FUSARIUM VIRULENCE AND PLANT RESISTANCE MECHANISMS: A PROJECT WITHIN THE AUSTRIAN GENOME PROGRAMME GEN-AU

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ABSTRACT

In 2002 the Austrian Federal Ministry for Education, Science and Culture has established the national genome programme GEN-AU (GENome Research in AUstria: http://www.gen-au.at/). The first call has brought together a broad spectrum of Austrian scientists (coordinator GA) focussing on the *Fusarium* problem. We proposed (as one part) that Austria should contribute 25% of the costs of sequencing the *F. graminearum* genome, but the panel felt that our proposal was too much dependent on the (in April 2002) not yet submitted proposal by US partners (Birren, Kistler, Xu, Trail). In the meantime a dramatically downscaled pilot-project (with only 5 of the initially 12 partner institutions remaining) has been funded (about 795.000 US dollar).

In the next two years researchers from the Center of Applied Genetics (CAG) of the University of Agricultural Sciences, Vienna, the Technical University (TU) of Vienna, the Institute for Agrobiotechnology in Tulln (IFA), the Austrian Research Center Seibersdorf (ARCS), and from the wheat breeding company Saatzucht Donau will collaborate on several aspects. The following principal investigators are involved: Josef Straub, Gerhard Adam (CAG) and Robert Mach (TU) will collaborate on the development of efficient gene disruption methods for *F. graminearum*. Mutants will be tested for altered virulence at the IFA Tulln (Marc Lemmens) and for altered metabolite production by LC-MS-MS (Rudolf Krska, IFA Center for Analytical Chemistry). Also analytical techniques and reference materials for “masked mycotoxins” will be developed. In the group of Gerhard Adam *Arabidopsis thaliana* genes encoding mycotoxin inactivating enzymes will be characterized, and Marie-Theres Hauser will explore the role of zearalenone in plants (CAG). The group from ARCS will establish wheat suspension cultures and work on the identification of differentially expressed genes in wheat and the development of DNA arrays. The genetic basis of so far uncharacterized highly *Fusarium* resistant wheat genetic resources will be elucidated by Hermann Büerstmayr (IFA), the knowledge gained will be utilized by the commercial partner (Julia Lafferty, Saatzucht Donau) in a marker assisted backcross breeding program (QTL pryamiding).

The aim of the GEN-AU program is “to secure and expand Austria’s competitiveness and ability to cooperate on an international level”. It may be of interest for US researchers, that the EU 6th framework programme is open to the participation of entities from nonmember countries on the project level on the basis of mutual benefit.

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.

ISOLATION AND CHARACTERIZATION OF *TRI16* FROM *FUSARIUM SPOROTRICHIOIDES*

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ABSTRACT

Many of the genes involved in the trichothecene biosynthetic pathway in *Fusarium* have now been identified within a 29 kb section of DNA. Within this cluster are 3 genes (*Tri4, Tri11, and Tri13*) encoding P450 oxygenases, a gene (*Tri5*) encoding sesquiterpene cyclase, a gene encoding an esterase (*Tri8*), two acetyltransferase genes (*Tri3 and Tri7*), a transport pump gene (*Tri12*), and two regulatory genes (*Tri6* and *Tri10*). One gene encoding an acetyltransferase, *Tri101*, is not located within the cluster. However, not all of these genes are functional in every *Fusarium* species. The *Fusarium* toxins can be divided into two groups based on the substitution of the A ring. *Fusarium sporotrichioides* produces A-type trichothecenes, such as T-2 toxin or 4,15-diacetoxyscirpenol, while *F. graminearum* produces B-type trichothecenes, such as deoxynivalenol (DON), that have a carbonyl at C-8. These differences in side groups are due, at least, to the non-functional *Tri7* and *Tri13* in B-type trichothecene producers. In the search for the remaining trichothecene genes, the use of an EST library from a toxin over-producing strain carrying an altered *Tri10* has identified *Tri16*, a gene believed to be involved with trichothecene biosynthesis. We isolated and cloned this gene from *F. sporotrichioides*, then formed disruption vectors through insertional disruption and truncated disruption. Insertional disruption vectors produced only single cross-over events when the vector was transformed into the host protoplasts thus producing a transformant with both a disrupted as well as an intact copy of the gene. Transformants carrying the truncated disruption vector were also tested by PCR and Southern hybridization for disruption events and analyzed for toxin production. Disruption of *Tri16* does not affect toxin production. Northern analyses suggest that *Tri16* is regulated like a secondary metabolite as it is turned on in later cultures like several of the other toxin biosynthetic genes. *Tri16* is physically located on linkage group 2 whereas the main trichothecene cluster is on linkage group 1. Even though *Tri16* is found in the EST library, these studies show that *Tri16* is not necessary for toxin production.

A SYSTEMATIC APPROACH FOR IDENTIFYING ANTIFUNGAL PROTEINS WITH ENHANCED RESISTANCE TO SCAB

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OBJECTIVES

1) Identification and purification of antifungal proteins from apoplastic fluids of wheat plants. 2) Expression of recombinant antifungal proteins and test the effectiveness of the purified proteins singly or in different combinations against scab using *in vitro* assays. 3) Incorporate the desired antifungal genes into elite germplasm of wheat using the transgenic approach.

INTRODUCTION

One of the strategies to enhance disease resistance in plants is to make effective use of their natural defenses such as pathogenesis-related (PR-) proteins. Many genes for PR- proteins were shown to be induced upon scab infection of wheat indicating their importance in plant defense (Li *et al*., 2001; Pritsch *et al*., 2001). In the past, we have utilized antifungal genes with different cellular targets in wheat transformation studies without experimental evidence that these proteins are actually effective against *Fusarium graminearum* (Chen *et al*., 1999; Anand *et al*., 2001). Even though some of these transgenic plants were moderately resistant under greenhouse trials, field evaluation did not show any significantly improved resistance to scab suggesting that these lines could not withstand continuous pathogen pressure encountered in the field where both the type I and type II resistance is required for survival (Anand *et al*., 2002 manuscript communicated). In order to speed up the effort to obtain transgenic plants with enhanced scab resistance and to improve the chance of success within a limited period of time, it will be useful to identify the genes encoding proteins that are effective against scab in preliminary *in vitro* assays. Thus a multi-pronged approach relying on identification of genes that are likely to have antifungal activity, isolating these genes from appropriate sources, and introducing them into wheat or barley plants has been developed.

MATERIALS AND METHODS

Field testing of the transgenic wheat plants- Field testing of transgenic and control lines were carried out in spring 2002 at the Plant Pathology Experimental Farm located near Manhattan, KS, USA. A randomized complete block design was used with 20 replicates for each treatment. Corn kernels (93 gm^{-2}) colonized by *F. graminearum* were applied to the soil.

Extraction of the Apoplastic fluid from wheat leaves- Ten grams of fresh leaves from mature plants were vacuum infiltrated with 100 mM sodium phosphate buffer (pH 6.8). The infiltrated leaves were dried on filter paper sheets and centrifuged at 5000 rpm for 30'. The supernatant representing the apopalstic fraction was collected and stored at -70°C.

Recombinant expression of antifungal genes- The wheat cDNA clones isolated from the fungus-infected plants of wheat (Li *et al*., 2001) or rice were used for expression. The coding region fragments (minus signal peptide) were moved into the *E. coli* expression vectors, pQE60 or pTOPO under the control of the *lac* promoter and induced with isopropyl thiogalactoside (IPTG) for different time intervals to optimize maximum expression.

RESULTS AND DISCUSSION

Greenhouse and field testing of transgenic plants- Greenhouse testing was carried out in spring 2001 and fall 2002 with 4 independent homozygous transgenic (see Table 1). The line over-expressing the 383 chitinase/ 638 glucanase transgenes (#32A) showed a delay in the development of disease symptoms, and was scored as moderately resistant, while three other transgenic lines did not show any elevated levels of resistance reaction to scab (Table 1). We suspect that there may be a requirement for a threshold level of PR-proteins in order to be effective against scab. The results of the field evaluation are presented in Table 2. The transgenic lines did not have any enhanced resistance against scab, suggesting that these lines could not withstand continuous pathogen pressure encountered in the field where both the type I and type II resistance is required for survival.

Table 1. Greenhouse trials with the homozygous progenies of different transgenic wheat lines.

Homozygous plants of #32A (383 chitinase and 638 glucanase), #32C (silenced line) used as an epigenetic control, Line #76 and #78 (289 glucanase and 383 chitinase) and line #82

Entry	Total no. plants inoculated	Days after inoculation	Mean infected spikelets / head
#32A	25	10	3.7[b]
#32C	31	10	6.6[a]
MN99112	24	10	2.0[c]
Bobwhite	42	10	6.8[a]
#32A	25	14	7.4[b]
#32C	31	14	12.5[a]
MN99112	24	14	4.2[c]
Bobwhite	42	14	13.8[a]
#76	44	10	6.5[bc]
#78	51	10	6.1[c]
#82	70	10	7.1[a]
MN99112	42	10	2.05[d]
Bobwhite	64	10	6.8[ba]
#76	44	14	11.8[d]
#78	51	14	14.0[ba]
#82	69	14	14.6[a]
MN 99112	42	14	3.1[e]
Bobwhite	62	14	13.0[c]

(638 glucanase) were tested along with MN99112 (resistant check) and non-transformed 'Bobwhite' plants (susceptible check) respectively.

Table 2. Field evaluation of transgenic plants and control plants in spring 2002

Entry	Symptom rating - 3rd day	Symptom rating - 6th day	Symptom rating - 10th day	Symptom rating - 14th day
BT-14-18	17.5[a]	25.6[a]	59.7[a]	65.0[ba]
32A	9.4[c]	23.0b[a]	51.0[b]	67.8[a]
32C	13.2[b]	16.5[c]	54.7[ba]	61.2[ba]
Bobwhite	12.6[b]	17.9[bc]	53[b]	60.8[b]
Wheaton	6.7[c]	12.2[c]	49[b]	52.7[c]
MN99112	0.5[d]	1.4[d]	9.0[d]	19.3[d]
Scab-7	0.5[d]	3.5[d]	32.5[c]	51.2[c]

BT-14-18, *TLP* transgenic line; 32A, transgenic line co-expressing 383 chitinase/638 glucanase; 32C, transgene-silenced line co-transformed with 383 chitinase/638 glucanase; 'Bobwhite', untransformed control; Wheaton, a susceptible check; MN99112, a resistant check; Scab-7, a resistant check.

Characterization of the apoplastic fluid and in vitro antifungal assays- Western blot analysis of apoplastic fluid from leaves of the line #32A (see Table 1, with lesion phenotype) indicated that in addition to the expected transgene-encoded chitinase and β-1,3-glucanase bands, these extracts contained several other PR-proteins, including TLP's. In the apoplastic (extracellular) fluid prepared from these leaves about 6-10 major bands could be detected (Fig. 1). Further analyses indicated that the majority of the PR-proteins (>85%) are secreted and are localized extracellularly. The apoplastic fluid from the *chi/glu* transgenic line, 32A, with about 100 μg of total protein showed a distinct inhibitory effect against *F. graminearum in ivitro* antifungal assays (data not shown). Less effective mycelial growth inhibition was detected with equivalent amounts of the apoplastic fluid of the *TLP* transgenic line, D34.

A spore germination inhibition assay using the conidial suspension of *F. graminearum* was utilized to confirm the results of the mycelial growth inhibition assay. No germination of the conidia could be detected in the presence of barley chitinase (4 μg) and apoplastic protein preparation (25 μg protein) after 4 h of incubation, while 75%-100% germination was detected in the presence of non-transgenic apoplast extracts and 4 μg of *M. sexta* chitinase (Fig. 2). It is likely that the inhibition of spore germination by the apoplastic fluid might be due an additive or synergistic effects between chitinase and other PR-proteins including β-1,3-glucanase and TLP, or other proteins present in the apoplastic fluid.

Recombinant expression of other PR-protein genes- The successful inhibition of *Fusarium graminearum* in the two *in vitro* assays has prompted us to use an alternative approach which involves identification of specific combinations of antifungal proteins effective against scab using *in vitro* assays prior to the utilization of their genes in transgenic studies. The coding region fragments (minus signal peptide) were insertd into the *E. coli* expression vectors, pQE60 or pTOPO under the control of the *lac* promoter and colonies expressing TLP,

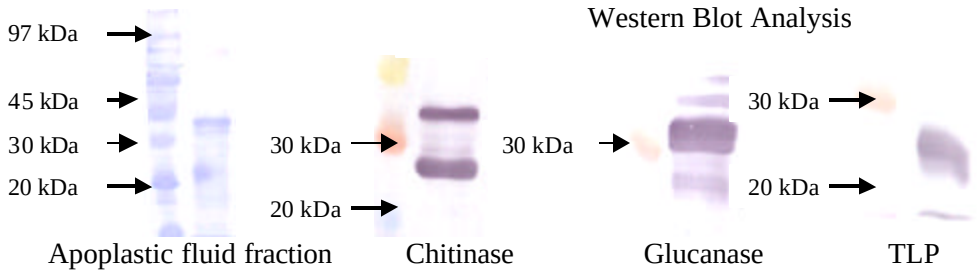

Figure 1. Detection of PR-proteins in apoplastic fluid prepared from the lesion-mimic transgenic wheat plant #32A. M = marker.

LTP and 194 chitinase upon induction with isopropyl thiogalactoside (IPTG) were identified. The recombinant LTP protein appears to be soluble and could be detected in the supernatant after sonication of the cultures while the rice D34 TLP and 194 chitinase proteins were in the pellet fraction.

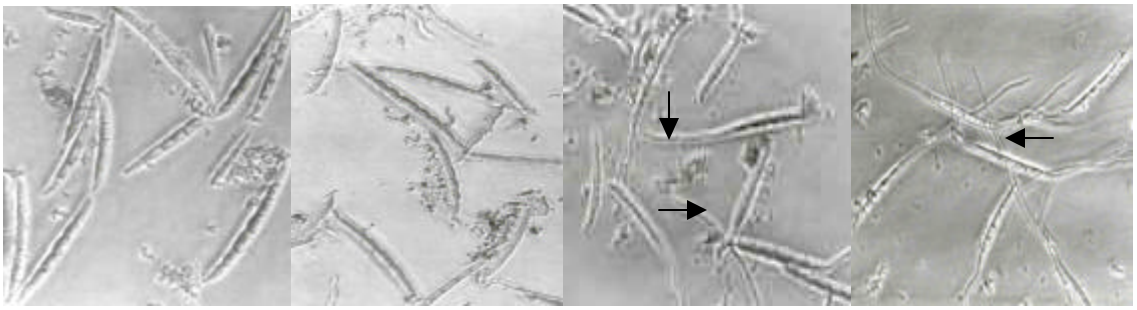

Figure 2. Spore germination inhibition assay with different protein preparations. Arrows indicate the germinating conidiophores.

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VERIFICATION OF MOLECULAR MARKERS LINKED TO FUSARIUM HEAD BLIGHT RESISTANCE QTLS IN WHEAT

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ABSTRACT

Molecular mapping led to the identification of QTL on chromosomes 3B and 5A of wheat (Anderson *et al*. 2001, Buerstmayr *et al*. 2002). The aim of this work was to verify molecular markers linked to these QTL in several spring by winter wheat crosses.

Crosses were initiated between FHB resistant spring wheat CM-82036 and several adapted European winter wheat genotypes. Populations of recombinant inbred lines in F4 to F8 generation were evaluated for resistance to FHB in replicated artificially inoculated field experiments.

The lines were genotyped with SSR markers mapping to one of the two putative QTL regions, 3B: GWM398, GWM533, GWM493, and 5A: GWM293, GWM304, GWM156.

Depending on testing year and population, markers on 3BS were more frequently associated with FHB resistance reaction than those on 5A, indicating that the QTL on 3BS has a larger and more consistent effect than the 5A QTL.

Not all 6 markers linked to a QTL in the model spring wheat population showed significant association in the verification populations. Further analysis should reveal the reasons for that.

Despite that, marker assisted selection for FHB resistance appears efficient in material segregating for resistance originating from CM-82036.

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WHEAT TRANSFORMATION FOR ENHANCED FUSARIUM HEAD BLIGHT TOLERANCE

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OBJECTIVES

To transform wheat lines with genes that may lead to improved tolerance of Fusarium head blight.

SUMMARY OF WORK

Fusarium graminearum is an important pathogen of wheat. Infection can result in significant yield losses and greatly reduce grain end-use quality due to detectable levels of the mycotoxin, Deoxynivalenol. To date, insufficient genetic resistance towards this pathogen has been identified within wheat germplasm. Biotechnology provides an avenue to introduce novel genes into elite wheat germplasm to complement an integrated program to manage *F. graminearum* pathogenesis. We have developed transgenic wheat with antifungal and anti-apoptotic genes using the *Agrobacterium*-transformation methods. Our preliminary data indicate that this approach will be effective in controlling FHB in wheat.

Antifungal genes: We have tested an animal lactoferrin gene for potential antifungal activity to *F. graminearum*. Lactoferrin (LF), an iron binding glycoprotein has long been reported to be active against a wide range of microorganisms including fungi (Zhang et. al. 1998). Lethal concentration of bovine LF, for instance, ranged from 18 to 100 μg/ml against yeast and 2 to 20 μg/ml against filamentous fungi (Bellamy et. al.; 1994). The lethal action of LF is believed to be due to the binding of the protein to the membrane and subsequent disruption of proton-gradient across the membrane. This results in membrane leakage and ultimately cell death. Lactoferrin contains an active antimicrobial domain lactoferricin (LFcin). Chong and Langridge (2000) recently demonstrated that LF protein expressed in potato tuber tissues has strong antimicrobial activities. The expression of non-plant antimicrobial genes such as LF in a transgenic plant has potential for broad-spectrum disease resistance.

In our previous work, the production of LF in tobacco cells indicated a potential for the development of disease resistant transgenic plants (Mitra and Zhang, 1994, Zhang et. al. 1998). In vitro analyses of total protein extracts from transgenic tobacco demonstrated strong anti *Fusarium graminearum* activity. Accordingly, transgenic wheat plants expressing the LF gene, were generated using both gene-gun and *Agrobacterium* mediated transformation methods. Among transgenic lines tested, five gene-gun generated lines and eight *Agrobacterium* generated lines consistently showed high levels of type II resistance (less than 10% infection) against scab in greenhouse experiments. Additional transgenic wheat plants

were generated expressing a short, synthetic LFcin gene (the 41 amino-acid long peptide from the amino-terminal end of LF) had a much stronger antimicrobial activity in tobacco than LF. Both LF and LFcin serve as membrane-disruptive agents when interacting with fungal membranes. Lactoferricin is substantially smaller and more cationic than LF; features that might help LFcin penetrate the fungal membrane more efficiently and protect it from further degradation by plant proteases. Nine western positive plants were obtained and 8-15 progeny of 3 transgenic lines were tested for type-II resistance in greenhouse. The plants were either 100% susceptible or had a high level of resistance (disease rating 10). The resistance correlated with the expression of the LFcin protein. In line 1, out of 15 plants, 6 were resistant (disease rating 10%) and 9 were susceptible (disease rating 100%); in line 2, out of 14 plants, 9 were resistant (disease rating 10%) and 5 were susceptible (disease rating 100%); and in line 3, out of 8 plants, 5 were resistant (disease rating 10%) and 3 were susceptible (disease rating 100%). Some PCR positive plants were susceptible, however, no lactoferrin protein could be detected in these plants. We have identified 9 LF and 6 Lfcin homozygous lines that showed consistent high level scab resistance in green house conditions.

The lactoferrin lines contained a human lactoferrin gene and the lactoferricin lines contained a synthetic human gene sequence. As a result, we decided to delay field trials of these lines and develop a parallel system using bovine lactoferrin gene. Significant amount of lactoferrin is present in milk and traces are also found in beef. Bovine lactoferrin will be safer and more acceptable to consumers and farmers. Accordingly, we have generated 18 primary wheat transformants containing the bovine lactoferrin (blf) gene and 7 primary wheat transformants with bovine lactoferricin (blfcin) gene. A preliminary assay showed anti-*Fusarium* activity of total protein extract from transgenic wheat with bovine constructs. Homozygous plants of these lines are being created for testing in green house and in field trials (pending authorization). As for intellectual property rights, we are authorized to use the full-length LF and the synthetic LFcin, and A-16 promoter belongs to us.

Antiapoptic genes: Our second major set of genes relates to apoptosis, or programmed cell death (PCD). Apoptosis is a highly regulated process whereby individual cells of multicellular organisms undergo systematic self-destruction in response to a wide variety of stimuli. Programmed cell death regulates normal cellular turnover, the immune system, embryonic development, metamorphosis, hormone dependent atrophy, and chemical-induced cell death (Pennell and Lamb, 1997; Ryerson and Heath, 1996; Jones and Dangl, 1996). It is believed that cell suicide responses evolved in response to viruses, providing a mechanism for limiting viral replication and spread. Most viruses have evolved genes encoding proteins that effectively suppress or delay PCD long enough for production of sufficient progeny. In addition, a growing number of viruses can induce PCD late in the infection process, which may promote spread of progeny to surrounding cells, while evading host immune inflammatory responses and protecting progeny virus from host enzymes and antibodies.

We have evidence that members of an animal anti-apoptotic gene family (Bcl-2) function in plants (Dickman et. al., 2001). Transgenic tobacco lines were generated harboring various anti-apoptotic proteins (human Bcl-2 and Bcl-X_L, nematode CED-9, and baculovirus Op-IAP). When several economically important fungal and viral pathogens were inoculated onto tobacco harboring these transgenes, the plants were highly tolerant and in most cases, completely resistant. We have now extended these findings to wheat, including having a number of elite

lines expressing resistance to scab. We believe the transgenes are functioning as expected and as they do in other plants for the following reasons. The observed resistance to the necrotrophic fungal (scab) pathogen is consistent with our previous observations in tobacco (Dickman *et al*, 2001). In addition, we have resistance in wheat to heat and salt stress which is also in accordance with results in tobacco. Thus, we are eager to evaluate these lines under field conditions. We now have homozygous (T5) lines of wheat carrying Op-IAP. We are creating homozygous lines for Bcl-xl and a mutant transgene Bcl-xl (G138A). The advantages of Bcl-xl, are: (i) the structure and mode of action is different then Op-IAP, although both genes are cytoprotective (anti-apoptotic); (ii) antibodies are commercially available for Bcl-xl, and (ii) we have a null mutant construct for comparative purposes. In addition, we are generating new wheat lines harboring Sf-IAP (Huang *et al*, 2000). Sf-IAP from the insect *Spodoptera frugiperda* is in the same class of proteins as OP-IAP. However, other transgenic plants (tomato and *Arabidopsis*) harboring this gene exhibit a number of interesting phenotypes (eg. delayed fruit ripening, delayed senescence) as well as fungal disease resistance.

Our Transformation Protocol: Introduction of a maize RIP into wheat as an example: A maize ribosomal inactivating protein (Genbank accession No. AF233881) was isolated from maize leaf (cv A188) via PCR. The PCR product was sequenced for authenticity and subsequently subcloned down stream of the maize ubiquitin promoter element coupled with its 5' intron. The resultant cassette was dropped into the binary plasmid pPZP212 (Hajdukiewicz *et al*., 1994). The final binary vector is referred to as pPTN285. The binary plasmid was mobilized into *A. tumefaciens* strain C58C1 carrying the Ti plasmid pMP90 (Koncz and Schell, 1986) via tri-parental mating (Ditta *et al*., 1980). Wheat transformations (cv Bobwhite) were set-up following a modification of the protocol described by Cheng *et al*. (1997). A total of ten lines representing eight independent transformants were recovered. Ten T1 seed per line were sown in the greenhouse. An npt II ELISA was conducted on the individuals approximately 20 day after planting using a commercial kit (Agdia Cat# 73000/0480) following the manufacturer's instructions. Northern blot analysis was conducted on a subset of the npt II positive individuals per line. A summary of the data is given in Table 1 below. T2 seed is being increased to identify homozygous lines for future screening.

Field Testing: In 2002, we undertook our first field trials of transgenic wheat. The trials were done at Mead, NE with APHIS approval. While the lines were misted to induce FHB infection,

Table 1. Segregation & Northern blot (N. blot) data in T_1 generation of wheat lines transformed with pPTN285

Line	Npt II Pos.	Npt II Neg.	N. blot
130-02-02-01	10	0	Pos
130-02-05-01	7	3	Pos
130-02-06-01	5	5	Pos
130-03-01-01	4	6	Pos
130-03-02-01	8	2	Pos
ye03-04-02-01	6	4	Neg
ye10-06-01-01	10	0	Pos
ye10-06-01-02	5	5	Pos
ye10-06-01-03	6	4	Pos
ye10-03-01-01	9	1	Pos

Npt II Pos and & Neg. columns refer to the total number of Ti individuals positive for npt II expression as determined by ELISA. N. blot column indicates whether the subset of npt II positive T1 individuals derived from the respective line were expressing the RIP transcript.

the climate was extremely hostile with severe heat after planting which "pushed" the plants to mature early and produce poor quality seed. We are currently evaluating the data, but it is expected that the main outcomes of this year's experiments were increase our seed and to learn how to meet the necessary regulations to take transgenic lines to the field.

Transfer of transgenes to elite wheat varieties: We are crossing the most FHB tolerant transgenic lines to Alsen (elite FHB tolerant line), to Wheaton (elite FHB susceptible line), and to Wahoo or Millennium, and Wesley, popular recent releases. Provided these efforts show promise, we will expand our crosses to additional lines and market classes. We are also making crosses among and between our elite antifungal genes and our elite antiapoptotic transgenic lines. We are interested in determining if pyramiding genes having similar mechanisms, and transgenes affecting the two mechanisms of antifungal activity that we are studying in combination may provide added protection against FHB.

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MOLECULAR CHARACTERIZATION OF SCAB RESISTANCE QTL IN WHEAT

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Profiling of scab resistance gene expression in wheat

The goals of this project are to discover new genes for scab resistance, identify the pathway involved in wheat resistance to scab infection and understand the genetic mechanism of Type II resistance in wheat. Three pairs (forward and reverse) of suppression subtractive hybridization libraries have been developed from infected spikes harvested 6, 36 and 72 hours after inoculation (HAI) by subtracting cDNA of bulked infected susceptible recombinant inbred lines (RIL) from bulked infected resistant RILs. The RILs generated from the cross between the resistant cultivar Ning 7840 and the susceptible cultivar Clark. So far, 2,306 differentially expressed sequence tags (ESTs) have been cloned and printed in glass slides for microarray analysis. About 80 clones were sequenced. For microarray analysis, several labeling and hybridization kits or protocols were experimented and only the kit from Genisphere produced reliable result with low background. Infected wheat spikes collected from both bulks at various time courses after inoculation will be used as probes for array hybridization to profile the scab resistance gene expression.

Mapping QTL from Wangshuibai

The objectives of this project are to: (1) discover new QTL for scab resistance from the Chinese landrace Wangshuibai which does not relate to Sumai 3, (2) investigate QTL effects on type II resistance in Wangshuibai, and (3) develop selectable markers for marker-assisted selection. F_8 recombinant inbred lines derived from the cross between Wangshuibai and the susceptible cultivar Alondra's were evaluated for Type II resistance in the greenhouse experiment. Total 15 plants were evaluated for each line. The frequency distribution of percentage of scabbed spikelets among 104 F8 RILs showed continuous distribution with one peak skewed toward resistant parent (Wangshuibai). The same distribution was observed for DON yield which was evaluated by Dr. Hart from Michigan State University. About 200 pairs of SSR primers were screened for the parents and about 30% showed polymophism. Based on the greenhouse scab evaluation, bulked resistant and susceptible lines were selected and are being used for SSR primers screening. The RILs will be further evaluated for scab resistance and DON yield next year and more SSR markers will be screened to identify closely linked markers to the QTL for scab resistance in cultivar Wangshuibai.

Marker-assisted selection

The goals of this project are to increase throughput of marker screening, reduce marker analysis cost, and develop breeder-friendly STS markers to facilitate marker-assisted selection (MAS). To improve efficiency of MAS, we optimized a fast DNA isolation protocol. In this protocol, NaOH is the DNA extraction buffer and Tris is used as DNA storage buffer. The

FastPrep system from Q.Biogene and a mini centrifuge are used for tissue preparation. DNA can be isolated with this method in any location where electricity is available. This method is suitable for MAS in conventional breeding programs since costs of equipment and reagents are low. With this method, about 200 DNA samples can be isolated daily by one person. Isolated DNA is good for STS and SSR even after 1 year of storage at –20°C. A Li-Cor Sequencer is used for SSR analysis. To increase throughput and reduce cost, primers of two flanking markers are labeled with two different fluorescence dyes (IR800 and IR700) and combined in one PCR reaction. The PCR is analyzed in one gel and produces images of both flanking markers. Each gel can be reused for 3-4 times. This method significantly reduces cost of MAS and has been successfully used for marker-assisted backcross to transfer 3BS QTL to Clark background.

To develop breeder friendly marker, one STS marker tightly linked to 3BS scab resistance QTL was converted from an AFLP marker and has been released to several breeding programs. This STS explained about 50% of phenotypic variation for Type II resistance in the population from the cross of Ning7840/Clark. It can be amplified with crude DNA isolated with the simplified method. An additional STS was developed from another AFLP marker, but the PCR condition still needs to be optimized before it can be used for MAS.

GENETIC DIVERSITY OF NEW FUSARIUM HEAD BLIGHT RESISTANT BARLEY SOURCES

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OBJECTIVE

To determine the genetic relatedness of new sources of Fusarium head blight resistance in barley to those already in use by the Minnesota barley breeding program.

INTRODUCTION

Since the outbreak of FHB in the Upper Midwest, there has been a major effort to identify FHB resistant sources and breed for FHB resistant malting barley. A number of barley varieties from around the world have been identified as potential sources of FHB resistance. All of these varieties exhibit partial resistance to FHB and, based on several mapping studies, it is known that resistance to FHB in barley is a quantitative trait controlled by a number of genes (Kolb *et al*, 2001). Thus, to obtain durable disease resistance, the best breeding strategy is to pyramid genes for FHB resistance from multiple resistant sources into one or more varieties. Many of the known FHB resistant barley sources are currently in use in variety development and/or genetic mapping studies. To date, no single source appears to provide sufficient resistance and additional new sources of resistance with novel FHB resistance genes are needed.

A major effort to identify new sources was undertaken by researchers at North Dakota State University (NDSU) who screened of over 8,200 accessions of six-rowed spring barley from the USDA Small Grains Germplasm Collection (Steffenson, 2002). These researchers identified some accessions with FHB resistance equal to or greater than that of Chevron; currently the standard six-rowed resistant check in most barley FHB research (Table 1). To more effectively utilize these new sources we wanted to determine how related these new sources are to those already in use.

MATERIALS AND METHODS

The objective of this research was to use SSR markers to determine the genetic relatedness of new sources of FHB resistance in barley (Table 1) to sources already in use by the Univ. of MN barley breeding program (Table 2). We obtained seeds of new accessions from Dr. Brian Steffenson at the Univ. of MN. Dr. Steffenson originally obtained seed from the USDA's Germplasm Resources Information Network (GRIN) and performed single head selection on each line. Plants were grown in the greenhouse and DNA extracted from a single plant. A genetic diversity study, previously conducted in our lab, included many of the parents our barley program has used in breeding for FHB resistance (Wingbermuehle, 2002). To combine the information from the Wingbermuehle study with the accessions screened here for the first time, we selected, for each SSR primer, a representative genotype for each allele that had been

previously identified. Then, for each primer, we re-ran these representatives along with the new genotypes. In this way, we could unambiguously assign SSR allele genotypes to each new accession. We used sixty-nine SSR markers in this study. For two of these markers (HvLTPPB and Ebmatc0028), two loci were used in analysis for a total of 71 polymorphic loci analyzed. Primers were chosen that both provided coverage spanning the barley genome and included eight markers that have been previously linked to FHB resistant quantitative trait loci (QTL) (de la Pena *et al*., 1999; Mesfin *et al*., 2003).

Table 1. Recently identified FHB resistant accessions from the spring six-rowed barley world collection.

Accession	Origin	Accession	Origin
CIho 2492	Sweden	PI 328642	Romania
PI 467654	Finland	CIho 9625	Ethiopia
CIho 6613	United States	PI 383090	Ethiopia
PI 371317	Switzerland	CIho 4530	China
PI 371308	Switzerland	PI 565567	China
PI 370919	Switzerland	PI 565583	China
PI 370984	Switzerland	PI 565854	China

Table 2. Midwest varieties, FHB resistant breeding lines, and FHB resistant varities.

Line	Description		Line	Description
AC Oxbow	FHB resistant variety, 2-row, covered hull, origin - Canada		FEG4-98	U of MN breeding line, 6-row; FHB resistance derived from Atahualpa
Atahualpa	FHB resistant variety, 2-row, hulless, origin - Ecuador		FEG6-28	U of MN breeding line, 6-row; FHB resistance derived from Kitchin
Chevron	FHB resistant variety, 6-row, covered hull, origin - Switzerland		Foster	6-row malting variety developed at NDSU
Frederickson	FHB resistant variety, 2-row, covered hull, origin - Japan		Lacey	6-row malting variety developed at the U of MN
Hor211	FHB resistant variety, 6-row, hulless, origin - Ukraine		Legacy	6-row malting variety developed by Busch Agricultural Resources, Inc.
Kitchin	FHB resistant variety, 2-row, covered hull, origin - USA		M100	U of MN advanced breeding line, 6-row
Zhedar1	FHB resistant variety, 2-row, covered hull, origin - China		M104	U of MN advanced breeding line, 6-row
CIho 9831	2-row, covered hull, origin – Japan		M105	U of MN advanced breeding line, 6-row
Conlon	2-row variety developed by North Dakota State University (NDSU)		M81	U of MN advanced breeding line, 6-row
Drummond	6-row malting variety developed at NDSU		M84	U of MN advanced breeding line, 6-row
Excel	6-row malting variety developed at the University of Minnesota (U of MN)		MAS2-002	U of MN breeding line, 6-row; FHB resistance derived from Kitchin
FEG14-119	U of MN breeding line, 6-row; FHB resistance derived from AC Oxbow		MAS2-054	U of MN breeding line, 6-row; FHB resistance derived from Kitchin
FEG2-26	U of MN breeding line, 6-row; FHB resistance derived from Zheddar #1		MNBrite	6-row variety developed by the U of MN, moderately resistant to FHB
FEG4-66	U of MN breeding line, 6-row; FHB resistance derived from Atahaulpa		Robust	6-row malting variety developed at the U of MN

All gels were run using the Global IR2 collection system with gel images recorded by E-seq software (LI-COR, Inc). GeneImagIR software (LI-COR, Inc) was used to size bands on gel images. Final band scores were determined visually with the aid of GeneImagIR. Data from the two studies were combined and organized using Microsoft Excel. Cluster and principal coordinate (PCO) analyses were conducted using NTSYSpc software (Exeter Software). Cluster analyses were calculated using the unweighted pair-group method, arithmetic average (UPGMA) algorithm.

RESULTS AND DISCUSSION

Using the full set of 71 SSR loci spanning the barley genome, we determined the genetic relationships of new FHB resistant barley accessions to resistant parent sources already in use and to existing varieties and breeding lines. From this analysis, larger clustering of groups is most evident with the PCO (Figure 1), while relationships between subsets of samples are easier seen with the cluster diagram (Figure 2). Both PCO and cluster analysis show genotypes falling into two major groups. The first group contains all of the six-rowed varieties and breeding lines. The second group contains the new resistant sources, the resistant sources already in use, and the two two-rowed varieties CIho9831 and Conlon.

Particularly interesting with respect to FHB breeding efforts are the relationships in the second group – those within and between the new resistance sources and those already being used. Within this cluster there is clearly more variation than within the variety and breeding line group (Figure 2). In addition, overall clustering does not appear to group genotypes by origin of accession. Toward the goal of combining different sources of resistance into a common variety, choosing new parent sources from those most different to any already being used is likely the best option.

Of the FHB resistance sources already in use as parents, Frederickson and Zhedar 1 (both two-rowed), are highly similar at 97%. All other parent sources appear to be relatively different. Based on a similarity index using the 71 SSR marker loci set, all other parent sources are less than 40% similar to one another (data not shown). Overall, the new FHB resistant accessions are also not highly similar to one another. This is with the exception of PI371308 and PI383090, which are 94% similar. Between the sources already in use and the new sources, two relationships stand out. First, Hor211 clusters closely with PI370919 and PI467654. Second, Chevron is most like accession CIho6613 at 74% similarity. While genetic dissimilarity does not insure that accessions will contain different genes for FHB resistance, maximizing the dissimilarity between new parents and those already in use, should increase the chance of identifying and combining different FHB resistance.

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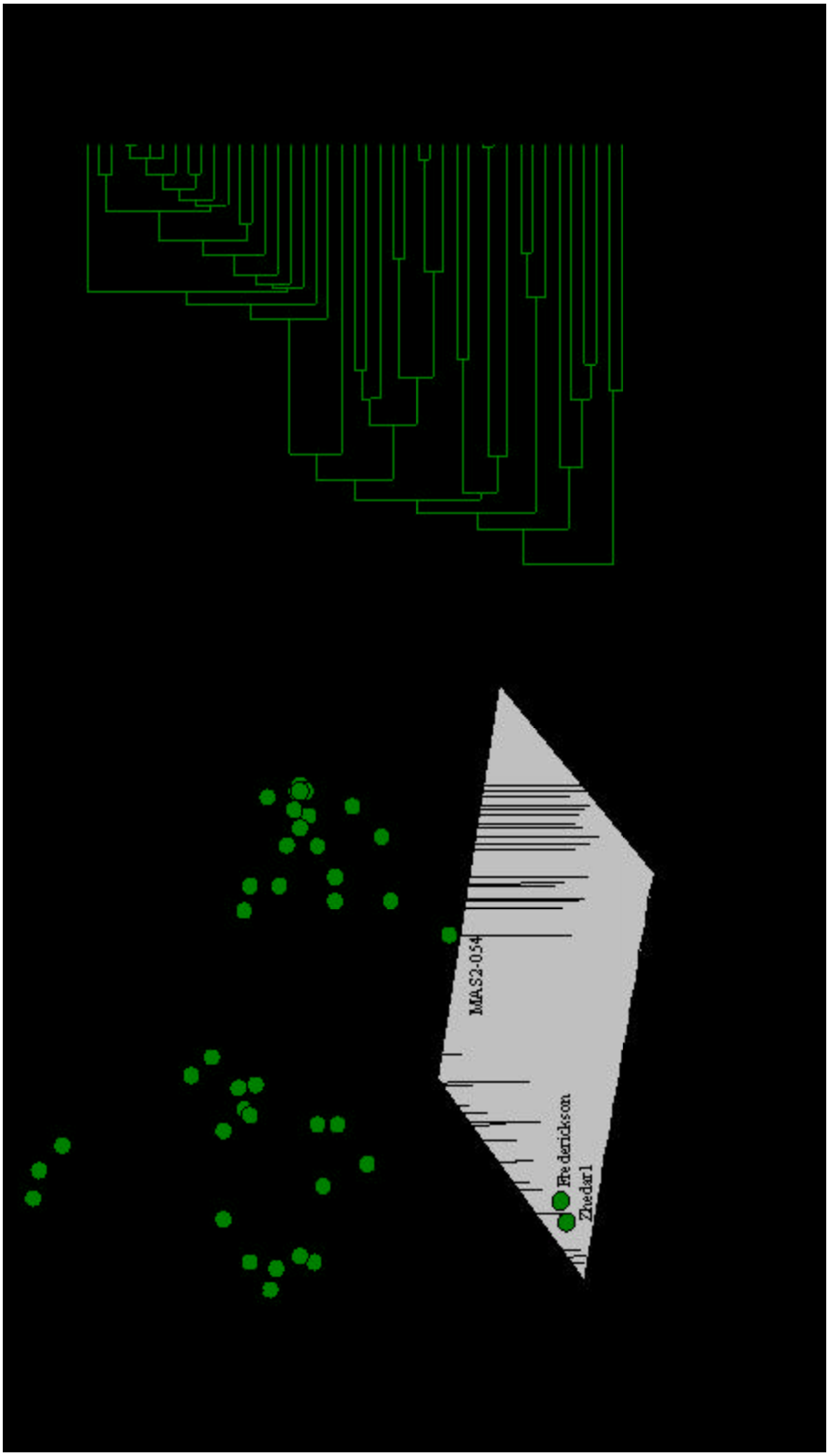
MAS2-054
Frederickson
Zhedar1

MAPPING FUSARIUM HEAD BLIGHT RESISTANCE QTL IN THE CHINESE WHEAT LINE FUJIAN 5114

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ABSTACT

Breeding for resistance to Fusarium head blight (FHB) is facilitated by the identification of different resistance lines and resistance QTL. A population of 78 recombinant inbred lines (RIL) was developed from the cross Fujian 5114/Norm, and was screened for FHB resistance in three field and two greenhouse experiments. Fujian 5114 is a spring wheat cultivar from the Fujian Province of China. Fujian 5114 has levels of FHB resistance similar to 'Sumai 3', but putatively differs from Sumai 3 in some resistance loci. The RIL population was evaluated for FHB severity and visually scabby kernels (VSK) in mist-irrigated, inoculated field trials in the summers of 2000 and 2001. The population was also evaluated for spread within the spikelet from point inoculations in two greenhouse trials in 2001. The results generally correlate well (r = 0.29-0.82 for correlations with p<0.05), with the best correlations resulting from the greenhouse experiments. In the field study, the proportion of variance due to RIL was 29% and 30% for field severity and VSK, respectively, and variance due to RIL X Environment was 34% and 12%. Heritability on an entry mean basis ranged from 0.90 in the greenhouse to 0.66 in the field FHB severity evaluations. Sixty microsatellite markers were mapped on the entire population and this information was combined with phenotypic data for QTL analysis. Interval analysis confirmed the presence of the 3BS resistance QTL in Fujian 5114. This QTL explained up to 28% of the phenotypic variation in FHB. An additional QTL was identified on chromosome 5BL, explaining up to 25% of the variation in FHB severity. The R^2 values of the two QTLs are higher for the two greenhouse experiments than those of the field experiments. The QTL on 5BL appears to be associated with delayed spread of the disease, as the corresponding R^2 values were reduced from the 15 to the 21 day greenhouse evaluations. These results indicate that Fujian 5114 contains some FHB resistance loci that differ from Sumai 3. Additional investigation of the 5BL QTL for breeding of increased resistance to FHB is warranted.

MOLECULAR MAPPING OF QTLS FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT

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INTRODUCTON

Aim of this work was to detect QTL for combined type I and type II resistance against FHB and estimate their effects in comparison to the QTL identified previously for type II resistance (Bai *et al*. 1999, Waldron *et al*. 1999, Anderson *et al*. 2001, Buerstmayr *et al*. 2002).

MATERIALS AND METHODS

Plant materials

The population of F_1 derived doubled haploid (DH) lines which was described in Buerstmayr *et al*. (2002) was used for this research. The resistant parent was 'CM-82036' (originating from Sumai3 x Thornbird) and the susceptible parent was 'Remus'. In total 364 DH lines were available.

Field experiments for evaluation of Fusarium head blight resistance

The lines were evaluated during two seasons (1999 and 2001) at the experimental field of IFA-Tulln. Trial location, seed treatment, plot size, sowing density and crop management were the same as described in Buerstmayr *et al*. (2002). Inoculation was done in separate experiments by spraying heads at anthesis with one *F. culmorum* or one *F. graminearum* isolate as described in Buerstmayr *et al*. (2000). Resistance reaction was assessed out in percent diseased spikelets per inoculated plot on days 10, 14, 18, 22 and 26 after inoculation.

Genotyping of the DH population with molecular markers

Genotyping of 239 DH lines was performed using 28 RFLP, 267 AFLP, 112 SSR, 3 storage proteins and one morphological marker. With the markers Barc75, Gwm389, Gwm1034, Gwm533, Barc133, Gwm493, Barc141, Barc40, Gwm304, Gwm293, Barc117, Barc186, and Barc1, which appeared to be close to one of the putative QTL regions, additional 122 DH lines were genotyped and included in the QTL mapping.

Statistical analysis

The FHB severity data were analyzed by ANOVA. Linkage maps were constructed using MAPMAKER 3.0b for MS-DOS (Lander *et al*. 1987). QTL analysis was done by simple interval mapping and composite interval mapping using PLABQTL (Utz and Melchinger 1996).

RESULTS AND DISCUSSION

The population showed significant quantitative variation for FHB severity readings (Figure 1). The genotype by isolate interaction was non-significant underlining the horizontal nature of FHB resistance in wheat.

QTL analysis revealed that two genomic regions were significantly associated with FHB resistance in that population, mapping to chromosomes 3B (*Qfhs.ndsu-3BS*) and 5A (*Qfhs.ifa-5A*) respectively (Table 1). The two QTL explained together 47 % of the phenotypic variance for visual disease severity. The peaks of the LOD profiles obtained by simple and by composite interval mapping were in the same regions (Figure 2). The two QTL on 3B and 5A mapped to the same genomic regions as in our previous study for type II FHB resistance (Buerstmayr *et al*. 2002), with the exception that we did not find a QTL after spray inoculation on chromosome 1B. Our results concerning the *Qfhs.ndsu-3BS* locus are in full agreement with Waldron *et al*. (1999), Anderson *et al*. (2001) and Zhou *et al*. (2000). A significant QTL in the *Qfhs.ifa-5A* region was also detected by D. Somers (AG Canada, Winnipeg, pers. comm.). In the present study using spray inoculation, the effects of the two QTL were in a comparable range. On the contrary, after single floret inoculation, the 3B QTL had a much larger effect than the 5A QTL (Buerstmayr *et al*. 2002). This is an indication that *Qfhs.ifa-5A* may contribute more towards type I resistance and to a lesser extent to type II resistance whereas *Qfhs.ndsu-3BS* appears to play a role primarily in type II resistance.

For both QTL the allele conferring resistance originated from the resistant parent ‘CM-82036’. The association of the two QTL on 3B and 5A with the phenotype is shown in Table 4. Lines with the ‘resistant’ allele (originating from ‘CM-82036’) at both QTL regions had a mean FHB severity of only 20 % compared to lines with the alleles from susceptible ‘Remus’ which reached on average of 58 % bleached spikelets after 26 days (Table 2).

Both QTL regions are already well covered by SSR markers. Marker assisted selection for the two major QTL appears therefore feasible and should help breeders to select for improved lines with combined type I and type II resistance.

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Table 1. QTL estimate for mean values of percentage of infected spikelets on day 26 after inoculation (FHB-26) over two years of the experiments in Tulln. QTL are described by chromosome location, logarithm of odds (LOD) and percentage of explained phenotypic variance (R^2). QTL analysis was carried out by composite interval mapping.

		FHB-26	
Map interval	QTL	LOD	R^2
Xgwm533 - Xgwm493	*Qfhs.ndsu-3BS*	29.1	31.6
Xgwm293 – Xgwm156	*Qfhs.ifa-5A*	20.5	23.2
Simultaneous fit			46.9

Table 2. Effect of alternative alleles at two QTL regions for mean percentage of infected spikelets 26 days after inoculation (FHB-26) for line means obtained in Tulln over two years.

QTL*)			FHB-26		
Qfhs.ndsu-3BS	*Qfhs.ifa-5A*	Number of lines	Median	Mean	Stderr.
CM-82036	CM-82036	87	19.8	21.9	8.5
CM-82036	Remus	74	34.1	34.8	13.6
Remus	CM-82036	73	37.5	39.6	13.1
Remus	Remus	110	58.3	57.7	16.1

*) Only lines with non-recombined *Xgwm533 – Xgwm493* (*Qfhs.ndsu-3BS*) and *Xgwm293 – Xgwm156* (*Qfhs.ifa-5A*) intervals were included in these calculations.

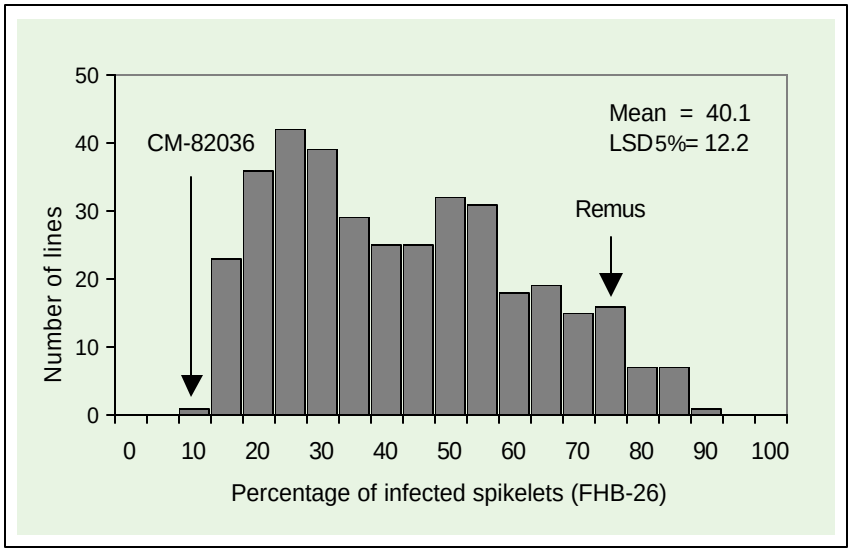

Figure 1. Frequency distribution of 364 DH-lines for mean values of FHB severity on day 26 after inoculation (FHB-26). Arrows indicate values of the parental lines. The overall population mean and the least significant difference for comparison of line means (a = 0.05) using the genotype by year interaction mean square as an error term are given also.

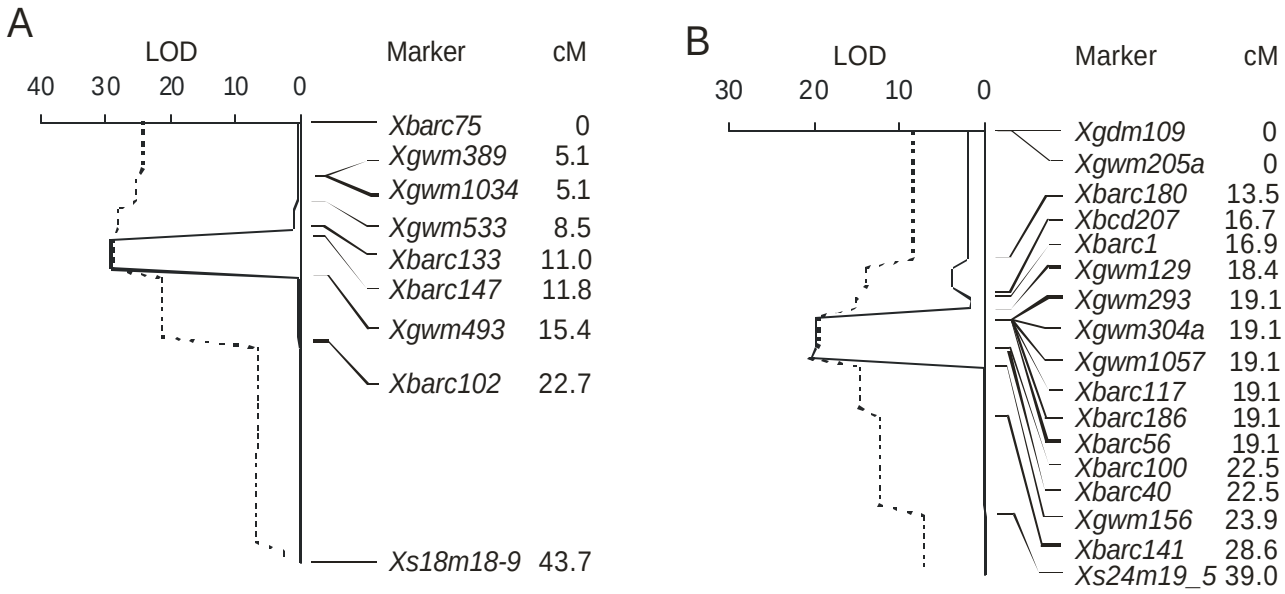

Figure 2. Interval analysis of QTL for percentage of infected spikelets on day 26 after inoculation (FHB-26) on linkage groups corresponding to chromosomes 3B (**A**) and 5A (**B**). LOD values were calculated by composite interval mapping (solid line) and simple interval mapping (dotted line).

QTL MAPPING AND SSR GENOTYPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN VIRGINIA TECH WHEAT BREEDING PROGRAM

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ABSTRACT

Mapping of quantitative trait loci (QTLs) associated with Fusarium head blight (FHB) resistance and application of marker assisted selection (MAS) can be used to accelerate development of FHB resistant wheat varieties and to provide for a better understanding of the mechanisms governing resistance. Two F_2 and two corresponding F_1-derived doubled haploid populations of Pion2684 x W14 and Madison x W14 were used in discerning the inheritance and identity of QTLs associated with FHB resistance in wheat line W14. In the F_2 populations, two complementary genes with major effects were postulated to govern FHB resistance. This was confirmed upon subsequent evaluation of doubled haploid populations in two independent experiments in 2001 and 2002. Microsatellite markers (SSRs) were used to identify QTLs associated with FHB resistance. Seventy six percent (152 out of 200) of SSRs detected polymorphism between parents. Among 36 pairs of primers used to date, a total of 45 loci on three chromosome regions (2BS, 3BS, and 5AL) have been comparatively mapped in one F_2 of W14 x Pion2684 and two doubled haploid populations of W14 x Pion2684 and W14 x Madison. Fifteen markers were significantly ($p < 0.05$) associated with FHB resistance, and explained 21%, 36% and 31% of the total variation of disease severity in F_2, $F_{2:3}$, and DH populations of W14 x Pion2684, respectively. These markers also explained 43% of total variation of disease severity in DH population of W14 x Madison. Nine of the 15 SSRs were used to genotype in 27 FHB resistant soft red winter wheat lines to determine the putative contribution of QTLs on these chromosomes to FHB resistance and the potential for using these SSRs in MAS. Among the nine SSRs loci genotyped, Xgwm 493 in the 3BS QTL region and Xgwm156 in the 5AL QTL region were the most commonly detected loci having the same fragment size as the FHB resistant wheat lines Sumai 3 and W14. In contrast, Xgwm 533 was detected in only five of 27 lines, while the other 22 lines have alleles at 2BS and/or 5AL QTL loci. The contribution of individual QTL towards FHB resistance will be evaluated further in doubled haploid and near-isogenic line populations.

INSIGHT IN THE DIFFERENTIALLY EXPRESSED GENES IN RESPONSE TO *FUSARIUM* MYCOTOXINS IN FHB RESISTANCE WHEAT NOBEOKABOUZU-KOMUG

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ABSTRACT

Fusarium head blight (FHB, scab) is a fungal disease of wheat and other small cereals that is found in both temperate and semi-tropical regions. FHB causes severe yield and quality losses, but the most-serious concern is the possible mycotoxin contamination of cereal food and feed. Breeding for FHB resistance by conventional selection is feasible, but tedious and laborious. This study was conducted to identify the response genes to *Fusarium* mycotoxins believed to be held by the Japanese variety Nobeokabouzu-komugi, which is highly resistant to FHB, and to construct a stressed ESTs library from this bread wheat variety. For this purpose, suppression subtractive hybridization (SSH) technique was used as it combines normalization (*suppression of abundant transcripts and enrichment of rare transcripts*) and subtraction (*isolation of differentially expressed transcripts*) and as it is a powerful approach to identify and isolate genes which are transcribed under a certain stress. It may as well help in understanding the complex regulating mechanism of resistance to FHB. Nobeokabouzu-komugi seeds were germinated in water; and then transplanted in a phosphorous solid solution with and without metabolite of *Fusarium graminearum* including 10 ppm DON. The root meristems were collected and used for total RNA extraction. cDNA was synthesized using Clontech kit and the SSH method was applied to generate differentially regulated cDNA probes. These cDNA probes were cloned by using TA cloning method. Approximately one thousand clones were isolated from the subtracted stressed plants. Out of these clones, 80 random ones were sequenced. Only one duplication was found, meaning that more that 98% are singletons. Further, sequence homology search using BLAST program from NCBI showed that 19 clones present high homology with some ESTs from Fusarium infected spike of another FHB resistance variety, Sumai 3; whereas others show homology to ESTs induced by different stresses specially (vernalization, ABA, dehydration, salt, etiolating, cold, and drought). These clones will be used for the differential display analysis by using micro array.

CONTROL OF SCAB WITH PUROINDOLINE-CONTAINING TRANSGENIC WHEAT

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ABSTRACT

Wheat and barley head blight or scab is a fungal disease caused by two species of *Fusarium* (*F. graminearum* and *F. culmorum*) causing premature ripening and white heads. The wheat puroindoline proteins (PINA and PINB), which are endosperm-specific and contribute to grain softness, also have *in vitro* and *in vivo* anti-fungal properties. These studies have been extended to include wheat *Fusarium* scab. The growth of both *Fusarium* species was negatively affected by PIN in *in vitro* bioassays. Control and transgenic HiLine wheat varieties that overexpress the *pinB* gene driven by the constitutive maize-ubiquitin promoter or by the endosperm-specific glutenin-promoter, were inoculated with *F. culmorum* in both field (summer 2001) and green-house (2001-2002) studies. The plants were analyzed for scab by visual inspection of the heads. The majority of Hi-Line control plants had between 40-70% infected spikelets/head. *PinB*-transgenic lines showed a large increase in plants with only 0-20% infected spikelets/head, a decrease in both the moderately and severely infected heads, and a decrease of the percentage of tombstones, when compared to the control. Experiments are in progress using *F. graminearum* as the fungal pathogen causing the scab on Hi-Line controls and on the *pinB*-containing transgenic wheat. These data suggest that PIN proteins may provide protection to wheat and barley against *Fusarium* scab.

GENETIC ANALYSIS OF TYPE II FUSARIUM HEAD BLIGHT (FHB) RESISTANCE IN THE HEXAPLOID WHEAT CULTIVAR 'WANGSHUBAI'

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ABSTRACT

The dramatic impact of FHB on the wheat production throughout the US has driven breeding and germplasm enhancement projects to search for new potential resistance sources. Chinese introductions show the most potential for resistance to the spread of the infection through the head (Type II). 'Sumai 3' is the most widely used of these introductions, being utilized for genetic studies and breeding purposes. In the Wheat Germplasm Enhancement project at NDSU we are interested in additional resistance sources that can be used in place of or in conjunction with Sumai 3. One of these new sources is the hexaploid wheat cultivar 'Wangshubai'. The level of resistance shown by Wangshubai in our greenhouse evaluations is 7-11%, compared to 15% for Sumai 3; this is consistent with reported results of others. Previous genetic diversity studies had detected no genetic relationship between Sumai 3 and Wangshubai, suggesting different loci or alleles for resistance. In order to dissect the genetic components of Wangshubai resistance to FHB spread, we have developed a population of 388 F6-derived recombinant inbred lines developed from a cross between Wangshubai and ND671 (a susceptible elite line from the HRSW breeding program at NDSU). We have phenotypic data from 4 greenhouse and 2 field evaluations. Infection in the greenhouse experiments was achieved through single floret inoculations at flowering, while the field experiments relied on natural infection. We are using a subset of 88 lines for preliminary QTL analysis. The molecular markers used for this purpose are SSRs previously mapped to specific wheat chromosomes. For confirmation purposes, the chromosomes will be anchored using RFLP markers. The remaining lines will be used for validation of QTL. Preliminary QTL analysis results using 75 SSR markers covering 13 wheat chromosomes shows the presence of major QTL in chromosome 3BS located close to the SSR locus *Xgwm533*. This QTL explained about 25% of the phenotypic variation, and its location is similar to that found in Sumai 3. The amount of phenotypic variation explained is comparable to that explained by the major QTL in Sumai3. However, the level of resistance in Wangshubai (7 to 11% of spread) is better than in Sumai 3 (15% of spread). This fact could be because: 1) both sources have different alleles of the gene/s for the QTL found in chromosome 3BS, or 2) Wangshubai has additional genes contributing to a higher level of resistance. Our plans include completing the genetic map for the population in this study to search for additional QTLs explaining more of the phenotypic variation and to study the possible relationship of resistance to FHB with other traits.

IDENTIFICATION OF SCAB RESISTANCE GENE EXPRESSION IN WHEAT FOLLOWING INOCULATION WITH *FUSARIUM*

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ABSTRACT

Fusarium head blight (scab), caused by fungus *Fusarium* species, is a worldwide disease of wheat (*Triticum aestivum* L.). Chinese cultivar, Ning 7480, is one of few wheat cultivars with resistance to scab. To identify the differentially expressed genes corresponding to scab resistance of Ning 7840, the pooled cDNA libraries at different time-points, 2hr., 4hr., 6hr., 12hr., 24hr., 36hr., 72hr. and 96hr., after inoculation with *Fusarium* were constructed using glume mRNAs from Ning 7480. We performed a PCR-selected cDNA subtraction using the pooled glume mRNAs in the tester (Ning 7480 inoculated with *Fusarium*) and the driver (Ning 7480 inoculated with water). The cDNA libraries were differentially screened by the forward subtracted cDNAs (the tester subtracted against the driver) and the reverse subtracted cDNAs (the driver subtracted against the tester) as probes. 24 cDNA clones were isolated based on their specific hybridization only with the forward subtracted cDNAs, and not with the reverse subtracted cDNAs. Real-time quantitative PCR showed that the known defense response protein, chitinase, was induced at 24 hours and reached maximal induction at 72 hours after inoculation with *Fusarium*. Also, the hypothetical defense response protein, XP_104345, was induced at 12 hours and showed high levels of induction at 72 hours. Two putative defense response genes, Sigma-E factor and a retroelement, were down-regulated early from 2 hours after inoculation in the treated tissue with maximal induction occurring around 72 and 96 hours. The slot-blots containing the above putative defense response genes were probed respectively with the cDNA pools from the tester and driver. The slot-blot analysis confirmed the presence of the cDNA induced with *Fusarium* in all of the four putative defense response genes. The location for these putative genes is proceeding based on nulli-tetrasomics analysis in our lab.

MAPPING GENES CONFERRING FUSARIUM HEAD BLIGHT RESISTANCE IN C93-3230-24

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ABSTRACT

The six-rowed line C93-3230-24, from the cross B2912/Hietpas 5, was identified by researches at Busch Agricultural Resources, Inc. (BARI) to have (FHB) resistance similar to Chevron, and better FHB resistance than either of its parent in a greenhouse test. The genetic background of C93-3230-24 appears to be completely different than that of any of the FBH resistant accessions identified. Thus, this line may have alleles for FHB resistance and DON accumulation not currently identified. The objectives of this study are: 1) to construct skeletal maps that includes RFLP and SSR markers for an F_1-derived DH mapping population developed from the cross Foster/C93-3230-24 and 2) determine the position of QTL controlling FHB resistance, DON accumulation, days to heading and maturity, plant height, and spike nodding angle. Field experiments were conducted in mist-irrigated FHB nurseries in 2001 and 2002 in North Dakota and Zhejiang Province China using 118 DH lines and parents. Single locus analysis using available marker data identified six regions in five chromosomes associated with FHB resistance. The regions are located in chromosomes 2H, 4H, 5H, 6H, and 7H. The region with the largest effect on FHB resistance appears to be in chromosome 2H. Associations between the markers and maturity and/or plant height were found in the same regions as FHB resistance. Results in this study are similar to those obtained in studies using the resistant six-rowed cultivar 'Chevron' and the ICARDA/CIMMYT cultivar 'Gobernadora'. Thus, preliminary results suggest that C93-3230, Chevron, and Gobernadora may have similar alleles for FHB resistance.

TARGETED SATURATION MAPPING OF *QFHS.NDSU-3BS* USING WHEAT ESTS AND SYNTENY WITH THE RICE GENOME

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ABSTRACT

A major QTL, *Qfhs.ndsu-3BS*, for resistance to Fusarium head blight (FHB) has been identified and verified by several research groups. However, the DNA marker density near this major QTL is less than required for map-based cloning. The objective of this project was to develop STS (sequence tagged site) markers from wheat ESTs to increase the marker density near this major QTL. On the basis of synteny between wheat chromosome 3BS and rice chromosome 1S, we initiated a strategy to identify wheat ESTs likely near this QTL. The sequences of BAC/PAC clones located on the distal portion of rice chromosome 1S were compared with wheat ESTs in GenBank using BLASTN search. Primers of STS markers were designed for non-redundant wheat ESTs with E values equal or less than e-15 and the length of identity greater than 100bp. Using wheat deletion lines for chromosome 3BS, 25 out of 79 STS markers were located to the chromosome bin 3BS 0.78-0.87, where this QTL is most likely located. Nine STS markers were mapped in a previously reported Sumai 3/Stoa mapping population. The STS marker *XSTS3B-138* explains 55% of the FHB variation of this mapping population. Therefore, this research strategy is useful for developing a high resolution map of this major QTL region, and may have broad applications for targeted mapping of other traits in cereal crops.

IDENTIFICATION OF QTL ASSOCIATED WITH SCAB RESISTANCE IN ERNIE

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ABSTRACT

Fusarium head blight (scab) in wheat is a major problem worldwide. No source of complete resistance is known. Sumai 3, a cultivar from China, is the major resistant resource across different breeding programs in US. A major QTL conditioning scab resistance in Sumai 3 has been identified on 3BS. Identification of different sources of resistance is critical to breeding scab resistant wheat to reduce the potential for genetic vulnerability. Ernie, a scab resistant cultivar, released from the University of Missouri, appears to have a different set of resistance genes. Using AFLP and SSR markers we have mapped the scab resistant QTL by 300 F_8 recombinant inbred lines (RILs) developed from a cross between Ernie and MO 94-317, a highly susceptible Missouri variety. The scab index (the ratio of infected spikelets to total spikelets of the inoculated head) in these lines ranged from 15.7 to 75.7%. Eight *Eco*RI and 8 *Mse*I primers forming 64 primer pairs were used to screen the parents. Over 80% of these pairs had polymorphic bands. The average number of polymorphic bands was 7 with a range of 2 to 21. Two hundred AFLP and SSR loci were used to construct a linkage map. MapMaker version 3.0 for Unix was used to construct the linkage map. QTL analysis was performed on the scab data using QTL Cartographer version 1.16. Two SSR markers per chromosome were used to anchor the AFLP markers to chromosomes. The QTL information will be useful in developing resistant materials by gene pyramiding.

OVER-EXPRESSION OF ANTI-FUNGAL PROTEIN GENES INCREASES RESISTANCE OF TRANSGENIC WHEAT TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Anti-fungal proteins (AFPs) such as ß-1,3-glucanases, chitinases, thaumatin-like proteins (tlps), thionins and ribosome-inactivating proteins (RIPs) are known to inhibit fungal growth via different mechanisms. Glucanases and chitinases degrade fungal cell walls, tlps and thionins degrade fungal cell membranes and RIPs inhibit fungal protein synthesis. Transgenic wheat (cv. Bobwhite), over-expressing these AFPs, has been generated using micro-projectile bombardment. We have developed 25, 25, 31, 24 and 15 transgenic wheat lines carrying a wheat -puro-thionin, aobarley tlp-1, a barley ß -1,3-glucanase, a barley RIP and a barley chitinase, respectively. In addition, we have developed 10, 11 and 11 transgenic wheat 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 resistance to Fusarium head blight. Four independent glass-house disease screens have been conducted on the tlp-1 lines and two of those lines consistently demonstrated an increase in resistance when compared to non-transgenic controls. Similarly, three disease screens have been conducted with our glucanase lines and four of these lines have performed well. In four disease screens, one -puro-thionin line performed α well in three of the screens, and two more lines performed well in two of the screens, and therefore, have been evaluated further. Molecular characterization of our lines shows that they are genetically independent and that they accumulate the appropriate AFP. In addition, preliminary disease screen data on the remainder of our AFP transgenic lines will be presented.

EFFECT OF CHEVRON ALLELES AT TWO FUSARIUM HEAD BLIGHT RESISTANCE QTL DETERMINED USING NEAR-ISOGENIC LINES

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OBJECTIVES

To evaluate near-isogenic lines, developed for chromosome 2H and chromosome 6H target QTL-regions, for FHB resistance and other confounding traits (heading date and plant height) associated with the disease severity.

INTRODUCTION

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, has caused significant yield and grain quality losses in barley (*Hordeum vulgare* L.) since 1993. Phenotypic selection for FHB resistance has been only modestly effective largely because FHB resistance is highly influenced by the environment and screening methods are laborious and expensive. Marker-assisted-selection (MAS) is a promising tool to augment current methods to breed for FHB resistance.

To utilize MAS for FHB resistance, the barley-breeding and genetics program at the University of Minnesota is engaged in mapping and validating QTL for FHB. We have previously identified FHB resistance QTL distributed across the genome using the source of resistance Chevron (de la Pena *et al*., 1999; Canci *et al*., 2000). We have also used Chevron-derived populations to validate 3 of the QTL; 2 on chromosome 2H and 1 on chromosome 6H (Canci, 2001; Gustus *et al*., 2001). These QTL regions are now candidate targets for MAS, but a significant problem is that they are coincident with QTL late heading date (HD) and tall plant heights (HT).

To further understand the association between FHB resistance and these two other traits (HD and HT) and also elucidate the genetic basis of FHB resistance, it is important to fine map the associated regions. For this purpose, we developed near-isogenic lines (NILs) for chromosome 2H and chromosome 6H target QTL-regions using both molecular marker-assisted backcrossing and heterogeneous inbred families (HIF) procedures. These lines will be useful for studying disease resistance. We have also used the BC-derived NILs as parents to create fine mapping populations.

MATERIALS AND METHODS

Development of the BC_3 NILs: We initiated the development of NILs using donor parents selected from the 101 $F_{4:7}$ progenies previously used for linkage mapping (de la Pena *et al*., 1999). The recurrent parent was the elite line M69. A marker-assisted backcrossing scheme was used to advance selected lines to the BC_3F_2 generation. Six BC_3F_2 lines carrying the FHB-resistance Chevron alleles at each target QTL region were selected and advanced by selfing to the BC_3S_4 generation.

Development of HIFs: Among the 101 $F_{4:7}$ families de la Pena *et al*. used for mapping FHB-resistance QTL in 1999, we selected 12 families segregating at marker loci associated with each target QTL region as HIFs. Ten segregating progeny lines grown from each selected HIF were genotyped with all available SSR markers in the target QTL region. Based on the marker data, two NILs contrasting at a specific marker locus were identified from each HIF and selected as pairs for field evaluation.

Field Evaluations of NILs: The 30 NILs comprising six chromosome 2H BC_3-derived NILs, six chromosome 6H BC_3-derived NILs, and 18 HIF-derived NILs together with parental lines, Chevron and M69, were evaluated at St. Paul and Crookston, Minnesota in the summer of 2002. At each location, entries were planted in 2.4 m long single-row plots spaced at 30 cm apart. The experimental design was a randomized complete block design with three replications. Nurseries at St. Paul were inoculated using the macroconidia inoculation technique (Dill-Macky, 2002). The initial inoculation was performed at heading so as to avoid confounding effect of differences in heading date and potential escape of the pathogen. A second inoculation was repeated three days after initial spraying. At Crookston, a grain spawn inoculation technique was used (Dill-Macky, 2002). Nurseries were mist-irrigated daily after inoculation until soft dough stage. We measured FHB severity, plant height and heading date. To measure FHB severity, 10 random spikes from each plot were examined and the number of infected spikelets from each spike counted and expressed as a percent of the total spikelets present. Heading date was determined as the number of days after planting to 50% emergency from the boot.

Statistical Analysis: Since all NIL pairs have different alleles only at the target QTL region, differences in phenotype can be attributed to the genes in those segments. To determine the effect of each NIL pair, the data were subjected to ANOVA using Proc GLM procedure (SAS Institute, 2000). Means were separated using LSD. A combined analysis across locations was conducted to determine genotype x environment interaction. The combined analysis showed that all measured traits had significant G x E effect. Therefore, results were presented for individual locations. The magnitude of the effect of alleles segregating at the target QTL regions were determined by comparing the means of NILs carrying different alleles at each locus.

RESULTS AND DISCUSION

Results of mean separations for the BC_3-derived NILs showed that NILs carrying the Chevron allele at the chromosome 2H QTL region reduced FHB by 44% for St. Paul and 41% for Crookston (Table 1). This same QTL region increased HD by six days confirming previous studies indicating that the chromosome 2H QTL region is associated with *Eam6*; a maturity gene that affects HD. On the contrary, there was no significant effect of the chromosome 6H region on either FHB or HD. The results for chromosome 2H QTL region are in agreement with Gustus *et al*. (2001), however Gustus found a small but significant reduction in FHB severity with the Chevron allele at the chromosome 6H QTL.

Based on the HIF-derived NILs, the Chevron allele at the marker *Bmag0140* on chromosome 2H had a similar effect as the BC-NIL reducing FHB by ~40% (Table 2). However, these NILs for this marker did not differ for heading date across the 2 locations (data not shown). Marker

Bmag0807 on chromosome 6H was associated with a 31-35% reduction in FHB. There was no association between HD and any of the analyzed markers on chromosome 6H. However, marker *Bmag0807* was associated with plant height in the two locations (data not shown).

The difference in results between the BC_3 and HIF NILs for chromosome 6H suggest that *Bmag0807* is closer to the FHB QTL than *Bmac0218*, which was used to develop the BC NILs. The general conclusion from this study is that the BC NILs for chromosome 2H should be useful for fine mapping FHB and HD. Since the BC NILs developed for chromosome 6H using *Bmac0218* did not appear to carry FHB resistance, we are looking back at BC2 lines generated in this project to see if they carry the Chevron allele at marker locus *Bmag0807*.

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Table 1. Means for Fusarium head blight (FHB), heading date (HD) and plant height (HT) for backcross-derived NILs and parents.

		FHB Severity (%)		**Heading Date**		**Plant Height (cm)**	
Genotype	No. of Lines	St. Paul	Crookston	St. Paul	Crookston	St. Paul	Crookston
Chevron	1	4.4c	1.7c	56.7a	54.7a	85.3a	105.5a
M69	1	55.6a	23.2a	48.0c	48.0b	75.3b	82.5c
BC_3Chr.2	6	30.9b	11.2b	54.6b	54.2a	76.9b	89.0b
BC_3Chr.6	6	65.7a	22.9a	49.6c	47.7b	75.6b	88.9b

Means within the same column followed by the same letter are not significantly different (P=0.05).

Table 2. Mean FHB severity of NILs derived from HIFs and contrasting at 5 marker loci

				FHB Severity (%)	
Chromosome	No. of NILs	Marker	Genotype	St. Paul	Crookston
2H	6	Bmag0125	A	31.5b	6.9b
			B	41.8a	10.8b
2H	6	Bmag0140	A	34.4b	8.3b
			B	61.5a	14.1a
2H	6	Bmac0093	A	36.1a	8.8a
			B	37.6a	8.5a
6H	6	Bmag0807	A	28.1b	7.0b
			B	43.5a	10.2a
6H	6	Bmag0870	A	26.2b	7.8a
			B	40.0a	9.1a

A=Chevron; B=M69.
Means within the same column followed by the same letter are not significantly different (P=0.05).

SATURATION GENETIC AND PHYSICAL MAPPING OF CHROMOSOME 3 FUSARIUM HEAD BLIGHT QTL REGION

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ABSTRACT

Complete resistance to Fusarium head blight (FHB), caused by *Fusarium graminearum* in the USA, has been a difficult goal to attain. To date, no single gene-for-gene resistance mechanisms have been discovered. Quantitative trait loci (QTL) for resistance against FHB have been mapped in several segregating populations. Eighteen of the 21 chromosomes in wheat and all 7 chromosomes in barley have been reported to be associated with resistance. In three or more mapping studies conducted using Chinese wheat cv. Sumai 3 as the resistant parent, QTL on chromosomes 3BS and 6BL were discovered. QTL for FHB resistance have also been mapped to chromosomes 2H, 3H, and 1H in several different barley populations. Because of the high level of synteny between grass species, it was determined that the QTL on 3BS and 3H reside in a syntenous position, between restriction fragment length polymorphism (RFLP) markers BCD907-ABG471. Using the resources provided by the Rice Genome Project sequencing effort, we have targeted phage artificial chromosomes (PACs) from rice chromosome 1 that are located in a syntenous position to 3HS in barley. By using the blastn function on the NCBI web site and limiting the search to genus *Hordeum*, barley expressed sequence tags (ESTs) can be identified with homology to the individual PACs. Eighty-seven unique barley ESTs were identified that covered 18 PACs. To date, 24 ESTs have been screened against the cv. Morex bacterial artificial chromosome (BAC) library. These 24 ESTs identified 193 BAC clones. We have genetically mapped 12 ESTs to date and 7 mapped in the target region on 3HS in the Steptoe/Morex DHL population and the Foster/CI4196 RIL population. The ratio of 7 out of 12 mapping to the target region is sufficient to expect to saturate the region since additional PAC clones are available from the target region. Another source of ESTs that we are experimenting with are the wheat ESTs mapped to group 3 using the wheat deletion lines (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi).

MICROSATELLITE GENETIC MAP IN WHEAT

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ABSTRACT

Genetic maps saturated with informative markers are of great importance for localizing and manipulating important genes or QTLs. In recent years, microsatellite loci, also referred to simple sequence repeats (SSRs) have proved to be a valuable source of highly polymorphic DNA markers. SSR polymorphisms are based on differences in the length of simple sequence repeats at loci defined by locus-specific PCR primers flanking the microsatellite. Currently, approximately 350 publicly available wheat microsatellite primer pairs have been reported in the peer reviewed literature (Röder *et al*. 1998; Korzun *et al.* 1997; Devos *et al* 1995; Pestsova 2000; Salina *et al* 2000). To date, we have developed more than 400 new SSR primer pairs, 209 of which generate PCR products which map to 225 loci[(a)] on the ITMI population. PCR products from an additional 137 primer pairs enable physical mapping of 142 loci. The poster associated with this abstract displays the latest version of a genetic/physical map containing over 1400 total loci including 367 Xbarc loci. Detailed information about primer pairs and the loci they amplify will be posted at: http://wheat.pw.usda.gov/ggpages/genomics.shtml

[(a)]Designated with the prefix "Xbarc", where "barc" in the acronym for "Beltsville Agricultural Research Center".

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STRATEGIES FOR COMBATING FUSARIUM IN BARLEY THROUGH GENE EXPRESSION TARGETING, METABOLIC PROFILING AND SIGNALING ANALYSIS

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ABSTRACT

Several basic studies must be undertaken in order to understand the interactions between *Fusarium graminearum* and its barley and wheat hosts: 1) Gene promoters are needed to target the expression of antifungal protein genes to organs that are initially colonized by *F.g.*, 2) Metabolic profiling must be developed to determine which metabolites are extracted from host tissues, and 3) It is important to understand the signaling pathway involved in host perception of *F.g.* invasion and attempts to mount an effective response. We have previously produced a gene promoter (*Lem1*) that is specific for the young lemma/palea. More recently, Tilahun Abebe has used the suppressive subtractive hybridization method to identify genes expressed in lemmas/paleas but not in flag leaves. This led to the development of the *Lem2*, which is specific to the lemma/palea of developing seeds during the period from endosperm elongation through the dough stage. Maria Laura Federico has developed a promoter (*EpiLTP*) that has preferential activity in the pericarp epithelium. A vector (*Ala/gfp*) was developed by Jianming Fu to test coding sequences of genes in a transient system, prior to their use in stable transformation. This has been applied to the expression of the anti-Fusarium gene *Hth1* of barley. Portions of the *Hth1* coding sequence were linked to a polyalanine bridge, followed by *gfp*. This showed that the failure of this endosperm protein to be produced in lemmas resides with sequences encoding the mature peptide. GC-MS by Cynthia Henson showed that early infection of the lemma and pericarp involves accumulation of metabolites that could be essential to fungal metabolism. In particular, metabolites known to be involved in appressorium turgor pressure (trehalose, mannitol and glycerol) were found. Our studies have shown that no alpha-amylase accompanies infection, even when infections are very heavy. We are examining whether the most obvious substrate (starch) is ever mobilized during infection, and we are attempting to develop a metabolic profile for infected tissue. Finally, it is not clear how barley reacts to the F.g. in the early stages of infection. Initial studies have shown that H2O2 is produced at the site of F.g. inoculation on the pericarp. Thus, barley may have the beginnings of a productive response that could be strengthened through breeding/molecular approaches.

TRANSGENE EXPRESSION IN SPRING WHEAT (*TRITICUM AESTIVUM* L.) TRANSFORMED WITH CANDIDATE ANTI-*FUSARIUM* GENES

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OBJECTIVE

To create transgenic wheat lines carrying novel co-dominant loci with the potential for conferring effective and durable resistance to Fusarium head blight (FHB).

INTRODUCTION

Host plant resistance is the most efficient and cost-effective way to protect the wheat crop from FHB. Our aim is to create new germplasm sources of *Fusarium* resistance by using genetic engineering to introduce novel anti-*Fusarium* (AF) genes into wheat. We have designed and constructed a set of transformation vectors that fuse the maize *Ubi*1 promoter to AF genes that target either the *Fusarium* cell walls or membranes or that mitigate the cellular toxicity of the mycotoxins synthesized by the fungus during infection (Table 1). To give our candidate AF genes the best chance of protecting the plant, they must be expressed at high levels in tissues encountered by fungus as it invades and spreads, i.e., young florets. Here we report semi-quantitative and tissue-specific data for expression of these transgenes in wheat. We also use a new technique to visualize transgene expression in wheat tissues and organs.

MATERIALS AND METHODS

Vector and plasmid constructs: The monocot expression vector pUBK (Okubara *et al*., 2002) consists of the *bar* gene conferring resistance to the herbicide bialaphos regulated by the promoter, first exon, and first intron of the maize *Ubi*1 gene (UBI) (Christensen and Quail, 1996). In the AF constructs, the *bar* gene was replaced by candidate AF sequences (Table 1).

Generation, selection and progeny analyses of transformants: Transformants made by particle bombardment of immature embryos of cv. Bobwhite were identified as described (Okubara *et al*., 2002). Transgene inheritance was followed and homozygotes were identified using PCR amplification of genomic DNA with a forward primer from the UBI region and reverse primers specific for each AF coding region.

Transgene expression analyses*:* Semi-quantitative RT-PCR was carried out with 5-600 ng of total RNA from endosperm (data in Table 1) or other organs (Fig. 1) as described (Okubara *et al*., 2002). Transcript-derived cDNA was amplified using a primer specific for the first exon of the *Ubi*1 gene and a reverse primer specific for each AF sequence. Actin amplification from 5 to 40 ng of total RNA served as the internal standard for RNA integrity (Okubara *et al*., 2002). For visual localization of AF and actin transcripts, we modified a method for *in situ* RT-PCR

(Kolti and Bird, 2000), adapting it for whole mounts of cereal organs and tissues (Somleva, unpublished).

RESULTS AND DISCUSSION

We used semi-quantitative RT-PCR to measure transgene expression in endosperm of hemizygotes and homozygotes from early generations of our initial set of transgenic lines (Table 1). Levels of transgene steady state mRNA varied among independent transformation events. In all, 16 hexaploid wheat lines have shown detectable levels of expression of the AF genes. Lines AB5-126 and C3-9 and C3-10, C1-3 and C9-25, C17-20, and AB8-7 and AB8-50 express the highest amounts of transgene constructs for *tlp-1*, FvExo, FvGlu and FvEndo, respectively. The highest *TRI101* transcript accumulation was observed in lines 156 and 176 (Okubara *et al*., 2002). Transgenes with coding regions of fungal origin were expressed at least 10-fold lower, on average, than the wheat *tlp1* transgenes from the same promoter. FvGlu exhibited about 100-fold lower expression than wheat *tlp* transgenes or endogenous actin.

Because of the possibility of gene silencing, there is no guarantee that primary transformants showing strong expression will produce progeny with the same characteristics. Therefore, we used semi-quantitative RT-PCR to measure mRNA levels in T_4-T_7 endosperm from homozygous progeny of some of our lines (Table 1). Nearly all of those tested had expression levels as high or higher than in earlier generations. Only one line, C17-21, had lost expression. Evidently transgene silencing did not occur in the majority of our lines, either in later generations or in the transition from the hemizygous to the homozygous state. Even transgenics containing the wheat *tlp* gene, which is completely homologous to wheat endogenous genes, maintained their expression levels. The increases in transgene expression in lines AB8-7 and C1-3 are higher than can be accounted for by the two-fold increase in gene copy number in homozygotes compared to early generation hemizygotes. A similar additive effect of transgene expression has been observed in homozygous rice plants (James *et al*., 2002).

To compare transgene expression levels among different parts of the plant, semi-quantitative RT-PCR was performed on mRNA from leaves, endosperm, anthers and ovaries of two FvExo lines (Fig. 1). Transcript levels were highest in endosperm and lowest in ovaries. This result shows that measurements of endosperm mRNA levels are not necessarily predictive of transgene expression in other organs. We plan to measure transcript levels in the outer tissues of the floret, since that is the first part of the plants encountered by the fungus. In addition, we are exploring the potential utility of other promoters to support stronger AF gene expression in floral tissues.

To more precisely localize expression from the UBI promoter, we have adapted a method of *in situ* RT-PCR (Koltai and Bird, 2000) for whole mounts of wheat tissues and organs. UBI-driven expression of various AF genes can be detected in lemma, pollen and stigma, but not in anthers (Fig. 2). These results agree with experiments using GUS fusions to report UBI activity in transgenic wheat (Stoger *et al*., 1999). However, the *in situ* method has the potential for more precise and construct-specific localization.

Table 1. AF gene expression in transgenic wheat.

Anti-*Fusarium* coding regions fused to UBI	**Line name**	**Expression results[6] RNA analysis in T_1-T_2 generations**	**RNA analysis in T_4-T_7 generations[7]**
wheat *tlp1*[1]	AB5-126	High[8]	n. a.
	C3-9	Medium[9]	High (T_5)
	C3-10	High	High (T_5)
Fs *TRI101*[2]	AB6-74	Low[10], some unspliced	n. a.
	AB6-176	Low-Medium, some unspliced	n. a.
	AB6-156	Low-Medium, some unspliced	n. a.
	B65-49	Low, some unspliced	n. a.
FvEndo[3]	AB8-7	Low	High (T_7)
	AB8-15	Low	n. a.
	AB8-50	Medium	High (T_5)
	AB8-108	Medium	n. a.
FvExo[4]	C1-3	Medium	High (T_5)
	C9-25	Medium	Medium-High (T_5)
FvGlu[5]	AB9-59	Low	n. a.
	C17-20	Low	Low (T_4)
	C17-21	Low	n. d.

[1]*T. aestivum* leaf cDNA encoding a thaumatin-like protein (Rebmann et al., 1991); [2]*Fusarium sporotrichioides* gene encoding DON acetyltransferase (McCormick et al., 1999); [3, 4, 5]*F. venenatum* cDNAs encoding an endochitinase, exochitinase, and glucanase (Berka, unpublished); [6]Relative levels based on RT-PCR of endosperm mRNA. [7]Homozygous plants. [8]RT-PCR amplification products of similar intensity to those from actin in 5-40 ng total RNA. [9]Detectable expression in 50-200 ng total RNA. [10]Expression was only detected in 600 ng RNA. n. a. = not analyzed; n. d. = not detected.

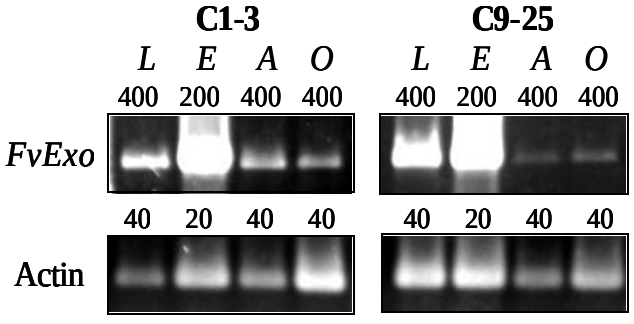

Figure 1. RT-PCR analyses of *FvExo* expression in four wheat organs. (*L* leaves, *E* endosperm, *A* anthers, *O* ovaries). Actin amplification was used as a standard for RNA integrity. Total RNA amount [ng] used in each assay is indicated above the bands.

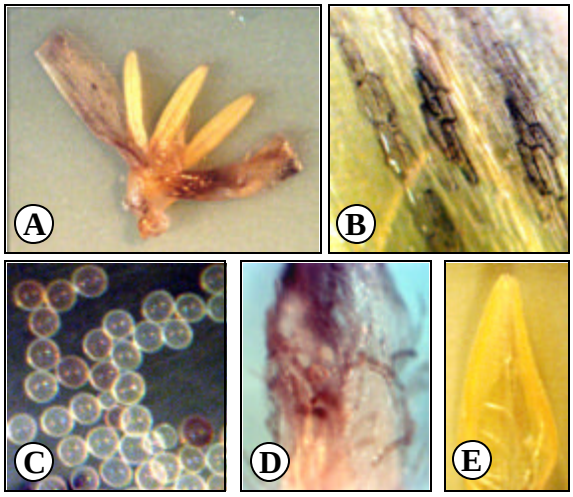

Figure 2. Localization of transgene mRNA by whole-mount *in situ* RT-PCR. Gene expression is visible as a *purple* (dark) precipitate. (**A**) Positive control - actin expression in a floret from a non-transformed plant (7x); (**B**) Wheat *tlp-1* expressing cells in the lemma (50x); (**C**) *TRI101* mRNA in pollen grains (90x); (**D**) *TRI101* transcripts in the stigma (90x); (**E**) A negative control – no expression of *FvEndo* was detected in palea after *in situ* RT-PCR without reverse transcription (7x).

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MOLECULAR MAPPING OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN THE SPRING WHEAT CULTIVAR FRONTANA

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ABSTRACT

Fusarium head blight (FHB, scab) may cause severe yield losses, but the most serious concern is the mycotoxin contamination of cereal food and feed. Breeding for FHB resistance by conventional selection is feasible but tedious and expensive. Despite that resistance originating from Sumai 3 is already well characterized (Anderson *et al*. 2001, Buerstmayr *et al*. 2002) only limited molecular genetic information is available on other sources of resistance.

A population of 210 doubled haploid (DH) lines originating from the F1 of the cross Frontana (moderately resistant) by Remus (susceptible) were evaluated for the expression of Fusarium head blight resistance traits in field trials in the seasons 1999 and 2001. Inoculation and evaluation methods used were similar to Buerstmayr *et al*. (2000).

The population was genotyped with more than 560 markers (SSR, AFLP, RFLP). QTL analysis revealed significant association of several genomic regions with FHB severity. The most prominent and consistent QTL effect was detected on chromosome 3A (LOD=5.3 R-square=13.3), associated with the SSR markers GWM1110 and GWM1121, and tentatively named *Qfhs.ifa-3A*.

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EXAMINATION OF MOLECULAR VARIABILITY OF *FUSARIUM CULMORUM* ISOLATES

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ABSTRACT

Fusarium head blight is the most important disease of wheat in Hungary. The main causative agents of this disease are *Fusarium graminearum* and *F. culmorum*. Mycotoxin contamination is the most serious effect of ear fusariosis, since the mycotoxins produced are harmful both to humans and animals. We examined the mycotoxin producing abilities and molecular variability of *Fusarium culmorum* isolates using different techniques. Altogether 11 Hungarian and 28 other *F. culmorum* isolates were involved in this study, together with *F. graminearum*, *F. crookwellense* and *F. pseudograminearum* strains. Mycotoxin producing abilities of the isolates were tested by thin layer chromatography. The mycotoxins tested involved deoxynivalenol (DON) and its acetylated derivatives, nivalenol (NIV), zearalenone and fusarenone X. Most of the isolates produced zearalenone. 28 isolates were found to belong chemotype I (producing DON and 3-acetyl-DON), while 8 represented chemotype II (producing NIV and/or fusarenone X) according to Miller *et al*. (1991). Among the Hungarian isolates, one produced NIV, while all other isolates belonged to chemotype I. Pathogenicity tests were carried out as described previously (Mesterházy, 1985). Isolates belonging to chemotype I were in general found to be more pathogenic in *in vitro* tests than those belonging to chemotype II. Phylogenetic analysis of random amplified polymorphic DNA (RAPD) profiles of the isolates obtained by using 40 different random decamers let us cluster the isolates into different groups, although the variability observed was relatively low. Most Hungarian isolates formed a well-defined cluster on the dendrogram. Sequence analysis of a putative reductase gene fragment of the isolates was also carried out. Strong correlation was observed between the geographic origin of the isolates, and their position on the cladogram produced based on sequence data. These observations are in agreement with the previous finding, that a similar correlation between geographic origin and sequence data exists in the case of *F. graminearum* isolates (O'Donnell *et al*., 2000). Correlation was not observed between sequence relationships and mycotoxin producing abilities or pathogenicity of the strains. Double-stranded RNA elements indicative of mycovirus infection were detected for the first time in 5 *F. culmorum* isolates. The sizes of the dsRNA elements varied between 0.6-3.9 kbp. Correlation was not observed between the presence of mycoviruses and geographic origin, mycotoxin production or pathogenicity of the isolates. Further work is in progress in our laboratory to reveal the structure of Hungarian *F. culmorum* populations, and to further characterize their mycoviruses.

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A NON-CODING WHEAT RNA MAY PLAY AN IMPORTANT ROLE IN WHEAT RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

To understand the molecular events underlying Fusarium head blight (FHB) resistance in wheat (*Triticum aestivum* L.), gene expression profiles (GEP) were compared between the *Fusarium graminearum*-inoculated and the water-inoculated (mocking inoculation) spring wheat cultivar Sumai 3 (FHB—resistant). One GEP, designated as G12 which is specifically expressed in the pathogen-inoculated Sumai 3, was identified, cloned and sequenced. Southern blot verified that G12 represented a wheat gene. The corresponding full-length cDNA, designated as G12-S, was cloned with 5'RACE technology and sequenced. Bioinformatic analyses indicated that the sequence of G12-S is similar to the minus strand of the wheat chloroplast gene encoding ATP synthase CF-O subunit I. No significant open reading frame is found in G12-S sequence, indicating that it may function at RNA level by directly targeting the complementary transcripts of the chloroplast ATP synthase CF-O subunit I gene and/or its likes to neutralize their ability to translation.

A PUTATIVE ACYL-COA-BINDING-PROTEIN OF *FUSARIUM GRAMINEARUM* MAY PLAY AN IMPORTANT ROLE IN THE FHB PATHOGENESIS IN WHEAT

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ABSTRACT

To identify the genes important to the pathogenesis of Fusarium head blight (FHB) in wheat, we compared the gene expression profiles (GEP) between *Fusarium graminearum* (isolate Fg4) infected and healthy spikelets, as well as between FHB-resistant cultivar Sumai 3 and FHB susceptible cultivar Wheaton. Several GEPs specific to *Fusarium*-infected Sumai 3 were identified, cloned and sequenced. Southern analysis indicated that GEP 4CL represents a *F. graminearum* gene. With 5'RACE technology, corresponding full-length cDNAs were cloned from the FHB-infected spikelets of Sumai 3 and Wheaton and from *F. graminearum* culture, respectively. Sequence polymorphisms were observed in the 5' untranslated region among the three full-length cDNA clones. Bioinformatic analyses indicated that the cognate gene may encode an acyl-CoA-binding-protein (ACBP) protein. The possible role of the putative ACBP protein in *F. graminearum*'s pathogenicity and the importance of the differential mRNA editing to FHB pathogenesis in wheat were discussed.

IDENTIFICATION OF CHROMOSOME REGIONS ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE IN BREAD WHEAT CULTIVAR SUMAI 3 WITH ITS SUSCEPTIBLE NILS BY USING DNA MARKERS

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ABSTRACT

Fusarium head blight (FHB) is one of the most destructive diseases of wheat by reducing the grain yield and quality. Several types of the host resistance to FHB have been described: resistance to initial infection (Type I), resistance to spreading of infection (Type II), and degradation the mycotoxin (Type III). Sumai 3, a Chinese wheat cultivar, is one of the most widely used resistant resources for FHB resistance in wheat breeding around the world. On the basis of molecular mapping, chromosomes 5A, 3BS, and 6BS seem to be likely locations for FHB resistant gene from Sumai 3. In this study, we reported the identification of chromosome regions associated with FHB resistance in Sumai 3 by using its susceptible near-isogenic lines (NILs).The plant materials used in this study were Sumai 3 and its four NILs (NILs-1, NILs-2, NILs-3, and NILs-4). The NILs were derived from a cross between Sumai 3 and Chuan980, a susceptible cultivar, followed by seven-times backcross with Sumai 3 and screened FHB susceptible lines in each generation by artificial inoculation. SSR and AFLP analyses were applied to screen the polymorphism between Sumai 3 and its four NILs. The detected polymorphic markers were mapped using a mapping population of double haploid lines (DHLs) derived from a cross between Sumai 3 and Gamenya. We examined 84 SSRs and 107 AFLP primer combinations that produce approximately 900 AFLP markers. Of these markers, two SSR (*Xgwm533-a* and *Xgwm389*) and five AFLP (ACT/CGAC118, AGA/CGAC136, AAC/CGAC285, AGT/CTGA225, and AGA/CTAT304) markers showed polymorphism between Sumai 3 and its four NILs. The NILs-1, NILs-2, and NILs-3 have different band pattern from Sumai 3 at all of the seven polymorphic markers while NILs-4 is different from Sumai 3 only at the AGA/CTAT304 marker, indicating that the genotypes of the different Sumai 3 NILs with susceptible to FHB is different. Six of the seven-polymorphism markers were mapped on chromosome 3BS where the resistance QTLs has been consistently detected in the populations including Sumai 3 or their derivatives. The AGA/CTAT304, which differ the NILs-4 from Sumai 3, was located on chromosome 2AL. The present study revealed that one FBH resistance gene locates on chromosome 3BS in Sumai 3 and Sumai 3 may have other genes that affect the FHB resistance.

TRANSPOSON-MEDIATED GENERATION OF MARKER-FREE BARLEY PLANTS EXPRESSING PUTATIVE ANTIFUNGAL PROTEINS

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ABSTRACT

The use of transposon-mediated repositioning of transgenes has been proposed as an attractive strategy to generate transgenic plants free of selectable marker genes (Yoder and Goldsbrough, 1994). In barley, previous research has demonstrated high transposition frequencies of a *Ds* element resulting from crosses of two transgenic plants, one containing *Ds-bar* and the other expressing *Ac* transposase. Expression of the relocated transposon-borne transgene is less prone to gene silencing than that of the transgene integrated at the original site as a result of bombardment (Koprek *et al*., 2001). Characterization of *Ds*-delivered transgenes in rice confirmed the stability of insertion site and the expression of the Cry 1B protein during generation advance (Cotsaftis *et al*., 2002). To date transposon-mediated repositioning of a value-added transgene has not been demonstrated in barley, although functionality of the maize As/*Ds* system as a gene-tagging tool has been described (Koprek *et al*., 2001).

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe (*teleomorph Gibberella zeae*) is a major disease for barley and wheat throughout the world (Parry *et al*., 1995). Introduction of putative, recombinant antifungal proteins into barley offers the potential to limit pathogen infection and growth. Transformation technologies for barley have been developed and subsequently improved (Wan and Lemaux, 1994; Cho *et al*., 1998; Bregitzer *et al*., 2000), setting the stage for the introduction of putative antifungal genes. To exploit potentially useful aspects of the maize *Ac/Ds* system, we are using this system to produce transgenic barley plants containing independent insertions of genes encoding putative antifungal proteins. Crossing of these plants to plants expressing *Ac* transposase will result in the excision and reintegration of *Ds*-bordered transgenes into new locations. This movement will result in the stabilization of transgene expression and the unlinking of the plasmid and selectable marker sequences needed to identify transgene-containing tissue from the transgene itself.

The putative antifungal genes chosen, *tlp1* (thaumatin-like protein) and *tlp4* from oat and two of the trichothecene pathway genes, *Tri101* and *Tri12*, isolated from *Fusarium sporotrichioides*, were placed in a *Ds*-bordered, maize *ubiquitin*- or rice *actin* promoter-driven expression cassette. The resultant *tlp* constructs, together with pAHC20 (*ubiquitin* promoter-*bar-nos*) or pActHpt4 (*actin* promoter-*hpt-nos*), were introduced via bombardment into scutellar cells of immature embryos or green, regenerative tissues of two spring cultivars of barley, Golden Promise, a 2-rowed variety, and Drummond, an elite 6-rowed variety. Plants derived from 3 putative *DsUbiTlp*1 lines and 3 *DsUbiTlp*4 transgenic GP lines were positive for *bar* and

further analyses for the presence of *tlp* and its expression is ongoing. Three, one, and five hygromycin-resistant lines were obtained from *DsActTlp*1-, *DsActTlp*4- and *DsActTri101*-transformed Drummond green tissue, respectively; these lines have been transferred to regeneration medium. During the transformation process, we found that bialaphos, used to identify *bar*- expressing tissue, is not suitable for selection of immature embryo and green tissue transformants in Drummond. Subsequently, hygromycin was used for selection. In addition, the *Ac*-transposase gene driven by its own promoter was transformed into Drummond green tissue; five hygromycin-resistant *AcTPase* lines were obtained and are under regeneration. *Ac*TPase was also introduced into Drummond by backcrossing *ubiquitin*- and *Ac* promoter-driven *Ac*TPase-containing Golden Promise lines that were previously isolated (Koprek *et al.*, 2001).

To assist in characterization of the level of transgene expression, antibodies to TLP1, TLP4, and the Tri101proteins are being developed. Genes for *tlp1, tlp4, tri101* and *tri12* were inserted in vector pGEX-4T3 and TLP1, TLP4 and Tri101 proteins were purified for antibody preparation. These three antibodies were tested for experimental efficiency. Antibody to Tri101 was efficient in detecting the Tri101 protein in western blots, while antibodies to TLP1 and TLP4 showed nonspecific binding to barley proteins. Nonspecific antibodies will be removed by passing serum through an affinity column bound with purified TLP1 and TLP4 proteins produced in *E. coli*. The *Tri12* gene was also cloned into pGEX-4T3, but protein expression was low. Subsequently the gene was inserted into other vectors pMAL-c2X and pMAL-p2X; however, the resultant MAL-Tri12 fusion protein expression was still weak. Low expression of the *Tri12* gene product may be due to its 14 transmembrane domains.

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