevron, M69, and pNIL were evaluated at St. Paul and Crookston, MN, in the summer of 2003 and 2004. The experimental design at each environment was a randomized complete block design with 3 replications. Entries were planted in 2.4 m

long single-row plots, spaced at 30 cm apart. At St. Paul, a macroconidia inoculation technique was used whereas at Crookston a grain-spawn inoculation technique was used (Mesfin et al., 2003). Nurseries were mist-irrigated daily to enhance disease. Entries were scored for % FHB severity by examining 20 random spikes from each plot and the number of infected spikelets from each spike counted and expressed as a percent of the total spikelets present. Heading date was scored as the number of days after planting to 50% emergence from the boot.

Statistical Analysis: The genotype x environment interaction effects were determined using Proc GLM (SAS Institute, 2000). The analysis revealed a significant G x E effect for both HD and FHB among rNILs. Further analyses were, therefore, performed on a per environment basis. Trait means for parental lines and rNILs were compared using protected LSD. The association between specific markers in the target QTL region and measured traits was determined by simple interval mapping (SIM) using PlabQTL (Utz and Melchinger, 1996).

RESULTS AND DISCUSSION

The rNILs carrying the Chevron allele at different segments at the chromosome 2H QTL region differed significantly for FHB and HD, indicating that the QTL for HD and FHB resistance were segregating amongst the rNILs. The pNIL used to develop the fine mapping population did not differ from the Chevron for FHB severity, but was slightly earlier than Chevron for HD (Table 1). This suggests the QTL for FHB on chromosome 2H is responsible for most of the resistance from Chevron. However, additional alleles at loci for HD outside the chromosome 2H target region likely contribute to the late heading of Chevron.

A total of 13 SSR markers, 9 from the linkage map of Canci et al., (2004), and four additional markers previously mapped in the same region by Ramsay et al., (2000) were genotyped for fine mapping (Fig. 1). Of these, *EBmac0849* mapped in the same location as *Bmac0093* and was subsequently dropped from the analysis. For the most part, the marker order was consistent with the original map. The total distance covered by the new map is 17.4 cM compared with the 44.9 cM distance covered by the updated Chevron x M69 map of Canci et al., 2004.

A major QTL for HD was detected between markers *HVBKASI* and *Bmag0015* (1 cM apart) at all the four environments tested and explained 40-80% of the phenotypic variation (Fig. 1; Table 2). A separate QTL for FHB flanked by markers *Bmag0140* and *Bmac0132* (3.5 cM apart) was detected 2 cM away from the HD QTL. This FHB QTL was detected in 2 of the 4 environments tested and explained 40-50% of the variance. Failure to detect FHB QTL in all environments was most likely due to poor disease levels experienced in the environments where QTL were not detected (Table 1). In conclusion, these data indicate that the association between FHB and HD is due to linkage (2 cM apart) rather than pleiotropy.

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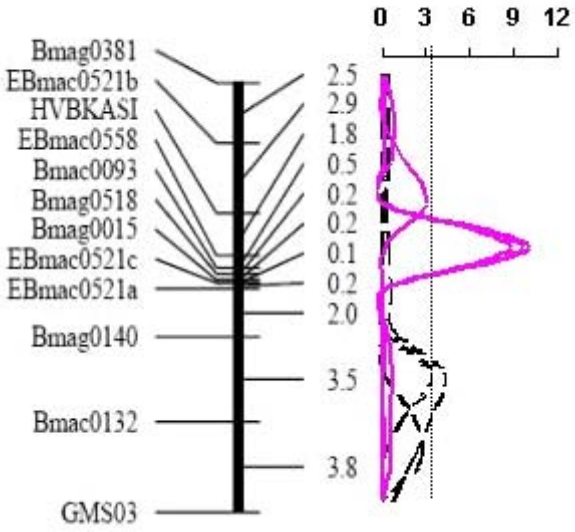

Figure 1. SSR fine map of the chromosome 2H FHB target QTL region and LOD score scan for the QTL associated with Fusarium head blight (FHB) severity and heading date (HD). Scans are shown for St. Paul 2003 (Sp03), Crookston 2003 (Cr03), St. Paul 2004 (Sp04) and Crookston 2004 (Cr04). Dark scan line is for FHB and dotted scan line is for HD.

Table 1. Means of the parents (pNIL, Chevron and M69) and recombinant near isogenic lines (rNILs) for percent Fusarium head blight severity (FHB) and heading date (HD).

Trait	Environment	pNIL	Chevron	M69	rNIL	
					Mean	Range
FHB	Sp2003	1.8a	1.3a	3.7b	2.4	1.0-4.4
	Cr2003	2.3a	3.8a	7.2b	5.5	0.7-13.1
	Sp2004	11.3a	8.5a	12.3a	9.4	1.9-20.6
	Cr2004	6.6a	1.6a	21.5b	17.6	4.6-59.7
HD	Sp2003	58.5b	60.0c	55.0a	58.0	54.0-61.0
	Cr2003	58.5b	61.0c	56.5a	58.1	54.0-61.0
	Sp2004	55.0b	55.7b	49.3a	56.7	48.3-57.0
	Cr2004	66.7b	68.7c	64.3a	66.0	62.3-68.7

Means within the same row followed by the same letter are not significantly different (P?0.05). Sp2003 = St. Paul, MN, 2003; Cr2003 = Crookston, MN, 2003; Sp2004 = St. Paul, MN, 2004; Cr2004 = Crookston, MN, 2004.

Table 2. Significant QTL (LOD>3.0) associated with Fusarium head blight (FHB) severity and heading date (HD) at four environments in the Chevron/M69 fine mapping population of recombinant near isogenic lines.

Trait/ Pos[1]	Marker interval	St. Paul 2003			Crookston 2003			St. Paul 2004			Crookston 2004		
		LOD	%Exp[2]	Add[3]	LOD	%Exp	Add	LOD	%Exp	Add	LOD	%Exp	Add
FHB													
11,13	Bmag0140-Bmac132				3.43	43.2	-2.82				4.21	50.0	-14.37
HD													
6	HVBKASI-EBmac0558										3.05	44.3	1.63
7	Bmag0518-Bmag0015	8.74	76.3	2.59	10.02	80.8	2.75	9.62	79.5	3.47			

[1]Pos = centimorgan position.
[2]% Exp = Percent phenotypic variance explained by QTL.
[3]Add = Additive effect of the Chevron allele on FHB severity and heading date expressed as regression coefficient.

A GFP REPORTER STRAIN FOR MONITORING TRI5 GENE ACTIVITY IN *FUSARIUM GRAMINAERUM*

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ABSTRACT

Fusarium graminearum is the most serious pathogen within the Fusarium Head Blight complex of fungal species. It produces the trichothecene mycotoxin Deoxynivalenol (DON) as a major virulence factor in the host-pathogen interaction. The TRI5 gene has a key role in the biosynthesis of trichothecene mycotoxins. We have developed a reporter system by transformation of *F. graminearum* TMW 4.0122 with the eGFP gene under control of the TRI5 promotor. A 926 bp fragment upstream of the TRI5 start codon containing the promotor region as well as a 342 bp portion upstream from the 3´end of the TRI5 coding region of a single spore isolate of *F. graminearum* TMW 4.0122 were cloned in *E. coli* DH5±. Fragments were excised and ligated via *Hin*dIII restriction sites newly introduced by modified primers. The ligation product was cloned into the pSM2 vector via *Pst*I and *Cla*I restriction sites to result in transformation vector pSM2GK1871, which was linearized by restriction of a singular *Hin*dIII site. Protoplasts of *F. graminearum* TMW 4.0122 were obtained by treatment of germinated conidia with driselase (Interspex) at 30 °C for 3 h. Protoplasts were separated and transformed with linearized pSM2GK1871. Selection on hygromycin B agar (150 µg/ml) revealed 88 transformants. Clones were subcultured on GYEP agar plates and inspected for expression of eGFP under the fluorescence microscope (Olympus). One clone (10/2/1) displayed intense green fluorescence emission at 510-550 nm upon excitation at 470-490 nm after 15 d of incubation at 25 °C. No such fluorescence was seen in the wild type strain grown under the same conditions. Fluorescence in the transformant was limited to a specific type of cells, which showed a characteristic yellow pigmentation when inspected under the light microscope. Such cells were also present in the wild type mycelium, with no green fluorescence emitted upon excitation at 470-490 nm. We are currently investigating whether trichothecene production in *F. graminearum* TMW 4.0122 might be restricted to specialized cells ("toxocytes"). Based on the results obtained during the current study we are using the TRI5 reporter strain to investigate the role of that gene in the barley/wheat-*F. graminearum* interaction and to learn more about the factors affecting and regulating production of DON under the conditions prevailing in the field and at processing of cereals, e.g. during malt production.

GLUCOSYLTRANSFERASES FROM *ARABIDOPSIS THALIANA* INACTIVATING THE *FUSARIUM* TOXINS DEOXYNIVALENOL AND ZEARALENONE

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ABSTRACT

During the infection of small grain cereals and maize *Fusarium graminearum* produces the mycotoxins deoxynivalenol (DON) and zearalenone (ZON). It has been demonstrated that the production of the trichothecene DON, which acts as an inhibitor of eukaryotic protein synthesis, contributes to the virulence of *Fusarium* (presumably by interfering with the expression of plant defense genes). ZON, which has very high estrogenic activity in animals, also seems to play a role in plant-pathogen interaction. We have searched for *Arabidopsis* genes which can inactivate these *Fusarium* toxins. A yeast strain highly sensitive to DON was used as a host for an *Arabidopsis thaliana* expression library and a UDP-glucosyltransferase (UGT) gene conferring resistance to DON (*DOGT1*) was identified (Poppenberger *et al.*, 2003). Overexpression of the *DOGT1* gene in *Arabidopsis* led to increased DON resistance of seedlings. The metabolite DON-3O-glucoside is inactive in inhibiting protein synthesis (tested with a wheat germ extract *in vitro* translation system). *DOGT1* is located in a cluster of 6 highly similar genes, but surprisingly the protein with the most closely related sequence is not protecting against DON. By making hybrid proteins and functional testing in yeast we characterized structural features essential for substrate specificity of these UGTs. Interestingly nivalenol, which has just one additional hydroxyl group, escapes detoxification.

We have also cloned an *Arabidopsis* UGT which converts ZON into ZON-4O-glucoside. While ZON shows strong binding to the estrogen receptor *in vitro*, this is not observed with ZON-glucoside. Expession of this UGT in an engineered yeast strain expressing the human estrogen receptor (hER), interferes with ZON-induced activation of hER-dependent reporter genes. This remarkable affinity can be exploited to produce ZON-glucoside in high yield by feeding ZON to the recombinant yeast.

We propose that the glucosides of DON and ZON produced by plants are a currently overlooked source of "masked mycotoxin". While the mycotoxin-glucosides escape standard analytical procedures, the toxic aglyca can be easily reactivated in the digestive tract.

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TRANSGENES IN WHEAT

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ABSTRACT

Our wheat transformation team at the University of Nebraska-Lincoln (UNL) employs an *Agrobacterium*-mediated protocol to deliver transgenes to the crop. We recently completed a survey of 30 spring wheat genotypes for enhanced transformation frequencies. From this work a hard white genotype Xin chun 9 was identified that displayed improved transformability over Bobwhite. Current efforts are focused on evaluating a series of novel *Agrobacterium tumefaciens* strains in a comparative study with both Bobwhite and Xin chun 9.

We have been using this transformation system to evaluate potential antifungal transgenes in support of a collaborative effort targeting Fusarium Head Blight (FHB) resistance at UNL. To this end a total of 48 transgenic wheat lines harboring three novel negative regulators of programmed cell death, or a ribosomal inactivating protein have been handed-off to our wheat breeding program. More recently transgenic wheat lines carrying two additional negative regulators of programmed cell death and a synthetic antifungal lytic peptide have been produced. Field trials were conducted in the spring of 2004 on 28 transgenic lines carry three negative regulators of programmed cell death genes, *ced9*, *IAP* and *Bcl-xl*, along with lines harboring the ribosomal inactivating protein. Field plots were inoculated with *F. garminearum* just prior to anthesis. Data has been ascertained on agronomic parameters, days to anthesis, vigor and plant height, in addition to FHB severity and incidence.

OPTIMIZATION OF AN *AGROBACTERIUM*-MEDIATED TRANSFORMATION SYSTEM FOR DURUM WHEAT

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OBJECTIVES

To optimize an *Agrobacterium*-mediated transformation system for durum wheat to facilitate incorporation of antifungal genes for resistance against Fusarium head blight.

INTRODUCTION

Durum wheat (*Triticum turgidum* L., $2n = 4x = 28$; AABB genomes) is an important cereal crop grown in the United States, Canada, and in some European countries. Several methods have been used for its genetic improvement. In recent years, genetic engineering has opened up new avenues for crop improvement and is a useful adjunct to conventional breeding. A prerequisite for application of such modern techniques is an efficient and reliable *in vitro* plant regeneration system. Gene transfers in plants have been achieved through direct DNA uptake, electroporation, microinjection, particle bombardment and *Agrobacterium*-mediated methods. We standardized an efficient regeneration system for commercial durum wheat cultivars (Bommineni and Jauhar, 1996; Satyavathi et al., 2004) and by using particle bombardment produced transgenic durum with marker genes (Bommineni et al., 1997) and antifungal genes (Satyavathi and Jauhar, 2003). Transgenic durum has now been produced in other laboratories (He et al., 1999; Pellegrineschi et al., 2002). In bread wheat, partial FHB resistance was achieved by expressing pathogenesis-related proteins using particle bombardment, but this technique was hampered by multiple copy gene insertions and gene silencing (Anand et al., 2003). Compared to direct gene transfer techniques, *Agrobacterium*-mediated transformation offers a number of advantages, including potentially low copy number and preferential integration into transcriptionally active regions of the chromosome (Hu et al., 2003). So far, an *Agrobacterium*-mediated transformation system has not been reported for durum wheat. Therefore, we attempted to optimize the conditions for *Agrobacterium* mediated transformation of the commercial durum wheat cv. Maier using marker genes.

MATERIALS AND METHODS

Plant material and preculture - An agronomically superior durum cultivar, Maier was used for transformation. Spikes were harvested 14 days post anthesis and the spikelets were surface sterilized and cultured as described by Bommineni and Jauhar (1996). The callus induction medium was supplemented with 2.0 mg L^{-1} dicamba. The cultures were incubated in the dark at 25 ± 2°C for 1-14 days depending on the experiment performed.

Agrobacterium tumefaciens strain, plasmid, and culture - A disarmed *Agrobacterium tumefaciens* strain AGL1 harboring pDM805 was provided by CSIRO Plant Industry, Canberra, Australia (Fig. 1). The binary vector pDM805 contains the phosphinothricin acetyltransferase (*bar*) gene under the control of the promoter from the maize ubiquitin 1 (*Ubi1*) gene and a terminator sequence from the *A. tumefaciens* nopaline synthase (*nos*) gene; the β-glucuronidase gene *uidA* (*gus*) under the control of the promoter from the rice actin 1 (*Act1*) gene and a terminator sequence from the rice ribulose bis-phosphate carboxylase/oxygenase (*rbcS*) gene. A full strength *Agrobacterium* suspension was obtained a day before transformation as described by Tingay et al. (1997).

Acetosyringone treatment and particle bombardment - Acetosyringone effects on trans-formation ef-

ficiency were studied by comparing *Agrobacterium* suspension without the chemical to suspension treated with 200 µM acetosyringone prior to infection. The explants were then infected for about 1 h. To study the effect of particle bombardment on the extent of infection, the scutella were precultured for 14 days and wounded by bombardment with gold particles. About 30-50 explants were bombarded with 0.3 mg of gold particles (1.0 µM) using a BioRad PDS-1000 biolistic device with 1,100 psi rupture disc and compared to unwounded explants.

Inoculation and co-cultivation - Isolated scutella precultured for 1 day and 14 days were used for transformation. The explants were immersed in full strength *Agrobacterium* suspension in a Petri dish for half an hour (1 h when acetosyringone was used in the suspension) and then transferred without rinsing, with scutellar surface placed in contact with the callus induction medium. Co-cultivation was carried out at 25 ± 2°C in darkness for about 2-3 days.

Selection and plant regeneration - After co-cultivation, the explants were washed thrice with sterile distilled water in a Petri dish and blotted on sterile Whatman filter paper. The explants were then plated on selection medium, which was same as callus induction medium but was supplemented with 150 mg L^{-1} Timentin and 5.0 mg L^{-1} bialaphos. Explants were maintained on selection medium for 3-4 weeks at 25 ± 2°C in darkness, after which they were transferred to regeneration medium (selection medium without growth regulators). The culture conditions and regeneration procedure were same as described by Satyavathi et al. (2004).

Histochemical GUS assay - T-DNA delivery into explant tissues was determined after 1-3 weeks of culture on selection medium using the histochemical GUS assay according to Bommineni et al. (1997). Explants with blue spots and the number of blue spots per explant were counted under a stereomicroscope.

Statistical analyses - For studying the effect of acetosyringone and bombardment on DNA delivery, we compared GUS expression among the explants. Each treatment had three replications and at least 50 explants (4 from each Petri dish) per treatment were sacrificed for GUS assays. As the data on the number of explants with GUS spots and the number of spots per explant for each treatment were not normally distributed, analysis of variance was done using PROC CATMOD (SAS version 8.2, 2001).

RESULTS

Preliminary tests were performed to compare the responses of the scutella that were precultured for 1 day vs 10-14 days. After 1-2 days of co-cultivation with *Agrobacterium* and on transfer to selection medium, only 10% of the scutella precultured for 1-day initiated calli. In the case of 10-14 day precultured scutella, about 70% of them developed callus over the cut surface within a week and later developed callus around the periphery of the scutellum. After 3 weeks on selection medium, 511 of 725 scutella co-cultivated were resistant to bialaphos selection. GUS assays done 7 days after co-cultivation showed GUS spots all over the scutellum surface but most of the spots were localized on the areas starting to form callus, usually at the periphery of the scutellum (Fig. 2A & B). In subsequent subcultures, the proliferating callus was selected and brown callus was discarded. After 4 weeks on selection medium, the embryogenic callus was transferred to regeneration medium supplemented with 5.0 mg L^{-1} bialaphos. Out of 725 scutella infected, only 3 plantlets were regenerated at the end of 3-4 weeks with a transformation frequency of 0.4%. Various treatments like increasing co-culture duration, adding acetosyringone in the *Agrobacterium* suspension, and bombarding the explants with gold particles before infection, had differential effects on transformation efficiency as follows:

Duration of co-culture - About 82% of the scutella that were co-cultivated for 2 days were resistant to bialaphos selection. When the co-cultivation was extended to 3 days, an overgrowth of the bacteria was observed on 50% of the scutella and the percentage of resistant scutella decreased to 56%.

Effect of acetosyringone - GUS expression was detected in all the tissues 3 wk after co-cultivation either in the presence or absence of the pretreatment.

The scutella that were treated with acetosyringone did not differ for GUS expression in terms of the number of explants with GUS spots and the number of GUS spots per explant compared to those infected with *Agrobacterium* suspension without acetosyringone (Table 1).

Effect of bombardment - The explants that were injured by bombarding with gold particles showed significantly greater number of explants with GUS spots ($p < 0.05$) and also significantly greater GUS spots per explant ($p < 0.01$) than those that were not wounded prior to infection (Table 1).

DISCUSSION

This work presents the first report on *Agrobacterium*-mediated transformation of durum wheat. A prerequisite for development of *Agrobacterium*-mediated transformation is the establishment of optimal conditions for T-DNA delivery into tissue from which whole plants can be regenerated. Based on our previous findings, we selected durum cultivar Maier for transformation with *Agrobacterium*. In general, model genotypes amenable to tissue culture or to microprojectile transformation have worked well for *Agrobacterium*-mediated transformation in several crops like wheat, maize, barley, and sugar cane (Cheng et al., 2004). The isolated scutella are known to be choice explants for many cereals including durum wheat and have been successfully used for regeneration and transformation experiments. In the present study, we found that 10-14 days preculture of explants increases transformation efficiency. Similar results were observed in wheat where longer precultures resulted in efficient T-DNA delivery (Cheng et al., 1997; Wu et al., 2003). We obtained a transformation frequency of 0.4% which is comparable to that reported in other cereals. In wheat, transformation frequencies ranged from 0.3-4.3% and were increased to 10.5% by desiccation of precultured embryos (Cheng et al., 2004).

Chemicals such as acetosyringone for *vir* gene induction are recommended in most of the monocot transformation protocols. We used a 200 µM acetosyringone treatment prior to infection. The presence of acetosyringone did not increase GUS expression. The addition of acetosyringone at a concentration of 150 to 200 µM during preculture or co-culture increased the number of transformed cells in rice (Hiei et al., 1994), barley (Tingay et al., 1997), and wheat (Cheng et al., 1997). In the present study, wounding precultured scutella with gold particles before infecting the explants with *Agrobacterium*, increased the GUS expression significantly. Similar results were observed in barley (Tingay et al., 1997).

The advantage of *Agrobacterium*-mediated transformation over particle bombardment is that this method is simple and cost effective. We optimized conditions for *Agrobacterium*-mediated transformation using scutella of the cultivar Maier and also studied the effects of various pretreatments that could enhance infection and T-DNA delivery. Further experiments are needed to increase regeneration from transformed callus.

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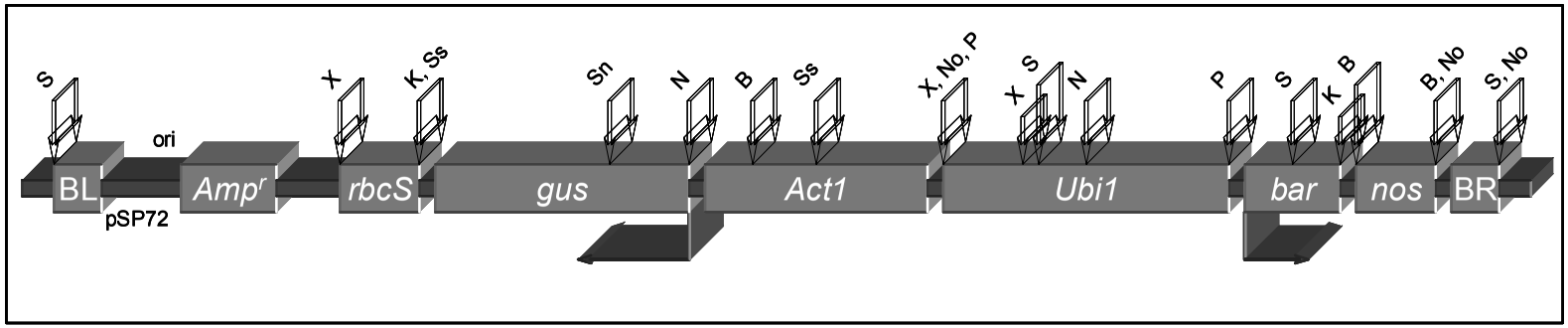

Figure 1. Structure and restriction map of the cereal transformation vector pDM805 (Tingay et al., 1997).

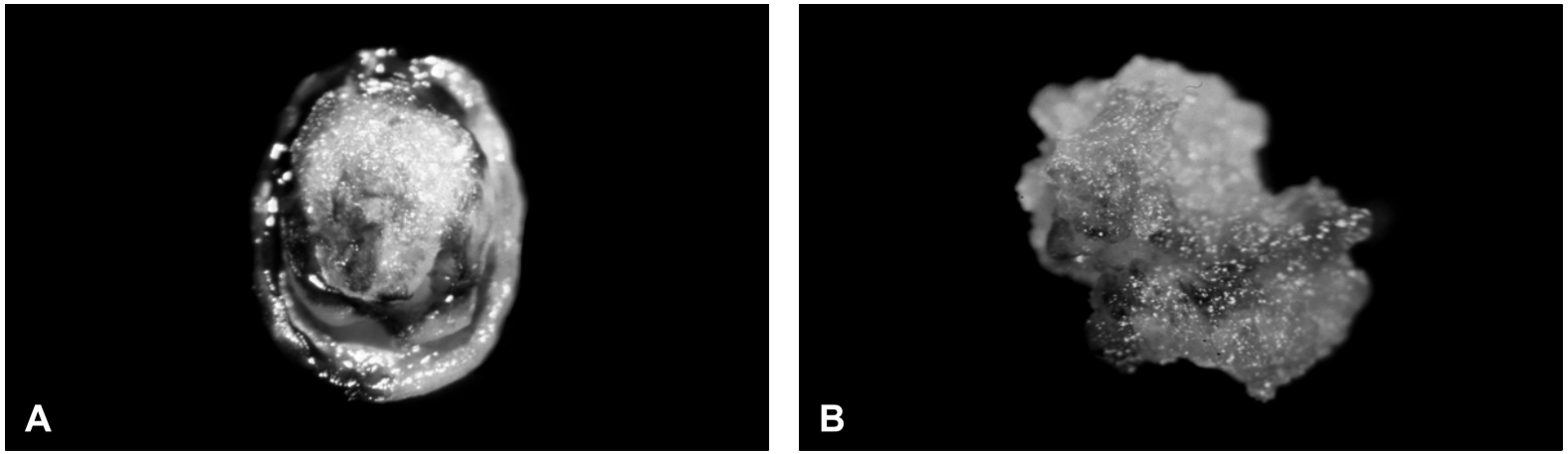

Figure 2. GUS expression in the scutellum one week after co-cultivation with *Agrobacterium.* GUS expression in callus that was resistant to 5.0 mg L-1 bialaphos three weeks after co-cultivation with *Agrobacterium*.

Table 1. Effect of different pretreatments on GUS expression in 14-day precultured durum wheat scutella infected with *Agrobacterium tumefaciens* strain AGL1 harboring pDM805.

Treatment	Number of explants	Proportion of explants with GUS spots (%)	GUS spots /explant
Acetosyringone			
Untreated	52	26/52 (50.00)	3.35
Treated	52	34/52 (65.38)	4.20
Bombardment			
Untreated	72	17/72 (23.61)	1.47
Bombarded	72	31/72 (43.05)*	2.54 **

* Chi-square value of 0.0194 was significant at p = 0.05 level
** Chi-square value of 0.0037 was significant at p = 0.01 level

DEVELOPMENT OF TISSUE-SPECIFIC PROMOTERS FOR TARGETING ANTI-FUSARIUM GENE EXPRESSION

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ABSTRACT

We identified lemma and pericarp epithelium tissues as rapidly infected by *Fusarium graminearum*. Genes specifically expressed in these tissues were identified and cloned so that promoters of selected genes could be used to express antifungal protein genes. These included a lipid transfer protein homologue (*Ltp6*), highly expressed in the pericarp epithelium but not in vegetative leaves, and a jacaline-like gene, *Lem2*, preferentially expressed in the lemma/palea, compared with the flag leaf. *Ltp6* is also expressed in coleoptiles and embryos; mRNA levels increase in response to salt, cold, abscisic acid and salicylic acid in a pattern distinct from other barley *Ltp*s. Transient expression analysis of the promoter showed that 192 bp of upstream sequence confers tissue-specific expression and retains most promoter activity. Stable barley transformants have been produced with a 247 bp promoter fused to a *gfp* reporter gene. In these, *gfp* expression is strong in the epicarp, embryo and coleoptile, but it is not found in other tissues. *Gfp* expression was detected during spike development, from early ovary differentiation through its final expression in the epicarp and during embyogenesis and germination in the coleoptile, reproducing the expression pattern of the native gene. *Lem2* is specifically expressed in the lemma/palea and coleoptile. SA induces *Lem2* within 4 h, suggesting that it is a defensive gene. Promoter deletion studies showed that the tissue-specificity and promoter activity are conferred by a short 5' proximal region from -75 to +70. Stable transformants were produced with the "full-length" 1414 bp promoter and 5' promoter deletions fused to *gfp*. G*fp* expression occurred in the lemma/palea and coleoptile, but it also unexpectedly occurred in the epicarp and ligules. Lack of methylation in the epicarp may account for expression in the epicarp.

TARGETING OF ANTI-FUSARIUM GENE EXPRESSION IN BARLEY

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ABSTRACT

We identified lemma and pericarp epithelium (epicarp) tissues as rapidly infected by a strain of *Fusarium graminearum* transformed with the green fluorescent protein gene (*gfp*). The fungus colonized the lemma in 48 h, but it colonized the brush hairs at the seed tip within 7 h and rapidly grew downward along the epicarp and more slowly inward through the cross cells (Skadsen and Hohn, PMPP 64:45-53, 2004). Genes specifically expressed in the lemma (Abebe et al., Crop Sci. 44:942-950, 2004) and epicarp were identified and cloned so that promoters of selected genes could be used to express antifungal protein genes in these susceptible tissues. Tissue-specific genes included a lipid transfer protein homologue (*Ltp6*), highly expressed in the pericarp epithelium but not in vegetative leaves, and a jacaline-like gene, *Lem2*, preferentially expressed in the lemma/palea, compared with the flag leaf. *Ltp6* is also expressed in coleoptiles and embryos; mRNA levels increase in response to salt, cold, abscisic acid and salicylic acid (SA) in a pattern distinct from other barley *Ltp*s. Transient expression analysis of the promoter showed that 192 bp of upstream sequence confers tissue-specific expression and retains most promoter activity. Stable barley transformants have been produced with a 247 bp promoter fused to a *gfp* reporter gene (Federico et al., PMB, in press). In these, *gfp* expression is strong in the epicarp, embryo and coleoptile, but it is not found in other tissues. *Gfp* expression was detected during spike development, from early ovary differentiation through its final expression in the epicarp, and during embyogenesis and germination in the coleoptile, reproducing the expression pattern of the native gene. *Lem2* is specifically expressed in the lemma/palea and coleoptile. SA induces *Lem2* within 4 h, suggesting that it is a defensive gene. Deletion studies showed that tissue-specificity is conferred by a short 5' proximal region from -75 to +70 (Abebe *et al*., Planta, in press) . Stable transformants were produced with the "full-length" 1414 bp promoter and 5' promoter deletions fused to *gfp*. G*fp* expression occurred in the lemma/palea and coleoptile, but it also unexpectedly occurred in the epicarp, perhaps due to a lack of methylation. In the lemmas, a developmental transition occurred wherein *gfp* was first expressed in the mesophyll cells; this was gradually replaced by expression in specialized cork cells of the epidermis. An additional promoter, *Lem1*, was produced by Sathish Puthigae and showed lemma/palea-specific expression in transient assays (Skadsen et al., PMB, 49:545-555, 2002).

A NOVEL STRATEGY FOR TRANSGENIC CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT

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ABSTRACT

Changes in agricultural practices (e.g., minimal tilling) during the past two decades combined with changing climate conditions have dramatically altered crop susceptibility to Fusarium head blight (FHB) or scab. FHB may lead to direct yield losses of 5-20% worldwide in average epidemic years, but losses as high as 60-70% have also been reported. Host plant resistance, the most cost-effective way to fight the disease, in available wheat germplasm is only partial and has been difficult to incorporate into cultivars adapted for regional growth in the U.S. Our goal is to achieve FHB resistance by employing plant genetic transformation, a potentially powerful tool for transgenic control of fungal diseases in cereals. We selected three candidate anti-*Fusarium* (AF) genes: *Aspergillus* glucose oxidase (*GO*) and barley peroxidases (*Prx7* and *Prx8*) based on their association with an array of naturally occurring plant defense mechanisms. Glucose oxidase is an apoplastic enzyme that catalyzes oxidation of β-D-glucose, generating H_2O_2, a compound with multiple functions in plant defense. Induction of the peroxidases Prx7 and Prx8 has been correlated with the appearance of antifungal compounds and papillae structures, respectively, in barley leaves exposed to powdery mildew. We inserted the coding regions of these genes into our vector that contains the barley *Lem1* promoter, which we have previously shown is active in the outer organs of transgenic wheat florets from anthesis to the soft dough stage of kernel development. This activity pattern makes it an excellent candidate for targeting AF gene expression to the path of *Fusarium* invasion. We have generated 100 transgenic wheat lines carrying the Lem1::PRX and/or Lem1::GO constructs. The *in situ* methods used for expression analyses of the primary transformants revealed that the transgene-encoded proteins are accumulated either in the extracellular space (GO and Prx8) or in the cells (Prx7) of the spike tissues and were not present in developing grain. The possible synergistic effect of these enzymes on improving host resistance to initial fungal infection and pathogen spread will be discussed. If our strategy is successful, the lack of recombinant proteins in the grain will minimize concerns about the safety of foods derived from these wheats and facilitate their approval by regulators and acceptance by consumers.

CHARACTERIZATION OF ORGAN SPECIFIC PROMOTERS IN TRANSGENIC WHEAT

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OBJECTIVE

To identify promoters suitable for targeting anti-*Fusarium* gene expression to wheat tissues surrounding the developing seed.

INTRODUCTION

Genetic engineering is the most promising approach to increase plant resistance to fungal pathogens, including *Fusarium*. The effectiveness of an antifungal gene *in planta* is determined by its expression levels in the crucial host tissues and by the timing of its expression such that suitable levels of the encoded protein accumulate before the infection (Dahleen et al., 2001). At present, constitutive promoters are widely used to achieve high expression levels throughout most tissues of the plant. If only specific tissues need to be protected or antifungal compounds need to be expressed at certain targeted sites, the use of specific promoters is recommended (Punja, 2001). Expression of anti-*Fusarium* (AF) genes in the glume and lemma is desirable for both wheat and barley, because these organs comprise the outer most protective barrier encasing the reproductive organs. In this study we present the organ- and developmental specificity of the promoter of a maize glutamine synthase gene, *GS*, and the promoter of a barley floret-expressed gene, *Lem1*, in stable hexaploid wheat transformants.

MATERIALS AND METHODS

Vector and plasmid constructs: The following plasmids were used for wheat transformation: pGS176 and pGS177 carrying a 664-bp fragment of the promoter of the maize $GS_{1\text{-}2}$ gene fused to the *uidA* coding region (GUS) and the first introns of the native gene and of the maize alcohol dehydrogenase (*ADH)* gene, respectively (Muhitch et al., 2002); and pBSD5sGFP carrying a 1400-bp fragment containing the *Lem1* promoter and a partial N-terminal coding region fused to the coding sequence for the green fluorescent protein (Lem1::GFP, Skadsen et al., 2002). For comparative studies of the promoter activity patterns, the plasmid pAHC15 carrying the *uidA* gene driven by the maize *Ubi1* promoter and first intron (UBI::GUS) was also used (Christensen and Quail, 1996).

Generation of primary transformants and monitoring of the reporter gene expression: Transient expression assays and wheat transformation were performed by particle bombardment of immature embryos of cv. Bobwhite. Stably transformed plants were identified as described (Okubara et al., 2002). GFP fluorescence in various tissues of transgenic plants and *in vitro* cultures was monitored using an Olympus SZX stereomicroscope equipped with an SZX-RFL fluorescence attachment and a DP11 digital camera. GUS activity was detected according to Hänsch et al. (1995).

Construction of cloning vectors: To make pBGS9Lem1, a 1067-bp fragment containing the *Lem1* promoter was PCR-amplified from the plasmid pBSD5sGFP (Skadsen et al., 2002) using Pfu Polymerase (Stratagene) and primers 5'-GATAAGCTTGGGATGTC-3' and 5'-ACGGATATCTGCGGTTGAAG-3' with 5' extensions to add *Hind*III and *Eco*RV restriction sites, respectively. After complete digestion with *Hind*III, the resulting fragment was ligated with a 3935-bp restriction fragment containing pBGS9 (Spratt et al., 1986) and the NOS 3' transcriptional terminator sequence. The latter piece of DNA had been prepared from the monocot transformation vector pUBK (Okubara et al., 2002). To make pBGS9Lem1ADHi1, the first in-

tron of the maize alcohol dehydrogenase gene, *ADH*, was PCR-amplified using the plasmid pGS177 (Muhitch et al., 2002) as a template. The resulting 601-bp fragment was inserted into the *Eco*RV site of pBGS9Lem1. A unique *Sma*I restriction site separates the *ADH* intron and the NOS 3' region.

RESULTS AND DISCUSSION

In wheat, the period of susceptibility to head infection by *Fusarium* lasts from anthesis (the time point when the anthers extrude from the spikes) through the dough stage of kernel development. To identify a promoter suitable for expression of anti-*Fusarium* genes, the activities of reporter genes GUS and GFP fused to the maize *GS* and barley *Lem1* promoters, respectively, were monitored during growth and development of primary wheat transformants and their progeny. The *GS* promoter is only expressed in the pericarp and in the scutellum of mature embryos (Fig. 1). Thus, it is not suitable for use in anti-*Fusarium* constructs. In transgenic plants carrying both Lem1::GFP and UBI::GUS, we compared the activity patterns of the two promoters by monitoring the expression of both reporter genes during plant development (Fig. 2). We observed no GFP fluorescence in vegetative organs, indicating that the *Lem1* promoter was not active in these tissues. This is in accordance with the data for its organ- and developmental specificity in barley (Skadsen et al., 2002). In floret tissues, we detected no GFP fluorescence before anthesis, demonstrating that the *Lem1* promoter did not function before this stage (data not shown). In contrast, strong UBI-driven GUS activity was detected in the young ovary and anthers (data not shown). At anthesis, UBI is active in the reproductive organs (Fig. 2A), while the *Lem1* promoter drove high levels of *gfp* expression only in the organs surrounding the developing floret (Fig. 2A). (The autofluorescence of the anthers was also seen in control plants.) These findings indicate that the *Lem1* promoter is active during the same period in spike development in transgenic wheat as it is in its native context in barley. No GFP fluorescence was seen in developing seeds (Fig. 2B and C). In contrast, GUS activity driven by the UBI promoter was detected in the seed coat during the earliest stages of grain development – watery ripe and soft dough stages (Fig. 2B and C, respectively).

The relative strength of the barley *Lem1* promoter was assessed in a transient assay (Fig. 3). Transient *gfp* expression was first observed in wheat embryos 10 h after bombardment (Fig. 3B), while *uidA* expression driven by maize UBI was detected within 2 h (Fig. 3A). This finding indicates that that *Lem1* is less active than UBI, which is one of the strongest of cereal promoters characterized to date. Approximately the same difference in *gfp* transient expression under the control of *Lem1* and UBI promoters was shown by Skadsen et al. (2002) in bombarded spikes of wheat and barley.

Results from these comparative studies suggest that, due to its organ specificity and moderate strength, the barley *Lem1* promoter would be an excellent choice to target anti-*Fusarium* gene expression to wheat tissues surrounding the developing seed at anthesis, while excluding transgene-encoded foreign proteins from the edible grain. To facilitate its use for this and other purposes, we constructed the cloning vectors pBGS9Lem1 carrying the *Lem1* promoter and the NOS 3' terminator sequence (Fig. 4A) and pBGS9Lem1ADHi1, in which the first intron of the maize *ADH* gene was fused to the *Lem1* promoter (Fig. 4B). Both vectors have unique blunt-end restriction sites that can be used for insertion of any coding sequence. Recently, we have successfully employed pBGS9Lem1 to express candidate anti-*Fusarium* genes in transgenic wheat.

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Figure 1. Activity of the *GS* promoter in developing florets and seeds in transgenic wheat. (*GUS activity visible in color photographs is indicated by* arrows.) **A** No GUS activity was observed in a young ovary and anthers. **B** GUS activity in a maturing ovary in a spikelet after pollination. There was no staining in the outer floral organs. **C** GUS activity in the pericarp. **D** and **E** No *uidA* expression was detected in immature embryos. **F** GUS activity in the scutellum of a mature embryo.

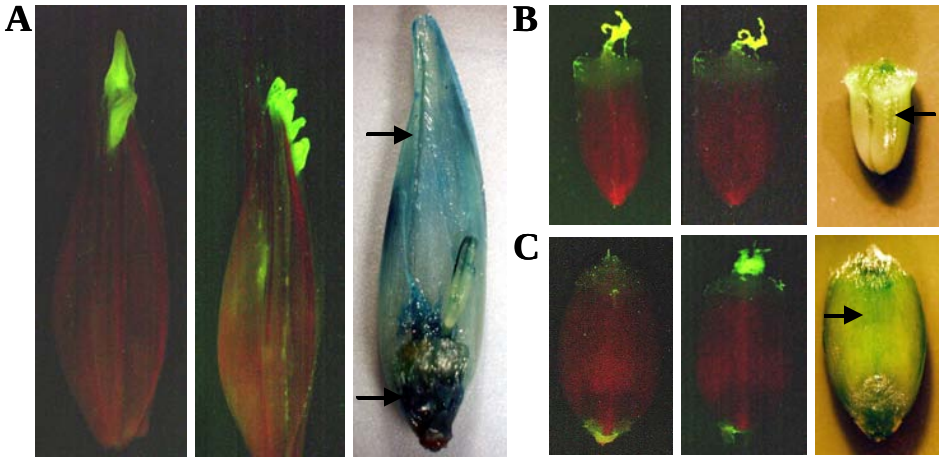

Figure 2. Activity patterns of the barley *Lem1* and maize UBI promoters during development of wheat florets and grain. Reporter gene expression was monitored in stable wheat transformants carrying Lem1::GFP and UBI::GUS, either by direct fluorescence (GFP) or by histochemical staining (GUS). Each panel shows developing florets and seeds from non-transformed plants under UV light (*left*) and GFP fluorescence (*center*) and GUS activity (*right*) in the same type of specimens from transgenic plants. (*GFP fluorescence is visible as very light areas. GUS activity visible in color photographs is indicated by* arrows.) **A** Florets at anthesis. Note the lack of GFP fluorescence in the outer floret organs of the control. **B** Developing kernels at a watery ripe stage. **C** Wheat grain at a soft dough stage. Note that the mature anthers and hairs of the caryopsis brush show strong autofluorescence under these conditions.

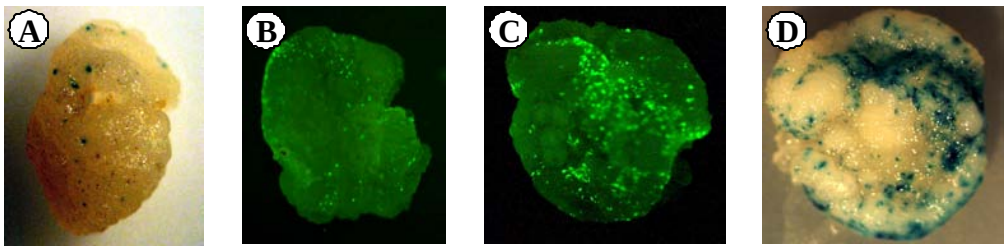

Figure 3. Activity of *Lem1* and UBI promoters during callus initiation and culture. Isolated zygotic wheat embryos 21 DAA were co-bombarded with Lem1::GFP and UBI::GUS. **A** GUS activity in an embryo 2 h after bombardment (*dark spots)*. **B** Expression of *gfp*10 h after bombardment *(lighter spots).* **C** GFP fluorescence in a callus after one week of culture on the recovery medium. **D** The callus shown in Fig. 3C after staining for GUS activity.

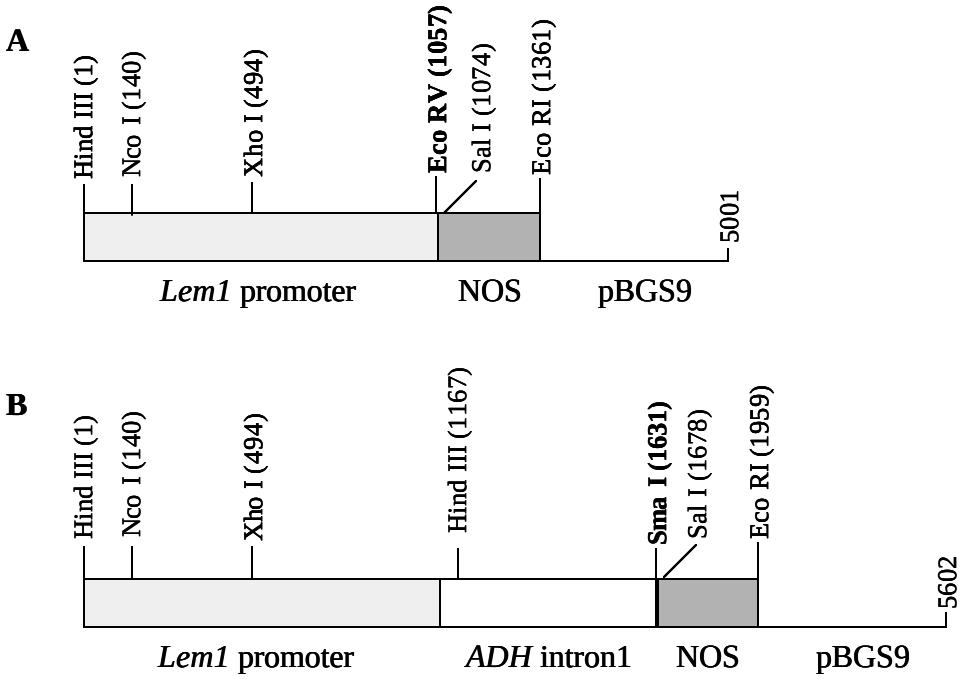

Figure 4. Linear diagrams of the cloning vectors. **A** The plasmid pBGS9Lem1 features the barley *Lem1* promoter and nopaline synthase terminator (NOS). **B** The plasmid pBGS6Lem1ADH1 contains the *ADH* first intron inserted between the promoter and NOS. The unique *Eco*RV (**A**) and *Sma*I (**B**) sites were created to facilitate insertion of any coding region as a blunt-ended fragment. The locations of some other restriction sites are shown. The pBGS9 portion is not to scale.

COMBINED EXPRESSION OF CANDIDATE ANTI-*FUSARIUM* GENES IN WHEAT SPIKELETS

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ABSTRACT

Fusarium head blight (FHB) or scab is one of the most destructive diseases of wheat, causing significant reductions in grain yield and quality. Although partial resistance has been identified in wheat varieties, no sources of immunity to *Fusarium* have yet been found. Genetic engineering is a promising method to create new sources of wheat germplasm with host plant resistance to scab. Expression of genes conferring resistance to FHB in wheat is desired in the glume and lemma, because these organs comprise the outer most protective barrier encasing the reproductive organs. Our objectives were to 1) introduce genes encoding recombinant antifungal proteins into bread wheat, *cv*. Bobwhite and 2) characterize their expression in *in vitro* cultures and stable transformants. A cloning vector carrying the barley *Lem1* gene promoter was constructed. We have previously shown that the *Lem1* promoter is active in wheat florets from anthesis to the soft dough stage of kernel development, making it an excellent candidate for targeting antifungal gene expression to the path of *Fusarium* invasion. Coding regions of genes selected for their ability to induce an array of naturally occurring plant defense mechanisms – the *Aspergillus* glucose oxidase gene and two barley peroxidase genes, *Prx7* and *Prx8* - were fused to *Lem1* and introduced into wheat immature embryos by particle bombardment. Functional analyses of the expression cassettes were performed by transient assays. The activities of the transgene-encoded proteins were studied in spike tissues of primary transformants and their progeny using enzyme assays. The employed *in situ* methods revealed that the recombinant peroxidases were present in the organs surrounding the developing floret at anthesis, but not in the developing grain.

EXPRESSION PATTERNS OF CHITINASE AND THAUMATIN-LIKE PROTEINS IN THREE TRANSFORMATION EVENTS OF BARLEY (*HORDEUM VULGARE* CV. CONLON)

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OBJECTIVE

To analyze the protein expression patterns in barley transformed with two antifungal genes.

INTRODUCTION

Fusarium head blight (FHB), predominantly caused by *Fusarium graminearum*, is a devastating disease in cereal grains including barley. Resistant sources available to plant breeders are multigenic and provide only partial protection from FHB. Resistance to FHB might be achieved by overexpressing pathogenesis-related (PR) proteins that degrade structural components of the fungal pathogen. Currently, there are no reports of barley genotypes that are highly resistant to FHB. Combinations of antifungal and antitoxin genes are likely to offer a greater degree of resistance than transgenic plants containing single genes. Delayed FHB development has been reported in transformed spring wheat overexpressing *tlp* (Chen et al. 1999), and in plants co-expressing chitinase and glucanase genes (Zhu et al. 1994, Jongedijk et al. 1995, Anand et al. 2003), demonstrating the potential role of antifungal proteins against FHB. The *tlp* is a membrane permeabilizing protein while *chi* catalyzes the degradation of chitin, a cell wall component of most filamentous fungi. Previously, the barley malting cultivar Conlon was transformed with two antifungal genes, *chi* and *tlp* by particle bombardment (Dahleen and Manoharan 2003) and 58 plants from three transformation events were obtained and analyzed for transgene integration and expression. In the the present report, T_3 progenies from the three transformation events were further analyzed for chitinase (*chi*) and thaumatin-like protein (*tlp*) expression in both leaf and spike tissues.

MATERIALS AND METHODS

Transformation and regeneration - Immature embryos from the malting cultivar Conlon (two-rowed barley) were cultured on callus induction medium (Dahleen and Bregitzer 2001) and transformed with the antifungal genes, *chi* and *tlp,* by particle bombardment (Lemaux et al. 1996). Plasmids used for co-bombardment, pAHRC-*tlp* harboring the *tlp* gene and pAHG11 containing the *chi* gene, were provided by Dr. S. Muthukrishnan (Kansas State Univ.). Both plasmids contain the *bar* gene for bialaphos selection. The *tlp* and *chi* genes are under the control of the maize *ubil* promoter and terminated by *nos*. Transgenic plants were regenerated as described by Manoharan and Dahleen (2002).

T_3 progeny analyses - DNA from leaf and spike tissues was prepared using a modified CTAB method. PCR was carried out to determine the presence of *chi* and *tlp* genes in homozygous transgenic materials. PCR products were separated by agarose gel (1%) electrophoresis. Southern hybridization was carried out to confirm the *chi* or *tlp* transgene integration pattern in the three events. Genomic DNA was digested with *Hin*dIII, electrophoresed on a 1% agarose gel and transferred onto Hybond N+ membrane. Probes used were full length inserts (both 1.1 kb) released from the plasmids by *Bam*HI/*Hin*dIII (*chi*) or *Pst*I (*tlp*) digests and labeled with ^{32}P dCTP using the Redi-Prime Labeling Kit (Amersham Pharmacia, Buckinghamshire, England). Total soluble protein was prepared from young leaf and spike tissues of transgenic and control plants. Western analyses were conducted using the Immun-Blot Colorimetric Assay (Bio-Rad, Hercules, CA). Antibodies for *tlp* and *chi* were provided by Dr. R. Skadsen (USDA-ARS, Madison, WI) and Dr. S.

Muthukrishnan (Kansas State Univ.), respectively. Reverse-transcriptase (RT)-PCR was conducted using the One Step RT-PCR System with Platinum Taq DNA polymerase (Invitrogen).

RESULTS AND DISCUSSION

PCR analysis for *tlp* on the T_3 progenies of three transformation events (Events 1, 2 and 3) was in agreement with the previous report of Dahleen and Manoharan (2003). All transgenic events showed the presence of the *tlp*. While *chi* was detected in Event 2, PCR detection of *chi* in Events 1 and 3 was not consistent (data not shown). Southern hybridization was carried out to confirm the transgene integration pattern in the three events. All transgenic lines tested from each event were found positive for *tlp* but only Event 2 showed stable integration of the *chi* transgene (Fig. 1).

Previous Western analysis of T_2 plants from three transformation events (Dahleen and Manoharan 2003) suggested that both transgenes were silenced in two events (Events 1 and 3). Our present results confirmed the presence of a 26 kD rice *chi* in Event 2 leaves and spikes that was not detected in transgenic lines from the other two events (Fig. 2a). Aside from a 35-kD putative barley *chi* found in leaves from all transgenic plants and the wildtype, other chitinase bands of 25 and 31 kD were detected. Although the 35-kD *chi* band found in leaves was also detected in Event 2 spikes, a smaller band (ca. 34 kD) was present in Events 1 and 3 spikes (Fig. 2b). In all three events including the non-transgenic Conlon spikes, at least one band of *chi* close to 25 kD was present.

The expected 23-kD protein of the rice *tlp* was highly expressed in Event 2 leaves but had lower levels in spikes of the other two events (Fig. 2c). In spikes (Fig. 2d), a native *tlp* of approximately 23 kD is expressed in wildtype Conlon and transgenics, which comigrates with the rice *tlp*, making transgene expression levels difficult to determine. A putative 24-kD protein native to barley as well as smaller proteins ranging from 13 to 18 kD were detected in leaves and spikes of all transgenic events and the wildtype.

It is possible that the bands detected in leaves or spikes other than the 26 kD rice *chi* or 23 kD rice *tlp* could be either isoforms that are native to barley or could have been derived by proteolytic processing. To further analyze the differential protein expression and isoforms in leaf and spike tissues of transgenic and wildtype Conlon, isoelectric focusing gel electrophoresis is currently underway. Northern blot analyses are being used to confirm the mRNA expression level of *chi* and *tlp* in all transformation events using gene specific probes. Initial RT-PCR showed *chi* transcripts in leaves for all events (data not shown) which could be due to the presence of another chitinase endogenous to barley since the primer pairs amplify a short (372 bp) region of the gene that contains 240 bases having 84% sequence similarity with a barley (cv. NK1558) chitinase gene. RT-PCR analyses are being conducted using pertinent primer pairs to discriminate rice *chi* and *tlp* transcripts from corresponding transcripts that are endogenous to barley. Work is underway to optimize conditions for isoelectric focusing and RT-PCR.

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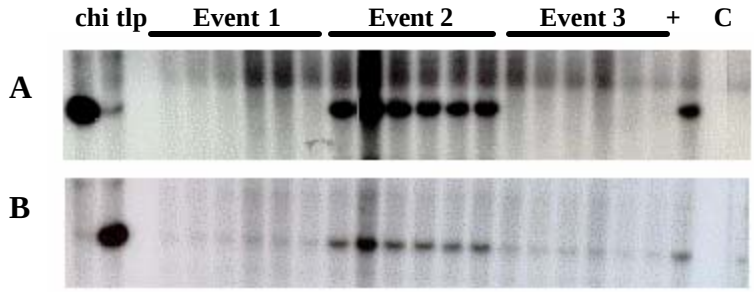

Figure 1. Southern analysis confirming the integration of (a) *chi* gene only in Event 2 (b) *tlp* in all three transformation events. Thirty µg of genomic DNA was loaded per la chi = wildtype Conlon DNA plus 250 pg chi (1.1 kb); tlp = wildtype Conlon DNA plus pg tlp (1.1 kb); + = positive control DNA; C = wildtype Conlon DNA.

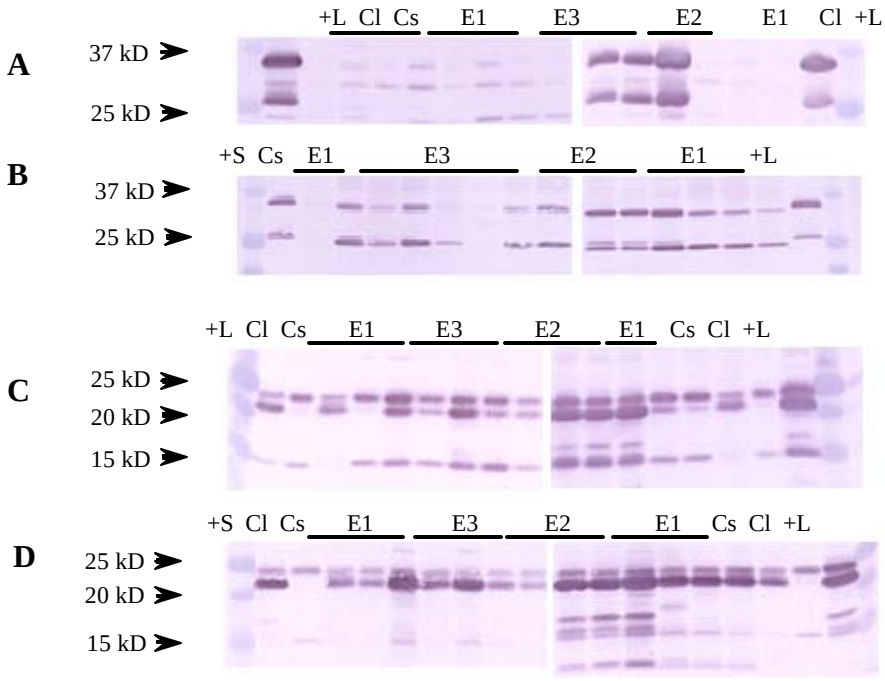

Figure 2. Western blots showing the 26-kD rice *chi* protein in (a) leaves and (b) spikes of Ever (E2) plants which was not present in Events 1 and 3 (E1, E3) transgenic and in wildtype Conlor 23-kD rice *tlp* protein was highly expressed in (c) leaves and (d) spikes of all Event 2 plants but similar band was detected in Events 1 and 3 plants. Fifteen μg of total soluble protein was loade per lane. +L = leaf, positive control; Cl = wildtype Conlon leaf; Cs = wildtype Conlon spike.

TOWARDS HIGH RESOLUTION TWO-DIMENSIONAL GEL ELECTROPHORESIS OF FHB INFECTED WHEAT SPIKE PROTEINS USING A PROTEIN FRACTIONATOR AND NARROW PH RANGE GELS

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OBJECTIVES

To enhance resolution of two-dimensional gel electrophoresis (2-D GE) and to improve detection of low abundant proteins in wheat spikes.

INTRODUCTION

Identification of proteins related to different phenotypes has been carried out in an extensive range of biological tissues since the mid 1970s' with the development of gel electrophoresis. Proteomics is the systematic analysis of the protein expression in a tissue, cell, or subcellular compartment. Ultimately, a proteome analysis should include most if not all the proteins from a biological sample. Two-dimensional gel electrophoresis (2-D GE) is the backbone technique of current proteomics because it enables to simultaneously separate complex mixtures of thousands of proteins that can be found in common biological samples. 2-D GE of proteins with broad range immobilized pH gradient (IPG) gel strips such as pH4-7 and pH3-10 is commonly used for displaying proteins A major setback of wide range IPGs is their limitation in visualizing less abundant proteins and in resolving proteins with either similar pI or molecular weight (Hoving et al., 2002). To enhance resolution and improve detection of low abundant proteins in wheat spikes, proteins extracted from wheat spikes were fractionated with a Zoom Fractionator prior to 2-D GE and then narrow range IPG gel strips with pH ranges matching that of each fraction of proteins were used for isoelectric focusing (IEF) before size separation.

MATERIALS AND METHODS

Plant materials - Wangshuibai, a Fusarium head blight resistant landrace from Jiangsu, P. R. China, was used in this study. Growth and inoculation of plants are described in the accompanying manuscript.

Protein extraction - Wheat spikelets inoculated with either H_2O or *Fusarium graminearum* were removed from spikes with a pair of forceps. About 15 treated spikelets from different spikes (5-8) were mixed as one sample and were ground in pre-chilled mortar with liquid nitrogen. Finely ground powder was collected into 2 ml microcentrifuge tubes and weighed. One ml of 10% (w/v) trichloroacetic acid, 0.05% (v/v) 2-mercaptoethanol in cold (-20°C) acetone was added to 0.3g of ground tissue. The samples were incubated for 2 h at -20°C to precipitate proteins and then centrifuged for 20 min at 16,000 g. The pellet of precipitated proteins was washed with 1 ml cold acetone containing 0.05% v/v 2-mercaptoethanol several times until the pellet was colorless. A 10 min centrifugation at 16,000g was used to pellet the proteins after each wash. Pellets were dried under vacuum for 10 min, and the proteins were resuspended with 1 ml of rehydration buffer (5 M urea, 2 M thiourea, 2% CHAPS, 20 mM DTT, and 0.5% carrier ampholytes pH3-10 (Invitrogen, Carlsbad, CA 92008, USA) for 1 h. After centrifugation at 16,000g for 10 min, the supernatant was collected, and a 10ml sample was removed for protein assay. The remaining supernatant was stored at -80°C until protein fractionation. Protein concentration of samples was determined using bovine serum albumin with the Bradford method (Bradford, 1976).

Protein fractionation - A Zoom® IEF Fractionator from Invitrogen (Carlsbad, CA 92008, USA) was used to fractionate isolated protein samples into different pH range fractions on the basis of isoelectric points (Zuo and Speicher, 2000). Six Zoom® polyacrylamide disks with pH values of 3.0, 4.6, 5.4, 6.2, 7.0, and 10.0 were used to form five chambers with successive pH ranges within the fractionation unit. A total of 2 mg protein of was loaded in the five chambers and separation was conducted as described in the users' manual. After fractionation, the five individual protein samples were removed from the individual chambers and stored to -80°C until loaded on IPGs gels

Protein Isoelectric Focusing and SDS-PAGE - Fractionated samples were loaded on IPGs gel strips on 11 cm pH3-10 or pH 4-7 IPGs gel strips according to the users' manual (Bio-Rad Ltd.), electrofocused and separated on small format polyacrylamide gels (10X12cm) for separation of samples based on their molecular size. On occasion, two 7 cm narrow pH range IPGs were run on large format gel (20X20 cm) to permit side by side comparison of samples. Alternatively when fractionation was omitted, 50 µg of solubilized proteins was mixed with 2 vol of rehydration buffer (6M urea, 2M thiourea, 2%CHAPS, 1% DTT and 0.5% ampholytes) and loaded on IEF gel strips according to the user's manual of the supplier. IEF was carried out according to the supplier's instruction. After IEF, the strips were either kept at -20ºC or directly used in SDS-PAGE. The strips were equilibrated in equilibration buffer I (6M Urea, 2% SDS, 0.05M Tris-HCl, (pH 8.8), 20% (w/v) glycerol, 2% (w/v) dithiothreitol) at ambient temperature for 15 min, and then in equilibration buffer II (6M Urea, 2% SDS, 0.05M Tris-HCl, (pH 8.8), 20% (w/v) glycerol, 2.5% (w/v) iodoacetamide) for another 15 min. After equilibration, the strips were positioned on top of the second-dimension gel and sealed with 1% agarose. SDS-PAGE was performed on 15% polyacrylamide gels. The small format gels were run for 1h at 200 V. The large format gels were run for 30 min at 30 mA followed by 60 mA for 6 h.

RESULTS AND DISCUSSION

Efficient protein fractionation by ZOOM® IEF Fractionator

About 2 mg protein extracted from spikes of Wangshuibai was applied on the ZOOM® IEF Fractionator. The 2D separation of fractionated samples on broad IPGs showed that wheat spikes contain a complex combination of polypeptides (Fig. 1A). Results showed that the ZOOM® IEF Fractionator was able to effectively separate this very complex mixture of proteins into several pH ranges as showed in Fig. 1B-E. Reproducible separation of complex protein samples into distinct liquid fractions was realized using this instrument. One disadvantage of ZOOM® IEF Fractionator apparent in our experiments was a protein loss of 40% during fractionation. This was likely due to an entangling of proteins in the polyacrylamide disks separating the different chambers.

Enrichment of low abundant proteins on narrow pH IPGs gel strips

The fractionated protein samples from Wangshuibai spikes were loaded on 7 cm IPGs gel strips with narrow pH ranges that were 0.1 unit wider than the pH ranges of each fraction at both ends. For example, 7 cm IPGs with pH range of 6.1 to 7.1 were used to load proteins in the fraction of pH 6.2 to 7.0, and two 7 cm IPGs were run on 20x20 cm PAGE in the second dimension (Fig.1F-G). Fractionation of protein samples using the ZOOM® IEF Fractionator enables enrichment for less abundant polypeptides (eg. spot #2 in Fig. 1F) thereby making them visually detectable. Spot 2 was not visible when 200 µg of whole protein sample was directly loaded on the same 11 cm IPG gel strips during the 1st dimension separation (Fig. 1A). The fractionation protocol permitted the identification of differentially expressed proteins between FHB infected and H_2O inoculated spikes such as spots #1 and 2 in Fig.1F-G.

In conclusion, the ZOOM® IEF Fractionator can separate wheat spike proteins into several fractions of different pH ranges. Narrow pH ranges IPGs matched with all fractions were available for 2D-GE analysis. Higher resolution of protein profiling, enrichment for less abundant proteins and identification of differentially regulated proteins were achieved in this experiment.

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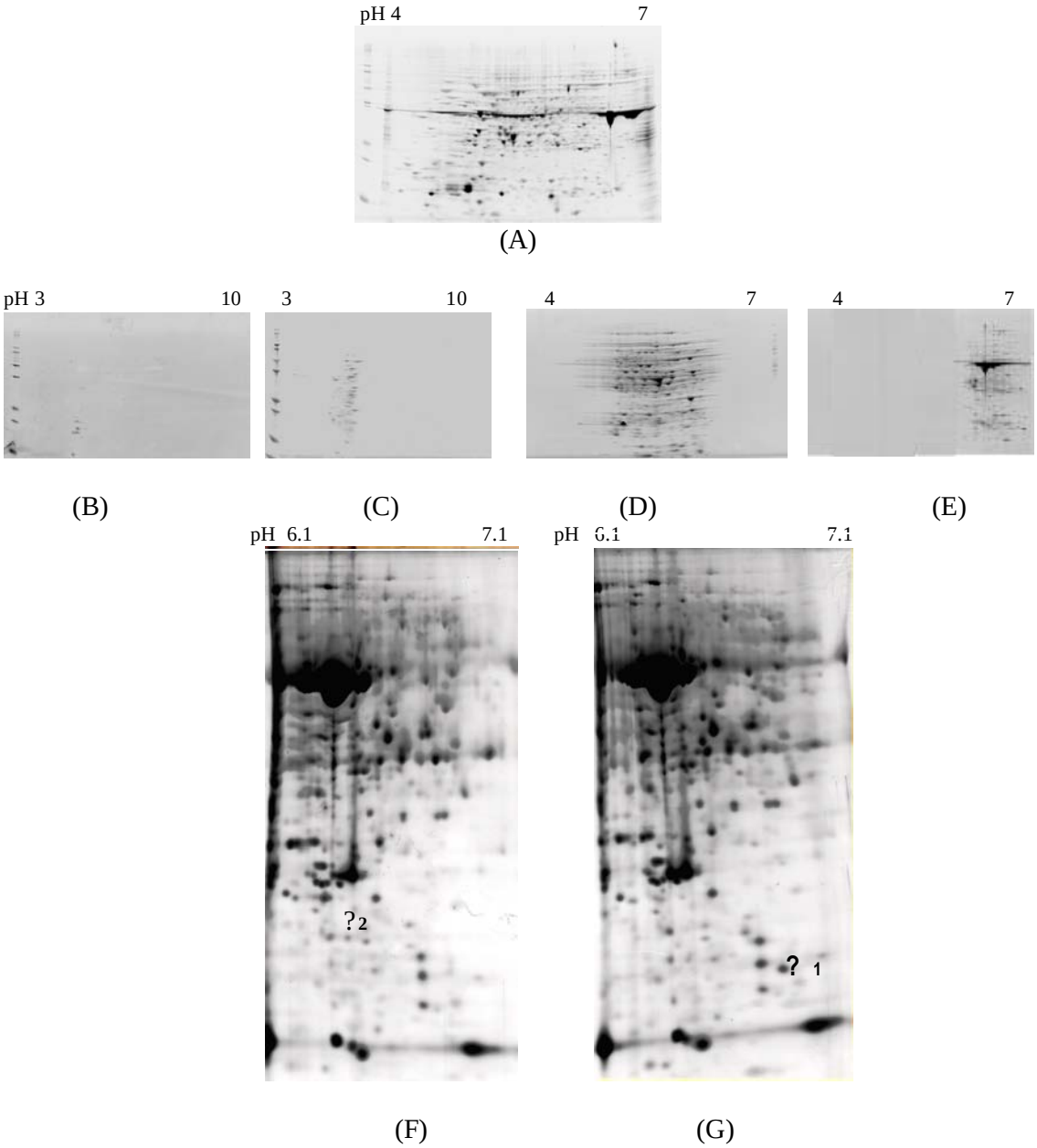

Figure 1: Two dimensional electrophoregrams of wheat spike protein samples. **A:** a whole protein extract (200 µg) separated directly on a 11 cm pH4-7 IPG gel strip in the first dimension; **B:** fractionated sample (10 µg) from pH3-4.6 range separated on a 11 cm pH3-10 IPG gel strips; **C:** fractionated sample (50 µg) from pH4.6-5.4 range separated on a 11 cm pH3-10 IPG gel strips; **D**: fractionated sample (50 µg) from pH5.4-6.2 range separated on a 11 cm pH4-7 IPG gel strips; **E:** fractionated sample (50 µg) from pH6.2-7.0 range separated on a 11 cm pH4-7 IPG gel strips; **F:** fractionated protein (50 µg) from Wangshuibai spikes 3 days after control inoculation from pH6.2-7.0 range separated on a 7 cm pH6.1-7.1 IPG gel strip. **G:** fractionated protein (50 ug) from Wangshuibai spikes 3 days after inoculation with FHB pH6.2-7.0 range separated on a 7 cm pH 6.1-7.1 IPG gel strip. Arrow 1 indicates a protein only present on FHB infected spikes. Arrow 2 indicates one protein present only in H_2O inoculated spikes. Gels A to E were stained with SYPRO-Ruby while gels F and G were stained with silver

TWO-DIMENSION DIFFERENTIAL DISPLAY OF PROTEINS ISOLATED FROM FHB INFECTED AND HEALTHY SPIKES OF WANGSHUIBAI

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OBJECTIVES

To identify proteins responsive to FHB infection from spikes of Wangshuibai, a FHB resistant landrace from China.

INTRODUCTION

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* Schwabe, and sometimes by other *Fusarium* species, is a severe disease of *Triticum spp*. and *Hordeum vulgare*, causing significant reductions in yield and quality in many wheat production regions around the world (McMullen et al., 1997). Although the genetics of FHB resistance have been well documented and resistant cereal cultivars have been developed to minimize FHB impact, there is a limited understanding of the molecular mechanisms involved in plant resistance against the infection and spread of *Fusarium graminearum*.

Proteomics techniques provide an important tool to study mechanisms of plant resistance against biotic and abiotic stress. Using two-dimensional electrophoresis (2D-GE) and proteomics techniques, specific proteins have been shown to be differentially expressed in salt- and heat-stressed wheat (Majoul et al., 2000, 2003; Ouerghi et al., 2000). One of the major advantages of this technique is that differentially expressed proteins can clearly and reproducibly be detected between sensitive vs. tolerant lines, or between infected (stressed) vs. uninfected (non-stressed) conditions. Proteins that are qualitatively or quantitatively different in their expression levels among treatments have a high likelihood of playing an important role in the response of the plant to a given stress. Further identification of these differentially expressed proteins by LC-MS/MS can provide powerful insight into the molecular mechanisms of resistance and underlying functions of these proteins in determining resistance or tolerance in plants.

MATERIALS AND METHODS

Plant materials - Wangshuibai, a Fusarium head blight resistant landrace from Jiangsu, P. R. China, was used in this study. Seeds of Wangshuibai were germinated in plastic trays filled with vermiculite. After seedlings emerged, plants were transferred to a vernalization chamber for 8 weeks at 4°C with a 16 hr photoperiod. Vernalized plants were transplanted into 15 cm pots and grown in a greenhouse. About 30 pots were placed randomly on a bench in a greenhouse maintained at 24°C with a 16 hr photoperiod (artificial lights were used to maintain light intensity over 300 watts/m^2 when it is necessary). The same water and fertilizer management were used for all materials during the entire growing period.

Wheat spikelets were inoculated with *F. graminarium* conidiospores or deionized water using two syringes on the morning when they were at the mid-anthesis developmental stage. About 1000 conidiospores in a volume of 10 µl were injected into two flowering florets of a spikelet. The same volume of deionized water was injected into flowering spikelets on a different plant to serve as a control. The inoculated spikelets were marked and the time and date of inoculation recorded. Inoculated plants were placed into a mist room immediately after inoculation. The humidity in the mist room was maintained at 90% using a computer controlled high-pressure mist system. The temperature in the mist room was 24 °C and with the same light intensity as in the growth room. Following inoculation, spikes were harvested by cutting with a pair of scissors 24 h, 48h and 72 h after inoculation Harvested

spikes were immediately placed on ice, and were transferred into a -80°C freezer for storage until protein extraction.

Protein extraction and quantitation - Standard protein extraction and quantification methods described in the accompanying manuscript in these Proceedings were employed.

Isoelectric focusing and SDS-PAGE - A solubilised protein sample (150 -500µg) was mixed with rehydration buffer from Bio-Rad (Hercules, CA, USA) to a total volume of 350 µl was loaded and focused on 17 cm Bio-Rad Ready Gel Strips as described by the manufacturer's manual. For the second dimension separation, the strips were positioned on top of the second-dimension gel and sealed with 1% agarose. SDS-PAGE was performed on 15% polyacrylamide gels. The gels were run for 30 min at 30 mA followed by 60 mA for 6 h. Sample separation was repeated three times.

Staining of PAGE gels - Three staining methods were used in this experiment. The silver stainning method was used for analytical purpose. The SYPRO Ruby stain method was used for quantitative analysis. For preparative gels, Colloidal Coomassie Blue (CBB) G-250 was used. Induced and differentially regulated protein spots were excised from CBB stained gels for LC-MS/MS analysis.

RESULTS AND DISCUSSION

2-DE display of proteins from spikes -

The separation of protein samples from the spikes of Wangshuibai that were harvested 1-, 2-, and 3-days after inoculation with *F. graminearum* or water are shown on Fig. 1. Acidic proteins were displayed on immobilized pH gradient (IPG) gel strips pH range 4-7, while basic proteins were displayed on IPG gel strips pH range 7-10. Collectively, these results represented proteins within pH range 4-10 that were extracted from wheat spikes. Analyses of results showed that under our conditions the 2-DE technique used was highly reproducible for proteins isolated from both FHB infected and healthy spikes.

Proteins displayed differentially between healthy and FHB infected spikes - Both qualitative and quantitative differences of protein expression were observed between healthy and FHB infected spikes on 3 time courses. In total, twelve protein spots ranging from 6 to 120 kilodaltons were detected only in proteins from FHB infected spikes. For example, two different protein spots from isolated spikes sampled 2- and 3-days after inoculation with FHB are shown in Fig. 2. More than twenty spots ranging in molecular size from 6 to 120 kilodaltons were also detected either as being up- or down-regulated following inoculation with FHB infection. These protein spots have been excised and we are waiting for the LC-MS/MS results to identify all these proteins with altered levels of expression caused by FHB infection.

The utilization of 2D-GE has enabled the reproducible identification of differentially regulated polypeptides and mass spectrophotometry results will permit protein identification whether these proteins originate from wheat or the pathogen and provide an indication of which biochemical pathways are involved following infection of the FHB wheat land race Wangshuibai.

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1- (A, B), 2-(C, D) and 3-day (E, F) after inoculation with *Fusarium graminearum* (B, D, F)or water (A, C, E). The small dashed box on 3-day after inoculation indicates the close-up region shown in Figure 2.

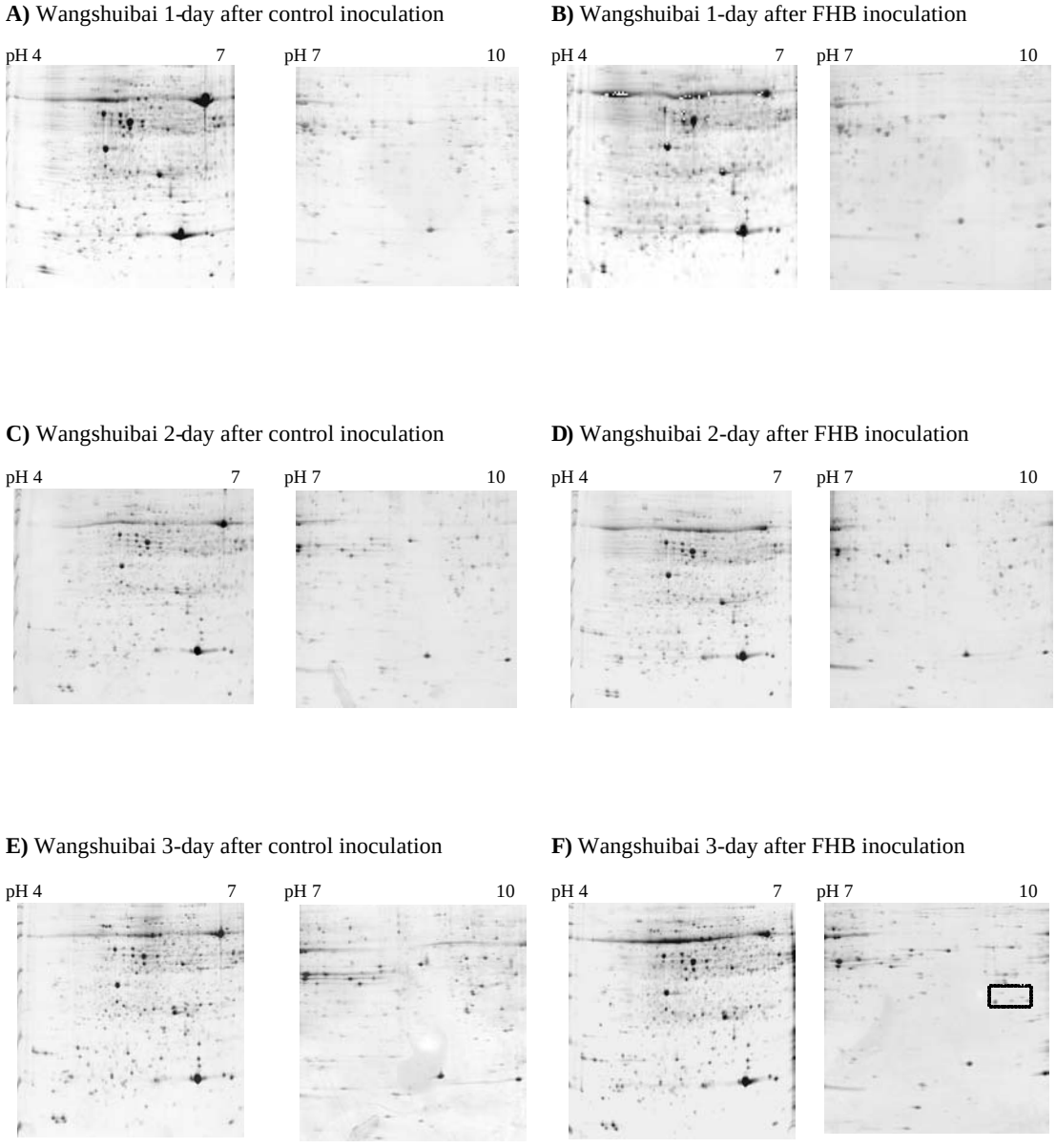

Figure 1. Two dimensional electrophoregrams of protein samples isolated from Wangshuibai wheat spike harvested

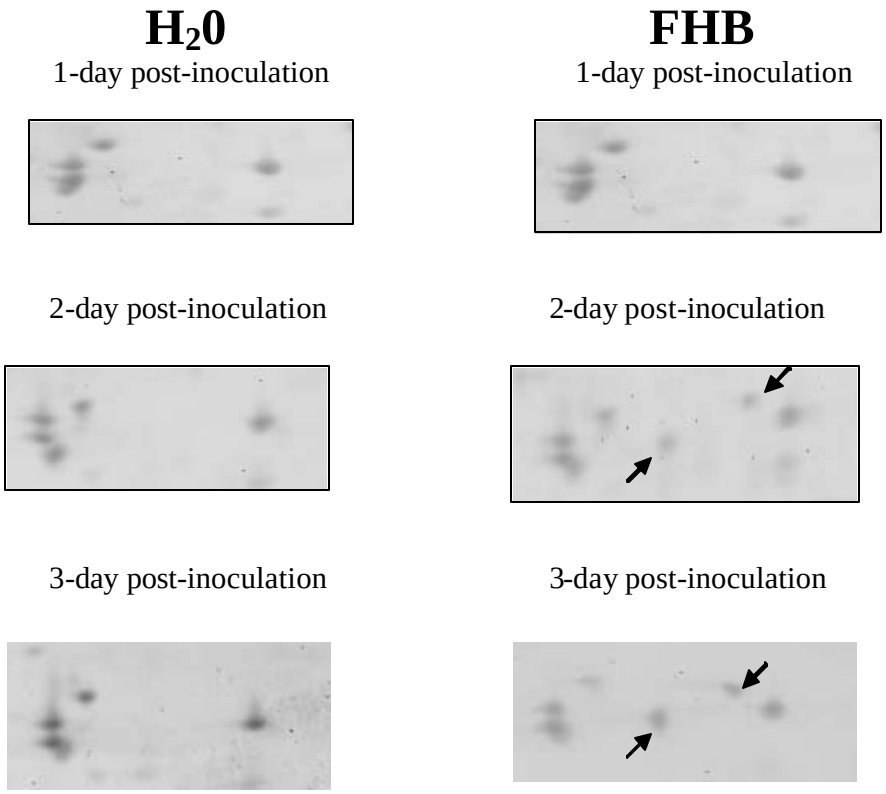

Figure 2. A close-up comparison of expression of some basic proteins in FHB inoculated and control spikes. Arrows point at spots that were only shown in protein samples isolated from spikes harvested 2- and 3-day after inoculation with *Fusarium graminearum.*