SELECTED GENES FROM THE FUSARIUM HEAD BLIGHT RESISTANT CULTIVAR FUNDULEA 201R

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ABSTRACT

The development of wheat varieties with resistance to Fusarium head blight (FHB) is slowly occurring through breeding techniques. The varieties so far developed provide improved resistance to the spread of infection within the spike (Type II resistance). Immunity to FHB (Type I) has not yet been reported, however, the correlation between the known components of resistance and toxin decontamination is not well-defined. Chinese germplasm has served as the source of resistance genes in the majority of varietal development. However, it is necessary to diversify the sources of resistance to FHB in order to increase the success rate of developing wheat varieties with good resistance. F201R, developed at A.R.D.I-Fundulea as result of complex crosses, is a FHB-resistant Romanian winter wheat whose resistance is not related to the Chinese resistant germplasm. F201R carries the FHB resistance associated with QTLs (quantitative trait loci) located on chromosomes 1B, 3A, 3D and 5A, unlike the Sumai3 resistance QTL which is associated with chromosome 3BS. In an effort to isolate individual genes involved with FHB resistance, particularly the reaction to the presence of the toxin DON, we have used a suppressive hybridization cDNA subtraction method (Clontech) to obtain differentially expressed messages. Libraries were made from F201R inoculated with water (control) to obtain plant immune response genes; inoculated with *F. graminearum* to obtain fungal genes, as well as plant response genes; and inoculated with DON (deoxynivalenol) to obtain plant genes that are turned on in response to toxin. Results indicate that libraries containing genes from water-inoculated and DON-inoculated F201R contained many of the same genes and, therefore, selection of specific genes turned on in the presence of DON should be accessible. BLAST comparison of sequences has found a hypervariable sequence from rice, a UDP-galactose 4-epimerase-like protein, a carbamoyl phosphate synthase, unidentified rice BAC clones, and some sequences that are unique. Further comparison of sequences from the libraries with publicly available sequences should lead to the identification of plant genes involved with resistance to DON and/or fungal invasion.

IMPROVEMENT OF FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY THROUGH *IN VITRO* SELECTION

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ABSTRACT

Infection of barley (*Hordeum vulgare* L.) by *Fusarium graminearum* (Schwabe) is associated with accumulation of mycotoxins such as deoxynivalenol (DON) which play a significant role in Fusarium head blight (FHB) pathogenesis. A study was conducted to determine the effectiveness of using such mycotoxins in anther culture system for doubled haploid (DH) production to select mycotoxin tolerant barley plants with improved FHB resistance in the field. Twelve crosses varying in FHB resistance were subjected to *in vitro* selection (IVS) using a mixture of 2 or 3 mycotoxins. All fertile IVS and control DH lines from 7 crosses involving "exotic" FHB resistance sources were evaluated for FHB resistance in the Brandon nursery in 2001and 2002, while 5 standard breeding crosses were evaluated in 2002. DON content was determined by the ELISA technique at Ottawa. Of 7 exotic crosses, only the two-row sub-group of Chevron/CDC Fleet cross showed significantly lower DON content of IVS vs. control group in 2001. Among the 5 standard crosses, only IVS lines from Rivers//Rivers/SB93806 cross had significantly lower DON content than control lines. Several IVS lines from both populations had substantially lower DON content than their parents. In conclusion, *in vitro* selection was effective in improving FHB in only some crosses but further testing is needed.

TRANSCRIPTION PROFILING OF WHEAT GENES FOR RESISTANCE TO *F. GRAMINEARUM* USING CDNA MICROARRAYS

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ABSTRACT

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* Schw., is a destructive disease of wheat (*Triticum aestivum* L.). Although several genes related to FHB resistance have been reported, global analysis of gene expression in response to FHB infection remains to be explored. To characterize differences in gene expression between FHB resistant and susceptible wheat cultivars with a view toward gaining insight into the genetic mechanism of Type II resistance in wheat, cDNA microarrays with 2306 ESTs from wheat subtraction libraries were used to determine Fusarium induced and differentially expressed cDNA in wheat resistant cultivar Ning 7840 and susceptible cultivar Clark. The subtraction libraries were made from *F. graminearum*-infected spikes of bulked resistant and bulked susceptible Ning 7840 x Clark F_{12} recombinant inbred lines. The dynamic change of wheat spike transcriptomes was monitored at a series of time courses (0h, 3h, 6h, 12h, 24h, 36h, 48h, and 72h) after imposition of pathogen stress. Microarray analysis with cDNAs from *F. gaminearum*-inoculated Ning 7840 and Clark as target revealed 170 ESTs with at least two-fold difference in gene expression level. There were more differentially induced genes than repressed genes in the resistant genotype during the first 24h after inoculation with the pathogen, but more significantly down-regulated genes were observed from treatments of 36h and onward. DNA sequencing to putatively identify the ESTs and real time PCR to confirm the result from microarray analysis is underway.

TRANSPOSONS AND MERISTEMATIC CULTURES: TOOLS TO IMPROVE TRANSGENE STABILITY, AGRONOMIC PERFORMANCE, AND CONSUMER ACCEPTANCE

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ABSTRACT

The production of transgenic barley plants expressing antifungal proteins (AFPs) is a potential tool for the introduction of novel sources of resistance to Fusarium head blight (FHB). For biotechnological approaches to be applied to practical production problems—such as fighting FHB—it is imperative that researchers keep in mind that the ultimate residence of their technology will be commercially useful germplasm. In deference to such needs, we are utilizing technological improvements in the production of transgenic barley that improve transgene expression and transgenic plant performance, and which also may facilitate consumer acceptance of transgenic crops.

The maize *Ac-Ds* transposable element system has been introduced into barley and has been shown capable of delivering transgenes into single copy regions which support stable transgene expression. Essential elements of the system are: 1) expression cassettes in which the AFP gene is flanked by the *Ds* terminal inverted repeat (TIR) sequences, and 2) transgenic lines expressing the maize *Ac* transposase (*AcTPase)* which mediates transposition of the *Ds*-flanked AFP. Hybridization of *AcTPase*-expressing lines with *Ds*-AFP lines mediates transposition of the *Ds*-AFP cassette, in some cases to unlinked locations which enable the segregation of *Ds*-AFP from the original site of insertion, thus eliminating bacterial and selectable marker sequences present in the original transformant. Studies of *bar* (herbicide resistance) expression have shown that transposition stabilizes and elevates transgene expression.

Meristematic cultures have been developed which, relative to standard embryogenic cultures, are characterized by greater levels of differentiation, long-lived regeneration, and a reduced level of somaclonal variation in regenerated plants. We are targeting our transformation efforts to meristematic tissues of the cultivar Drummond, a 6-rowed malting cultivar recently released by North Dakota State University. Direct transformation of such germplasm will facilitate the production of commercially competitive cultivars for the midwest because backcrossing from unadapted germplasm is obviated. A more immediate benefit is that such transformants, relative to those produced from unadapted cultivars such as Golden Promise, will possess morphological features similar to the midwestern cultivars in which FHB resistance is most needed, and which have been shown to influence FHB infection and severity.

To date, *Ds*-flanked AFP expression cassettes have been constructed for two oat-derived thaumatin like proteins (*tlp1* and *tlp4*), and two trichothecene pathway genes from *F. sporotrichioides, TRI101* and *TRI12*, driven either by maize *ubiquitin* or rice *actin* promoters. Antibodies have been prepared against both *tlp* genes and from *TRI101*. Because working with meristematic cultures of Drummond is technically difficult, preliminary experiments were conducted in which these constructs were introduced into scutellar cells of Golden Promise. The experiments have resulted in plants from 3 transgenic lines containing *tlp1* and

tlp4 which have been confirmed to be transgenic via PCR. Additional lines putatively encoding these genes, and also *TRI101* and *TRI12*, are awaiting additional molecular characterizations.

Subsequent transformation efforts have resulted in the production of three transgenic Drummond lines containing *tlp1*, *tlp4*, or *TRI101*. Additional lines putatively encoding these genes are awaiting additional molecular characterizations. We have also produced Drummond-derived plants expressing *AcTPase*. To our knowledge, these are the first 6-rowed barley plants produced which have been confirmed to contain AFP genes introduced via transformation.

T_1 plants derived from both Drummond and Golden Promise transformants will be developed into homozygous lines and characterized for protein and/or mRNA production. Promising lines which produce measurable transgene products will be tested for FHB resistance by collaborating pathologists. In addition, they will be crossed to *AcTPase*-expressing plants to initiate transposition of the *Ds*-AFP cassettes. Ultimately, we intend to provide comprehensive characterizations of these materials with respect to AFP production, FHB resistance, the inheritance of these transgene-encoded traits, and the agronomic performance of lines derived from these materials.

MOLECULAR MAPPING AND MARKER ASSISTED SELECTION OF QTLS FOR FUSARIUM HEAD BLIGHT RESISTANCE IN CHINESE WHEAT LINE W14

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OBJECTIVES

Objectives of the current study were to identify QTLs in addition to 3BS in Chinese wheat line W14 and to evaluate contribution and feasibility of selecting these QTLs in a marker assisted selection (MAS) assay.

INTRODUCTION

Fusarium head blight (FHB) is one of the most destructive diseases of wheat causing significant reductions in grain yield and quality. Deployment of resistant varieties is an effective, economical and environmentally safe way to control FHB in wheat. Development of commercially viable FHB resistant varieties via traditional breeding methods has been hindered because resistance of currently used sources is quantitative in nature and incomplete. In addition, most resistance sources are not adapted, susceptible to other prevalent diseases and have poor combining ability. Significant environmental effects and laborious disease screening techniques also impede progress. Use of molecular markers offers an efficient alternative to phenotypic selection for FHB resistance. A QTL on 3BS has been identified as being a robust QTL for FHB resistance in mapping studies of several related sources including Sumai 3 (Anderson et al., 2001), Ning7840 (Zhou et al., 2002), and CM-82036 (Buerstmayr et al., 2002, 2003), but only recently has it been used for MAS in breeding programs. Minor QTLs on other chromosomes also have been postulated (Zhou et al., 2002; Otto et al., 2002; Buerstmayr et al., 2003; Shen et al., 2003; Steiner et al., 2003), yet their significance needs validation as results vary among labs, genetic background, and environments.

MATERIALS AND METHODS

Genetic populations - W14, an improved type II resistance source developed by recurrent selection (Jiang, 1997), was crossed with two FHB susceptible soft red winter (SRW) wheat cultivars Pioneer 2684 and Madison. Two doubled haploid populations (DH1: 96 lines, and DH2: 62 lines) were developed using wheat by maize hybridization method (Chen et al., 2001). Floret inoculation method was used to assay disease severity and type II resistance in greenhouse tests. Severity (percentage of infected florets) was assessed 7, 14 and 21 days after inoculation. Severity ratings made on 21st day were used in mapping analyses. For both DH populations, two independent greenhouse experiments were conducted, and entry means of DH lines from the two experiments were used in subsequent analyses. W14 and another related source Futai8944 were backcrossed four times to adapted FHB tolerant cultivars Roane and Ernie. The BC_4F_1 and BC_4F_2 generations were used to develop NILs via MAS.

SSR mapping – Linkage groups previously postulated as possessing QTLs for FHB resistance include 1B, 2B, 3B, 3A, 5A, 6A, 6B, 6D, and 7B. Therefore, 308 SSRs known to be located on these chromosomes

were selected and used to survey DNA polymorphism among parents W14, Pion2684 and Madison as well as four extreme bulks using BSA analysis (Michelmore et al., 1991). Sequence information of 100 SSR primers was available from Röder et al. (1998), and the remaining 208 were kindly provided by P. Cregan, USDA-ARS, Beltsville, MD. DNA extraction, PCR amplification and SSR assays were conducted as previously described (Saghai Maroof et al., 1994; Bryan et al., 1997; Röder et al., 1998). Simple and multiple regression analysis were conducted using Agrobase (Agronomic Software, INC. 1999). Linkage maps were constructed using MAPMAKER 3.0b for MS-DOS (Lander et al., 1987). Markers were grouped using a threshold LOD > 3.0.

RESULTS AND DISCUSSION

QTLs detected in W14 mapping populations (data and maps will be presented at the meeting) – While 71% (218 out of 308 SSRs) of the primers were polymorphic among parents W14, Pioneer 2684 and Madison, only 18 SSRs showed putative association with FHB resistance in one or both of the DH populations based on BSA analysis. Twenty-six pairs of primers, including the 18 primers above were mapped in DH1 and DH2 populations. Five QTLs were detected on 1BL, 2BS, 3BS, 5AL, and 7AL chromosome regions. Eighteen markers in these regions were significantly ($P < 0.05$) associated with FHB resistance. These markers explained 42% and 62% of the total phenotypic variation in DH1 and DH2, respectively. According to the peaks of the LOD profiles, the QTL on 3BS had a much larger effect than the 5AL and 2BS QTLs in both DH populations, and therefore confirmed as being a major QTL explaining 29% and 42% of phenotypic variation in DH1 and DH2, respectively. QTL position of 3BS is in agreement with Anderson et al. (2001), Zhou et al. (2002), and Buerstmayr et al. (2002 & 2003). QTL position of 5AL is similar to Buerstmayr et al. (2003). QTL position of 2BS is in disagreement with Zhou et al. (2002). Two minor QTL regions (1B and 7A) were detected in DH2 and are being saturated with more markers.

Application of MAS for FHB QTLs in Backcrosses - Eighteen SSRs were used for MAS in BC_4F_1 and BC_4F_2 generations. A total of 84 recombinants were selected in four BC_4F_2 populations having allele or allele combinations of the two resistant parents (RP) at four SSR loci (BARC75-Xgwm533a-BARC133-Xgwm533b) in 3BS QTL region, two SSR loci (BARC18 and BARC91) in 2BS QTL region, and two SSR loci (BARC100 and Xgwm156) in 5AL QTL region (Table 1). These recombinants were also identified as having alleles of the two recurrent parents (RCP) for the other ten SSRs (data not presented in Table 1). The 3BS QTL was found to be more frequently transferred into recurrent backgrounds than the 2BS and 5AL QTLs using phenotypic selection for type II resistance; however, recurrent parent background did affect efficiency in selection of this QTL. The 3BS QTL spans a relatively large interval of five SSR markers (BARC75-Xgwm533a-BARC133-Xgwm493-Xgwm533b) in our map. The intervals in this QTL region have been divided into smaller segments in a Roane backcross population in which 23 homozygous NILs having different allelic combinations in this region have been derived. The precise location and composition of the 3BS QTL will be further delineated via evaluation of these NILs for resistance to FHB compared to their marker composition.

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Table 1. Haplotypes and mean disease severity (%) of 84 selections among 213 plants in four BC_4F_2 populations (Total plants in the four BC_4F_2s were 91, 58, 32, 32, respectively. The number in parenthesis represents the total number of plants having same allele type).

		BC_4F_2 populations (No. of plants)			
Haplotypes*	Allele type**	W14 x Roane	Futai8944 x Roane	W14 x Ernie	Futai8944 x Ernie
1	3, 3, 3, n, 1, 1	21.7 (2)	19.3 (4)		
2	2, 2, 2, n, 1, 1	18.4 (3)	18.2 (10)		
3	1, 1, 1, n, 1, 1	19.7 (2)	16.3 (3)		
4	2, 3, 3, n, 1, 1		36.1 (1)		
5	1, 2, 2, n, 1, 1		20.6 (1)		
6	2, 2, 1, n, 1, 1		10.5 (1)		
7	3, 3, 1, n, 1, 1	50.0 (1)			
8	1, 1, 2, n, 1, 1	47.4 (1)			
9	1, 1, 3, n, 1, 1	17.5 (1)			
10	1, 3, 3, n, 1, 1	32.4 (1)			
11	1, 3, 3 ,3, 1, 1	10.5 (1)			
12	1, 2, 2, 3, 1, 1	33.3 (1)			
13	1, 1, 1, 3, 3, 1	17.2 (5)			
14	1, 1, 1, 3, 2, 1	18.8 (2)			
15	1, 1, 1, 3, 1, 1	18.1 (6)			
16	1, 1, 1, n, 3, 1	47.6 (7)			
17	1, 1, 1, n, 2, 1	21.1 (1)			
18	3, 3, 3, n, n, 1				15.3 (8)
19	3, n, 3, n, n, 1			14.9 (12)	17.4 (9)
20	3, n, 3, n, n, 2				32.1 (1)
21	3, n, 3, n, n, 3				33.3 (1)

*Haplotypes were characterized based on allele and allele combinations of two parental lines for six SSR marker loci ordered by BARC75, Xgwm533a, BARC133, Xgwm533b, BARC18, and BARC100. ** Allele types are scored as 3 = W14 and/or Futai8944 type, 1 = Roane and/or Ernie type, n = null allele type of Roane and/or Ernie, 2 = heterozygous type of two parental alleles. Haplotype of W14 and Futai8944 = 3,3,3,3,3,3, Roane = 1,1,1,n,1,1, Ernie = 3,n,3,n,n,1. Disease severity of the four parental lines were: 12.0, 13.5, 31.6 and 33.4%, respectively.

SATURATION MAPPING OF A MAJOR FUSARIUM HEAD BLIGHT RESISTANCE QTL REGION IN TETRAPLOID WHEAT

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ABSTRACT

Previous screening for Fusarium head blight (FHB) resistance identified a *Triticum dicoccoides* accession carrying FHB resistance genes. Using *T. durum* cv. 'Langdon'-*T. dicoccoides* chromosome 3A recombinant inbred chromosome lines (RICLs), a major quantitative trait locus (QTL) *Qfhs.ndsu-3AS*, that explains 55% of the genetic variation for FHB resistance, and a microsatellite locus, *Xgwm2*, tightly linked to the highest point of the QTL peak have been identified (Otto et al. 2002). This QTL region spanned a 29.3cM interval on chromosome 3A. The objective of this study is to saturate the QTL *Qfhs.ndsu-3AS* region and identify recombinants for a smaller donor chromosomal segment carrying this QTL.

Screening of the *T. monococcum* bacterial artificial chromosome (BAC) library with the DNA-based probe *NDSU.fhb.3A* derived from the microsatellite marker *Xgwm2* has identified 15 BAC clones. We are isolating low or single copy sequences from these BACs to generate more markers for saturating the QTL region. A novel marker technique Target Region Amplification Polymorphism (TRAP) has also been used to generate markers in this study (Hu et al. 2003). We designed 50 fixed primers based on the ESTs mapped on the short arm of chromosome 3A (3AS) and the conserved domain leucine rich repeat (LRR) of disease resistance genes. Nine polymorphic markers were generated with these fixed primers in combination with random primers. All these 9 markers were mapped on chromosome 3A. In addition, we have designed 9 pairs of microsatellite primers based on the 3' and 5' sequences of the ESTs mapped on 3AS and have been trying to generate more markers within the QTL region. Two microsatellite markers, *Xgwm493* and *Xgwm389*, mapped in a major FHB resistance QTL *Qfhs.ndsu-3BS* region, showed no polymorphism between the two parents of the RICLs. Fourteen out of 28 STS primer pairs developed from the ESTs mapped on chromosome 3B by Dr. J. A. Anderson (Liu et al. 2003) showed polymorphism between the two parents of the RICLs. Six STS markers generated through this approach have been mapped on chromosome 3A. Based on microcolinearity between wheat homoeologous group 3 chromosomes and rice chromosome 1, we have been identifying the rice genomic sequences in the collinear region and using these sequences to screen the ESTs that have not yet been mapped in the wheat genomes. Positive ESTs from this screening will be used to develop STS markers for saturating the QTL region. Concurrently, a large F_2 population has been developed by crossing Langdon with a RICL carrying a smallest *T. dicoccoides* chromosomal fragment spanning the *Qfhs.ndsu-3AS*. This population is being employed to identify more recombinants within the QTL region for fine mapping.

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TRANSFORMATION OF BARLEY WITH TWO ANTIFUNGAL GENES

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ABSTRACT

Insertion and expression of multiple antifungal genes has the potential of increasing resistance to a variety of fungal diseases. Using particle bombardment and bialaphos selection for the *bar* gene, multiple barley plants containing both a rice thaumatin-like protein (*tlp*) gene and a rice chitinase (chi) gene were regenerated from three transformation events. Southern analysis confirmed integration of the transgenes into the barley genome. Northern analysis of T_0 plants indicated that event 1 did not contain RNA from either gene. Plants from event 2 expressed both genes at high levels, while plants from event 3 showed small amounts of *tlp* RNA but no chi RNA. Western analysis of T_1 progeny confirmed that event 2 lines expressed both genes and event 3 lines expressed only *tlp*. Homozygous T_2 lines have been identified and are being examined for gene expression levels for both transgenes.

EXPRESSION OF THE YEAST L3 AND THE POKEWEED ANTIVIRAL PROTEIN GENES CONFERS RESISTANCE TO TRICHOTHECENE MYCOTOXINS

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ABSTRACT

Trichothecenes are a highly diverse class of toxic, sesquiterpenoid secondary metabolites that are produced mainly by plant pathogenic fungi. The contamination of important agricultural products, such as wheat, barley or maize with the trichothecene mycotoxin, deoxynivalenol (DON) due to infection with *Fusarium graminearum* and *F. culmorum* is a worldwide problem. Trichothecene mycotoxins interact with the peptidyltransferase site of eukaryotic ribosomes and inhibit eukaryotic protein synthesis. Ribosomal protein L3 (*RPL3*) participates in the formation of the peptidyltransferase center. Mutations in the *RPL3* gene (called *TCM1*) were initially identified by conferring resistance to trichodermin, a trichothecene mycotoxin that inhibits the peptidyltransferase reaction.

To determine if expression of the yeast *RPL3* gene will confer resistance to trichothecene mycotoxins, we generated transgenic tobacco plants expressing either the wild type or mutant forms of the yeast *RPL3* alone or together with pokeweed antiviral protein (PAP), a ribosome inactivating protein that inhibits viral and fungal infection. Transgenic plants containing the wild type yeast *RPL3* and PAP or a mutant form of the yeast *RPL3* and PAP were phenotypically normal. Similarly, transgenic lines expressing the yeast *RPL3* genes alone were indistinguishable from wild type plants. To determine if transgenic tobacco plants expressing the yeast *RPL3* genes are resistant to trichothecenes, seeds from transgenic and wild type plants were germinated on MS medium, containing 1 μM DAS or 10 μM of DON and their root length was measured at the end of six weeks. Plants from all transgenic lines showed resistance to DAS and DON compared to the wild type plants. However, the highest level of resistance was observed with transgenic plants expressing the yeast *RPL3* genes together with PAP. To confirm that yeast *RPL3* genes are expressed in these plants, we carried out real time PCR analysis using primers specific for the yeast *RPL3* genes, which do not hybridize to the tobacco L3 genes. The results confirmed the expression of the yeast *RPL3* genes in the transgenic lines. These results demonstrate that we can obtain phenotypically normal transgenic plants that show high levels of resistance to DON by coexpressing the wild type or mutant forms of the yeast *RPL3* together with PAP in transgenic tobacco plants.

WANGSHUIBAI: A HEXAPLOID WHEAT RESISTANT TO THE SPREAD OF FUSARIUM HEAD BLIGHT

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INTRODUCTION

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

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

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

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

Other sources of type II resistance to FHB have been studied and several QTLs have been identified on chromosome 3A of *Triticum dicoccoides* (Otto et al., 2001), chromosome 2D of 'Wugham', 3BS of 'Maninga' (Somers et al., 2003), and chromosomes 1B, 3A, 3D and 5A of 'Fundulea 201R' (Shen et al., 2003).

Other potential sources of type resistance to FHB are Wangshuibai (the subject of this paper) from China, cltr9445 from China, PI157593 from S. Korea, PI362463 from Yugoslavia, and cltr9429 from China.

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

these data and the high level of resistance it is logical to contemplate the possibility of Wangshuibai carrying different resistance genes than Sumai 3.

In this study our objectives were to 1) identify the chromosomal location of genes responsible for the resistance to FHB of Wangshuibai, 2) estimate the effect of these genes, both in single locus and epistatic models, and 3) identify PCR markers closely linked to these genes, so they can be used in a marker assisted selection (M.A.S.) breeding scheme to develop resistant cultivars.

MATERIALS AND METHODS

An F_6 derived population consisting of 388 recombinant inbreed lines (RIL) was developed by SSD from the cross between Wangshuibai and ND671 (an elite HRSW line from the NDSU-HRSW breeding program). A random subset of 88 lines was used in this study.

Phenotyping of these lines was done in 3 greenhouse replicated trials during the years 1999, 2000, and 2001. Lines were grown in 36X21 cm buckets with two rows of five plants/bucket. At the time of anthesis, heads were single point inoculated in a floret in the middle of the spike with 10 ¼l of a suspension with 50000 spores/ml. After inoculation, the spikes were covered with plastic bags and misted for 3 days to ensure high humidity conditions. Temperature in the greenhouse was kept between 25 and 30°C. Disease scores were taken 14 and 21 days after inoculations. The NDSU variety 'Alsen' (derived from Sumai 3) was used as a resistant check.

Genotyping of these lines was done using 2 sets of SSR PCR primers, GWMs (Röder et al., 1998), and BARCs (http://www.scabusa.org/pdfs/BARC_SSRs_011101 .html). Amplification was done in accordance to those described by the developers of both sets. Amplified products were separated using either 6% acrylamide non denaturing gel, or 6% acrylamide denaturing gel visualized with silver staining. Several STS markers developped by Liu and Anderson (2003) were also included in the linkage map. In addition, several Target Region Amplified Polymorphisms (TRAPs) were developed from wheat EST sequences. In total, the linkage map contains 185 loci across the genome.

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

RESULTS AND DISCUSSION

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

After scanning the genome for significant QTL peaks, we found a major peak on chromosome 3B, directly over *Xsts138* (Fig 2). This peak explains 17% of the total phenotypic variance for 14 DAI, and 31% 21

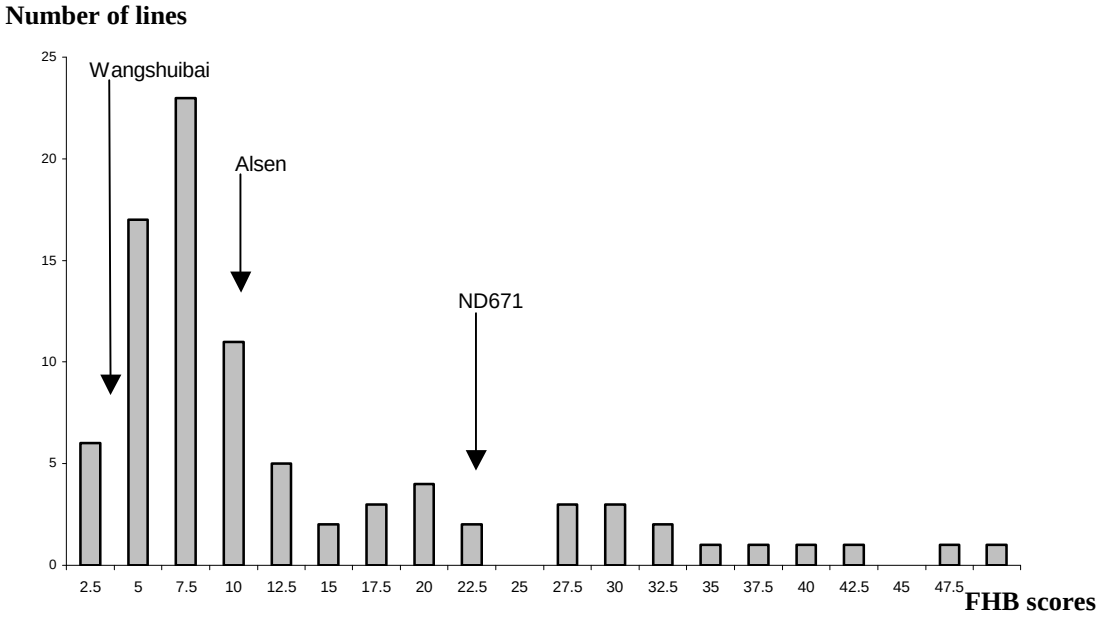

Fig.1: Frequencies histogram for FHB 21 DAI.

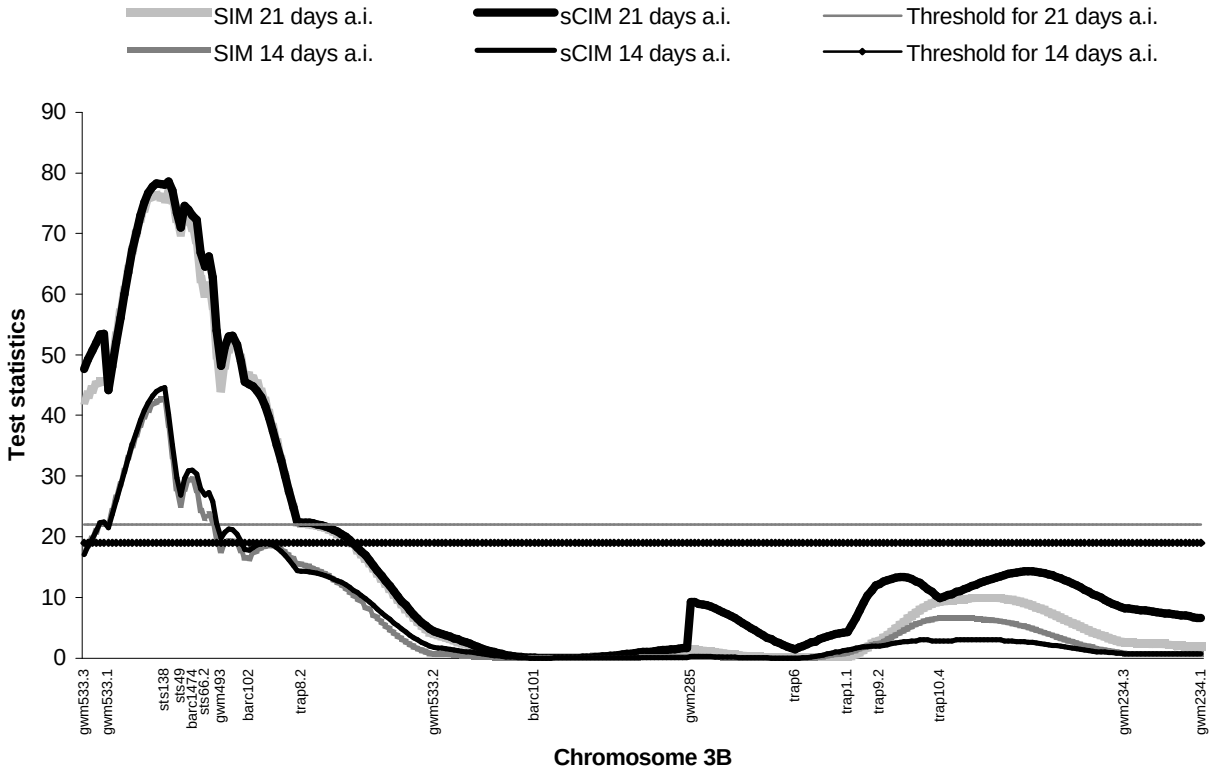

Fig.2: Simple interval mapping (SIM) and simplified composite interval Mapping (sCIM) graphs on chromosome 3B for readings 14 and 21 DAI.

In the case of 14 DAI interactions were identified between *Xsts138(3B)* and *Xgwm304(5A)*, *Xbarc117(5A)* and *Xgwm192(4B)*. The estimates of the effects of the interactions ranged between 40 to 53%, compared to a 17% of single locus model with *Xsts138*. In the case of 21 DAI interactions were identified between *Xsts138(3B)* and *Xgwm2(3A)*, *Xgwm333(7B)*, *gwm165(4B)*, *Xbarc101(3B)*, *Xbarc1033(6B)*, and *Xtrap9.2(3B)*. The estimates of the effect of these interactions ranged between 47 to 67%. Given the population size, only one-way interactions were considered, however the data suggests the possibility of 3-way interactions. This will be tested with the remaining 300 individuals.

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

Locus	PhenotypicVariance explained (%)	Time of observation (DAI)
Xgwm2 (3A)	67	21
Xgwm333 (7B)	47	21
Xgwm156 (4B)	55	21
Xbarc101 (3B)	51	21
Xtrap9.2 (3B)	59	21
Xbarc1033 (6B)	55	21
Xgwm304 (5A)	53	14
Xbarc117 (5A)	42	14
Xgwm192 (4B)	40	14

The results of this study clearly indicate the significance of the 3BS QTL. Additionally, epistatic interactions seem more critical than previously believed in determining strong resistance to FHB. An important region to note in this regard is that on chromosome 3A, which together with that on 3BS could explain up to 67% of the phenotypic variance. The results of this study further confirm field data on the degree of the 3BS QTL effectiveness.

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PLANT AND FUNGAL GENOMICS OF FHB/GIBBERELLA EAR ROT

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ABSTRACT

In order to further develop robust disease resistance to *F. graminearum* (*Fg*) in wheat and maize, we are using genomic and proteomic approaches to investigate the host/pathogen interaction. High and low density maize arrays (6.6K unigene or targeted arrays) are being used to establish how susceptible and resistant maize inbreds respond to attack by *Fg*, by comparing gene expression between fungal-inoculated or mock-inoculated kernel and silk tissues. Many genes, including PR proteins, genes from the terpene biosynthesis pathways, and genes with unknown function, are being induced or up regulated by *Fg*. Proteomic analyses by 2-D gel separation are being carried out in parallel on the same tissue samples. Comparison of the kernel tissues as identified a novel 35 kDa protein which was unique to infected tissues from the susceptible hybrid. Analyses with the silk tissues are in progress. Our collection of *Fg*-challenged wheat ESTs has been combined with a unigene set of about 1500 rye ESTs (enriched in genes induced under cold stress) to produce a "5K stress" small grain chip and array hybridizations are underway to compare gene expression in susceptible and resistant wheat cultivars during *Fg* infection.

We have released the majority of our ~7400 *Fg* ESTs to assist the annotation of the *Fg* genome sequence (Gb#AACM00000000). Electronic northerns have been conducted using in-house and public *Fg* EST databases to identify *Fg* pathogenicity gene candidates. We are collaborating with the USDA to determine *Fg* gene function through directed gene disruption. Disruption of the gene represented by contig Fg1A2287 has demonstrated that this sequence encodes a cytochrome P450 responsible for oxygenation at carbons 8 and possibly 7 in the trichothecene mycotoxin biosynthetic pathway.

IDENTIFICATION OF GENES UPREGULATED IN BARLEY IN RESPONSE TO INOCULATION WITH *FUSARIUM GRAMINEARUM*

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ABSTRACT

We used the barley Affymetrix GeneChip to examine transcript profiles in barley in response to inoculation with *Fusarium graminearum* (*Fg*). The barley GeneChip contains over 22,800 unique barley transcripts. Three replicate experiments were performed to compare transcript accumulation between *Fg*-inoculated and mock-inoculated spikes of the susceptible cultivar Morex at 24, 48, 72, 96 and 144 hours after inoculation. Barley plants were grown in six-inch pots in a growth chamber with 16h light, and 8h dark at 20°C and 18°C, respectively. Plants were inoculated by spraying conidiospores (100,000 spores/ml) onto spikes 2-3 days after they emerged from the boot. Total RNA for each time point/treatment was extracted from 8 spikes and checked for quality on an Agilent Bioanalyzer before labeling and hybridization to the Gene Chip. Comparisons between transcript levels in *Fg*-inoculated and mock-inoculated plants from each time point were performed using Gene Data Expressionist software. Transcripts showing differential accumulation were not detected at 24 h after inoculation, but were initially detected at 48 h, and also at 72, 96 and 144 h. The detection of transcripts with differential accumulation at 48 h coincides with the timing of a change in *Fg* pathogenicity from hemi-biotrophism to necrotrophism, which was observed by others. The induction of some classes of genes has been confirmed by RNA blot analysis. A description of these genes, their expression profiles and their possible role in defense will be presented.

EXPRESSION OF ANTI-APOPTOTIC GENES IN SPRING WHEAT CONFER RESISTANCE TO NECROTROPHIC PATHOGENS (*FUSARIUM GRAMINEARUM*) BY INHIBITING HOST-CELL DEATH

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ABSTRACT

Fusarium graminearum, a necrotrophic fungal pathogen of cereals, has caused severe damage to crops during the last decade. In particular, Fusarium head blight epidemics have caused an estimated $3,000 million in damages throughout the North-Central United States during the 1990s (Windels, 2000). As *Fusarium graminearum* resistant germplasm is limited, traditional breeding practices used to confer resistance have resulted in limited progress. Recombinant DNA technology for *Agrobacterium*-mediated wheat transformation has become sufficiently developed to be a practical means for introgression of genes conferring beneficial agronomic traits. We have previously demonstrated the efficacy of using a trans-kingdom approach for conferring resistance to necrotrophic pathogens, by generating tobacco plants harboring animal anti-apoptotic genes and showing that these transgenic plants are resistant to necrotrophic pathogens (Dickman et al. PNAS 2001) as well as abiotic stresses (unpublished). Apoptosis is a genetically regulated process that results in the decomposition of non-essential and non-functional cells and tissues during the growth and development of organisms. Importantly, during these fungal diseases of tobacco, markers associated with mammalian apoptosis were observed, but absent from transgenic resistant plants. We therefore explored the use of these cell survival genes in wheat. We have previously reported greenhouse and field trial evaluations of advanced-homozygous events expressing significant increases in resistance. Wheat plants expressing heritable resistance to necrotrophic pathogens through animal anti-apoptotic genes; Bcl-xL (chicken), CED-9 (nematode) and Op-IAP (baculorvirus) have demonstrated resistance/reduced PCD in various independent events (>10/transgene). We then evaluated whether scab disease exhibited characteristics associated with mammalian-programmed cell death. Following head inoculation, wild type (Bob White) wheat showed DNA fragmentation, DNA laddering and TUNEL positive staining cells, indicative of an apoptotic-like response. Scab tolerant transgenic wheat expressing anti-apoptotic genes, when inoculated resulted in minimal DNA fragmentation and nuclear staining, suggesting that the apoptotic-like response was inhibited. As reduced levels of apoptosis during head inoculation were detected, effects of the anti-apoptotic genes in transgenic wheat were subsequently studied under other conditions. Due to reports showing increase resistance to abiotic stresses in tobacco expressing anti-apoptotic genes, we also tested for increased resistance in the transgenic wheat. When placed under conditions of high salinity, Op-IAP transformed wheat did not show characteristic DNA laddering while all transgene-containing events demonstrated a reduced level of TUNEL positive nuclei. These results demonstrate that expression of anti-apoptotic genes in spring wheat results in broad-spectrum resistance to abiotic and biotic stresses.

GENETIC STUDIES OF SCAB RESISTANCE IN THE SOFT RED WINTER WHEAT 'ERNIE'

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OBJECTIVES

This research was designed to: (1) identify QTL associated with type II resistance in Ernie and to determine if these QTL differed from those in Sumai 3; (2) map the QTL for other agronomic traits and to study the associations between scab resistance and these traits; and (3) estimate the gene number and genetic effects conditioning scab resistance in Ernie.

INTRODUCTION

Fusarium head blight (FHB), also called scab, caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)], is a disease that affects wheat (*Triticum aestivum, and T. durum*) and barley (*Hordeum vulgare* L.) in warm, humid areas of the world. Genetic studies on host plant resistance using both molecular and traditional methods have been emphasized (Kolb et al., 2001). Research to date has mainly focused on resistance in the Chinese cultivar Sumai 3 and its derivatives, and the Brazilian cultivar Frontana (Zhou et al., 2002), which have been widely used in the breeding programs globally. More recently, scab resistance in cultivars from Europe, such as Fundulea 201R, have also been studied (Shen et al., 2003).

Incidental sources of scab resistance have been identified from routine screening in U. S. breeding programs. The soft red winter wheat Ernie, released in 1995 by the University of Missouri (McKendry et al., 1995) has a moderately high level of scab resistance and is now used in U. S. breeding programs as a complementary source of resistance to the Sumai 3 source; however, the genetics of its scab resistance have not been studied. This information should enable breeders to more efficiently exploit this source of resistance.

MATERIALS AND METHODS

A set of 244 F_8 recombinant inbred lines (RILs) was developed for QTL analysis from the cross Ernie / MO 94-317. Eight plants/RIL were planted in a greenhouse environment, arranged in a randomized complete block design with three replications. For conventional genetic analyses, F_1 (and reciprocal, 50 plants/generation/replication), F_2 (200 plants/replication) and backcross generations (120 plants/generation/replication) were also developed from the same cross. Plants were planted in the greenhouse, arranged in a completely random design with 3 replications. Phenotypic data for *F. graminearum* type II resistance and related traits were collected. Data collected included disease spread (the number of diseased spikelets on the inoculated head), spread with wilt (the number of diseased spikelets plus those spikelets wilted due to disease), spike length (the total number of spikelets on the inoculated head), Fusarium head blight index (FHBI) (spread/spike length), FHBI with wilt (spread with wilt/spike length).

AFLP procedures followed manufacturer's recommendations from the AFLP System I Kit from Invitrogen (Carlsbad, CA). Sixty-four EcoRI/MseI primer pairs and 420 SSR primers were used to screen parents for polymorphisms. Sequence information of SSRs was from Roder et al. (1998) and Q. J. Song and P. Cregan USDA-ARS, Beltsvile, MD (personal communication). The chromosome locations of these SSR markers were from Roder et al. (1998) and JR. Shi and R. Ward, Michigan State University (personal communication). Analyses of variance and QTL multiple regressions were done using SAS Version 8.0. MapMaker 3.0 was used to construct the linkage maps. Composite interval mapping was done using QTL Cartographer 1.16 model 6. Generation means analyses were conducted according to Mather and Jinks (1977).

RESULTS AND DISCUSSION

A total of 139 markers including 94 SSR and 45 AFLP markers were mapped on 19 chromosomes. Two chromosomes, 4D and 6D had only one marker. The order and distance of most mapped markers were consistent with the reference map (Roder et al., 1998; Shi and Ward, personal communication). Based on composite interval mapping at LOD =3.0, five QTL were identified associated with both FHBI and FHBI with wilt on chromosomes 2B, 3B, 4B, 5A, and 5D (Table 1; Figure 1). Among them, the QTL on 5A was also associated with spread and spread with wilt. The major QTL for scab resistance in Ernie were located on 4B, 5A, and 5D, and explained 9.7 to 33.3% of phenotypic variation. All resistance alleles were from Ernie. Most Chinese cultivars have the 3BS major QTL from Sumai 3, which is located on telomeric region of 3BS (Zhou et al., 2002). The minor QTL on 3BS in Ernie was located near the centromere. We concluded that the 3BS QTL identified in Ernie differed from that in Sumai 3. Compared with mapped QTL in other resistant sources, the QTL on 2B and 5D appeared to be new QTL. QTL identified on 3B, 4B, and 5A need further study to determine if they differ from those identified in Wuhan-1 and Fundulea 201R (Somers et al. 2003; Shen et al. 2003). Digenic interactions were found for spread with wilt, FHBI, and FHBI with wilt that explained additional phenotypic variation.

Conventional genetic analysis agreed with molecular analysis. Four genetic factors were estimated for FHBI while 2 factors were associated with disease spread. Gene action conditioning scab resistance was primarily additive, however, a small but significant dominance effect was detected. Additive x dominance epistasis was also significant for both disease spread and FHBI. These data suggest recurrent selection could be important in pyramiding genes for scab resistance in breeding programs utilizing Ernie.

Table 1. QTL associated with type II scab resistance the soft red winter wheat cross Ernie x MO 94-317.

Traits	Chromosome location	Markers	QTL peak position	LOD score	R^2 (%)	Additive effect	Source of alleles
Spread	5A	Xbarc056	38.67	4.1	10.4	-0.77	Ernie
Spread with			36.67				Ernie
wilt	5A	Xbarc056		5.2	12.5	-1.37	
FHBI	2B	Xgwm319	118.62	3.9	5.0	-3.98	Ernie
	3B	E8M1_1	123.71	4.3	10.3	-5.00	Ernie
	4B	Xgwm495	0.01	10.3	14.7	-6.02	Ernie
	5A	Xbarc165	42.67	5.8	13.9	-5.71	Ernie
	5D	Xgwm182	27.84	3.3	16.8	-6.28	Ernie
FHBI with			118.45				Ernie
wilt	2B	Xgwm319		4.0	5.4	-5.60	
	3B	Xgwm077	98.80	3.0	5.1	-4.95	Ernie
	4B	Xgwm495	0.01	6.7	9.7	-6.69	Ernie
	5A	Xbarc165	40.67	8.7	23.5	-10.10	Ernie
	5D	Xgwm182	32.84	3.0	33.3	-12.10	Ernie

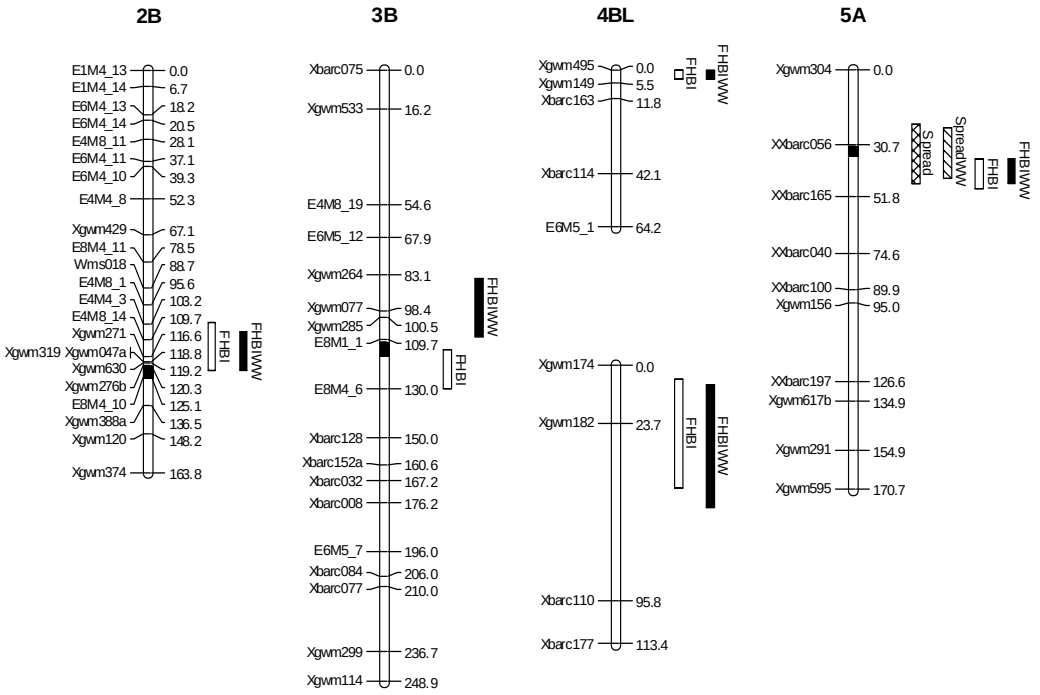

Figure 1. QTL associated with type II scab resistance in the soft red winter wheat cross Ernie x MO 94-317. Abbreviations are defined as follows: FHBIWW and FHBI = Fusarium head blight resistance index with and without wilted spikelets; SpreadWW and Spread = number of diseased spikelets with and without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

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COMPLEX MICROSYNTENY AMONG WHEAT, RICE AND BARLEY AT THE *QFHS.NDSU-3BS* REGION

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ABSTRACT

A major wheat QTL, *Qfhs.ndsu-3BS*, for resistance to Fusarium head blight (FHB) has been identified and verified by several research groups. The objectives of this study were to construct a high resolution map of the *Qfhs.ndsu-3BS* region, and to examine the microsynteny among wheat, rice and barley at this genomic region. Sixteen hundred F_2 plants derived from a single F_7 plant heterozygous for the *Qfhs.ndsu-3BS* region were screened for recombinants with two SSR markers, gwm533 and gwm493, and 192 recombinants were identified. Two additional SSR markers and six STS (sequence-tagged site) markers developed from wheat ESTs were used to genotype the 192 recombinants, and a fine genetic map was constructed. Except for two STS markers that cosegregate, the genetic distance between adjacent markers ranges from 0.2 to 1.5 cM. An inversion was revealed by comparing the order of STS markers and their counterpart genes on three overlapped rice PAC clones. It was previously reported that the microsynteny at this genomic region between rice and barley was interrupted by insertion of six additional barley genes. Six STS markers were developed from wheat ESTs homologous to each of the six barley genes. One STS marker was placed on the high resolution map, and an inversion between wheat and barley was revealed. Therefore, microsynteny among wheat, rice and barley at the *Qfhs.ndsu-3BS* region is complicated by microrearrangements such as inversions and insertion/deletions.

A MODEL CULTIVAR FOR TRANSFORMATION OF WHEAT TO IMPROVE RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Transformation of wheat is proving to be an effective method of introducing new sources of scab resistance into existing germplasm. However, transformation is a time-consuming process and transgenic lines require at least three greenhouse screens before it is prudent to field test the lines. Thus, the time taken to develop, test and move a transgenic line into a breeding program is several years. The cultivar Apogee is a fast-growing dwarf wheat. It flowers significantly faster than Bobwhite, the current cultivar of choice for wheat transformation. We have tested the regenerability of Apogee and have produced transgenic plants with the pAHC25 construct, carrying the *uidA* and bar genes under the control of the maize ubiquitin promoter. Moreover, we have shown that Apogee is susceptible to Fusarium head blight. Histochemical analyses of the expression of the *uidA* gene throughout our transgenic plants are presented along with data relating to the growth of Apogee in growth chambers and the greenhouse. Our data show that Apogee is a model cultivar for developing and testing transgenic wheat for scab resistance.

A TRANSGENIC APPROACH TO ENHANCING THE RESISTANCE OF WHEAT TO FUSARIUM HEAD BLIGHT

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ABSTRACT

We are developing and testing transgenic wheat for resistance to Fusarium head blight (FHB). Anti-fungal proteins (AFPs) such as chitinases, thaumatin-like proteins (tlps) and ribosome-inactivating proteins (RIPs) are known to inhibit fungal growth via different mechanisms. Chitinases degrade fungal cell walls, tlps degrade fungal cell membranes and RIPs inhibit fungal protein synthesis. Transgenic wheat over-expressing these AFPs were generated using micro-projectile bombardment. We developed 17 and 7 lines carrying a barley chitinase and a barley RIP, respectively. In addition, we developed 4, 11 and 5 lines expressing a combination of chitinase/RIP, chitinase/tlp-1 and RIP/tlp-1, respectively. These combinations each employ two of the three different mechanisms of fungal growth inhibition. We screened these lines for FHB resistance in the greenhouse 2-3 times. Results of these screens are discussed.

OVER-EXPRESSION OF ANTIFUNGAL PROTEINS INCREASES THE RESISTANCE OF WHEAT TO FUSARIUM HEAD BLIGHT

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ABSTRACT

We are developing and testing transgenic wheat for resistance to Fusarium head blight (FHB). Anti-fungal proteins (AFPs) such as ß-1,3-glucanases, thaumatin-like proteins (tlps) and thionins are known to inhibit fungal growth via different mechanisms. Glucanases degrade fungal cell walls while tlps and thionins degrade fungal cell membranes. Transgenic wheat over-expressing these AFPs were generated using micro-projectile bombardment. We developed 25, 25 and 31 lines carrying a wheat a-puro-thionin, a barley tlp-1 and a barley ß-1,3-glucanase respectively. Three to five independent glasshouse screens were conducted to assess these lines for FHB resistance. Two tlp-1 lines, four glucanase lines and one a-puro-thionin line consistently performed well. Molecular characterization of our lines has shown that they are genetically independent and that they accumulate the appropriate AFP. These 7 lines will be screened in the field in the summers of 2004 and 2005.

NPR1: A CANDIDATE TO ENHANCE BROAD SPECTRUM SCAB RESISTANCE IN WHEAT

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ABSTRACT

In recent years, Fusarium head blight (FHB) or scab has re-emerged as a devastating disease of wheat and barley, severely limiting productivity. Breeding has been the mainstay in developing wheat with improved resistance to scab. Biotechnology offers an alternative approach for rapidly developing scab resistant wheat. The *NPR1* gene which coordinates expression of several defense-associated genes in *Arabidopsis thaliana* is a regulatory gene that offers promise in developing plants with broad-spectrum resistance to scab and other fungal diseases. Overexpression of the Arabidopsis *NPR1* gene confers enhanced resistance in transgenic Arabidopsis and rice plants. We have generated transgenic wheat plants which overexpress the Arabidopsis *NPR1* transcript from the ubiquitously expressed maize Ubi1 promoter. Currently T_2 progeny derived from some of these transgenic lines are under evaluation for resistance to scab.

In parallel, we have identified three BAC's that contain the wheat homolog (WhNPR1) of the Arabidopsis and rice NPR1 gene. WhNPR1 has been mapped to chromosome 3. In addition, we have cloned a partial cDNA for WhNPR1 from a rust-infected Lr21 wheat cDNA library. The predicted WhNPR1 protein exhibits 80% similarity to the rice NPR1 protein. Expression of WhNPR1 is elevated in the flag leaves of scab-inoculated plants. We are presently reconstructing the full-length WhNPR1 cDNA by RACE. Since, interaction with other plant proteins is essential for the NPR1-mediated resistance, we hypothesize that in comparison to the Arabidopsis NPR1, overexpression of the WhNPR1 may confer higher level of disease resistance. To test this hypothesis we will generate transgenic wheat plants, which overexpress the WhNPR1 protein. We will present the current status of our research with the WhNPR1 gene and the transgenic wheat plants expressing the Arabidopsis NPR1 gene.

REGENERATION AND GENETIC TRANSFORMATION OF DURUM WHEAT

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ABSTRACT

Durum wheat (*Triticum turgidum* L.) is an important cereal crop used for making pasta and semolina. Efforts are in progress to improve durum wheat through gene transfer technology for characteristics such as disease resistance, especially for Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe). A major constraint is the lack of an efficient, reproducible and reliable method of genetic transformation of durum wheat. We have established an efficient and reproducible regeneration system with the cv. Monroe. Murashige and Skoog (MS) medium with different concentrations (1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) of picloram (4-amino-3,5,6-trichloropicolinic acid) or 2 mg/L 2, 4, 4-dichlorophenoxy acetic acid (2,4-D) were used to culture immature embryos for their morphogenetic response. Embryogenic calli proliferated on 2.0 mg/L picloram but was less frequent on 2,4-D containing media. Picloram at 2.0 mg/L also regenerated more plants than either 2,4-D or the other picloram concentrations. For genetic transformation, the calli were bombarded with the pathogenesis-related gene thaumatin-like (*tlp*) from rice, and a modified *Tri101* gene, along with the *bar* gene for selection. PCR and Southern analysis indicated the regenerated plants contained the transgenes and the western analysis confirmed the expression of the *tlp* in the durum wheat cv. Monroe.

FINE MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE AND HEADING DATE QTL IN BARLEY

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ABSTRACT

Previous mapping studies in barley using populations derived from the Fusarium head blight (FHB) resistant cultivar Chevron have positioned a QTL for FHB resistance on chromosome 2 (2H) in a genomic region (~20 cM) flanked by SSR markers *Bmag0140* and *Ebmac0521a*. A coincident QTL for heading date (HD) is also located in this region creating uncertainty as to whether this association is due to linkage or pleiotropy. We are fine mapping the target QTL region using a population of five hundred and thirty two F_2 plants derived from a cross between a BC_5 line carrying the Chevron alleles in this region and the recurrent parent M69. Forty-four recombinants identified in this population were genotyped with 13 SSR markers previously mapped between *Bmag0140* and *EBmac0521*. A linkage map was constructed using GMendel v3.0 software. An average marker interval of 1.3 cM was observed. The 44 recombinants were advanced to the $BC_5F_{2:4}$ and evaluated in replicated field trials for FHB and HD at St. Paul and Crookston, MN, in the summer of 2003. Using trait means from individual locations, data were analyzed for presence of QTL via simple interval mapping (SIM) model (PlabQTL v1.0 software) and single marker analysis of variance. A QTL for HD was detected between markers *Bmac0093* and *EBmac0558* (0.2 cM apart) at St. Paul (LOD=6.7; R^2=85.6) and at Crookston (LOD=6.7; R^2=85.4). An FHB resistance QTL was found to be closely associated with EBmac0521b (located 4.7 cM away from the HD QTL) both in St. Paul (*P*=0.03) and Crookston (*P*=0.05). These preliminary results suggest that FHB resistance and late HD are closely associated due to linkage rather than pleiotropy.

INVESTIGATING THE GENETICS OF RESISTANCE TO FHB IN SIX-ROWED, HULLESS, BARLEY ACCESSION HOR211

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ABSTRACT

Development of Fusarium head blight (FHB) resistant barley varieties will require breeding with diverse sources of resistance that carry different resistance genes. All FHB mapping studies in barley to date have identified a significant quantitative trait locus (QTL) for FHB on chromosome 2(2H) in many cases linked to heading date. On the basis of cluster analyses and selective genotyping analyses, we identified the Ukrainian line Hor211 (six-rowed, hulless) as a genotype that is genetically dissimilar from other known sources of FHB resistance. The overall objective of this study is to identify QTLs for FHB resistance and to determine the genetic relationship between FHB and other associated traits (heading date, plant height, DON concentration, and presence of a hull). Hor211 was crossed with Lacey (University of Minnesota cultivar) to develop a $F_{6:8}$ mapping population. The Hor211/Lacey population was evaluated in three field environments (St. Paul 2002, Hangzhou, China 2002, St. Paul, 2003). We found significant variation among the lines for FHB severity, plant height and heading date. Thus far, linkage maps have been created for chromosome 1(7H) and 2(2H) using SSR markers. QTL analysis has identified a QTL for heading date on chromosome 1(7H) (R^2 = 14.5%, 24.5%) and on chromosome 2(2H) (R^2 = 12.7%, 13.0%) detected in two environments. No QTLs for FHB resistance were found on either chromosome 1(7H) or 2(2H). This suggests that Hor211 likely carries resistance that is not associated with the major QTLs that have been previously identified. Continued mapping in this population should yield new information about FHB resistance in barley.

IN VITRO REGENERATION OF COMMERCIAL DURUM CULTIVARS AND TRANSFORMATION WITH ANTIFUNGAL GENES

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OBJECTIVES

To standardize an efficient *in vitro* regeneration protocol for commercial durum wheat cultivars and to incorporate antifungal genes to produce resistance to Fusarium head blight.

INTRODUCTION

Durum wheat (*Triticum turgidum* L., $2n = 4x = 28$; AABB genomes) is an important cereal used for human consumption worldwide. Scab or Fusarium head blight (FHB), caused primarily by the fungus *Fusarium graminearum* Schwabe, is a devastating disease inflicting heavy losses to wheat growers especially in the Northern Plains of the United States (Windels, 2000). There is no reliable source of FHB resistance in current durum cultivars. Through sexual hybridization with wild grasses, coupled with manipulation of chromosome pairing, we have produced FHB-resistant durum germplasm (Jauhar and Peterson, 2001; Jauhar, 2003). Although such chromosome-mediated gene transfers have resulted in genetic improvement of both bread wheat and durum wheat (Friebe et al., 1996; Jauhar and Chibbar, 1999), this technique of germplasm enhancement is often tedious and time-consuming. Genetic engineering provides an efficient means of directly incorporating disease resistance genes into the wheat genome. FHB resistance in wheat was achieved by expressing antifungal genes, including *TRI101*, *PDR5*, and thaumatin-like protein (TLP) genes that degrade structural components of the fungus and/or interfere with biochemical and metabolic processes in the pathogen (Chen et al., 1999; Anand et al., 2003).

A major impediment to durum wheat transformation has been the lack of an efficient method of *in vitro* regeneration. Earlier, we standardized a rapid regeneration protocol for four durum cultivars (Bommineni and Jauhar, 1996) and using this regeneration system, we produced the first transgenic durum wheat (Bommineni et al., 1997). Since we showed that durum is amenable to genetic transformation, there are a few more reports of production of transgenic durum (He et al., 1999; Pellegrineschi et al., 2002). Incorporation of the gene(s) of interest directly into modern cultivars will facilitate development of new germplasm in a relatively short time. Because of genotypic differences in regenerability, it is important to determine optimal *in vitro* culture conditions for current commercial cultivars before selecting one for transformation. Therefore, we studied the effects of three growth regulators (2,4-D, picloram, and dicamba) on callus induction and plant regeneration from scutellum cultures of four commercial durum cultivars, Ben, Maier, Munich, and Lebsock. Having selected the best responding cultivar, Maier, we are incorporating antifungal genes into it.

MATERIALS AND METHODS

Plant material - Four agronomically superior cultivars, Ben, Maier, Munich, and Lebsock, were selected to study their *in vitro* culture abilities.

Callus induction and plant regeneration - Scutella were isolated from the four cultivars and regenerated on Murashige and Skoog medium according to the method described by Bommineni and Jauhar (1996). Four different concentrations (0.5, 1.0, 2.0, and 2.5 mg L^{-1}) of each of 2,4-D, picloram, and dicamba were used in the modified MS medium. The experiment was replicated three times with 20 scutella per concentration. The scutella were incubated in the dark at 25±2°C for a period of 4 weeks for callus induction. Four week-old calli were transferred to auxin-free MS medium and incubated at 25°C with a 16-h photoperiod. The regenerated plants were transferred to peat pellets and, when established, were grown to maturity in the greenhouse.

The data gathered on the number of explants callusing and the number of calli showing green shoot buds were analyzed (4 cultivars × 3 growth regulators × 4 concentrations) using a logistic regression model (Hosmer and Lemeshow, 1989). For plant regeneration, a Poisson Regression Model was used. SAS (version 8.2, 1999-2001) procedures were used for statistical analyses.

Chromosomal studies - Root tips from regenerated plants were fixed and somatic chromosomes were studied using conventional and fluorescent genomic *in situ* hybridization (fl-GISH) techniques standardized earlier (Jauhar et al., 1999).

Transformation - The most regenerable cultivar Maier was selected for transformation experiments. Two-week old calli cultured on 2.0 mg L^{-1} dicamba-containing medium were subjected to microprojectile bombardment. The plasmids (pAHC25/pAHRC-TLP) containing *bar* alone or both *bar* and rice *tlp* were co-bombarded with the pUBK-Bgl containing modified *TRI101* gene. All the genes are under the control of ubiquitin promoter and nos terminator. Transformations were done according to Bommineni et al. (1997).

Preliminary screening of transformants was done using PCR analysis. Genomic DNA was isolated from leaves of putative T_0 and T_1 transformants as well as from non-transformed (control) plants using the method of Dellaporta et al. (1983). PCR analyses were carried out using *bar* and *TRI101* gene-specific primers. For Western blot analysis, a polyclonal anti-TLP-antibody was used as a primary antibody (Chen et al., 1999) for confirming the expression of *tlp* gene. For Southern hybridization, genomic DNA (15 µg) was digested with enzymes *Eco*RI or *Hin*dIII and electrophoresed following standard procedures. The *bar* gene fragment (1.4 kb) released from pAHC25 was used as a probe. The *bar* gene was biotin-labeled using random priming method. Hybridization and detection of the signal were carried out according to Boehringer Mannheim manual instructions.

RESULTS AND DISCUSSION

Initiation of callus was apparent as a white translucent tissue within 3-7 days in all four cultivars. The callus induction rates among cultivars varied from 13-93%. The embryogenic calli differentiated into somatic embryos within 3-4 weeks on auxin containing medium. On transfer to media devoid of growth regulators, the embryos differentiated as green shoot buds and developed into plantlets when exposed to light, as observed earlier by Bommineni and Jauhar (1996).

The logistic regression model showed significant effects of cultivar, growth regulator, and concentration of growth regulator on callus induction ($p < 0.001$) and shoot bud formation ($p < 0.05$). Poisson regression analysis revealed significant differences among cultivars, growth regulators, and their concentrations for plant regeneration.

Among the four cultivars, Lebsock showed the highest callus induction, while Maier gave highest number of plantlets per explant. We found dicamba to be more effective for callus induction and subsequent plant regeneration compared to other two growth regulators, 2,4-D and picloram. Dicamba has been reported to equal or surpass 2,4-D in inducing shoot formation in many cereals including wheat, maize, and barley (Mendoza and Kaeppler, 2002).

All of the regenerated plantlets grew to maturity without any apparent morphological changes and had the expected chromosomal number of $2n = 28$. Fluorescent GISH proved that the chromosome complement of regenerants was intact, with 14 A- and 14 B-genome chromosomes.

Two week-old calli of Maier bombarded with *bar* or *tlp* along with *mTRI101* gene were selected on medium containing 5 mg L^{-1} bialaphos. About 50% of the calli turned either brown or remained watery and did not grow further. From the 650 scutella co-bombarded with genes *bar* and *mTRI101*, 117 plantlets (18%) were regenerated, and out of 720 scutella co-bombarded with *tlp* and *mTRI101*, 120 plantlets(16.7%) were derived.

Preliminary screening of the transformants was done by PCR analyses using *bar* and *TRI101*-specific primers. Western blot analysis using specific antibody to Tlp protein showed an expected protein band (~25 kDa) in some of the transformants that was absent in the untransformed control. By over-expressing a rice *tlp* gene in a transgenic wheat line, Chen et al. (1999) observed moderate resistance to scab.

We have raised 20 T_1 progeny from each T_0 putative transformant. Southern hybridization is being done to confirm the inheritance of the transgene and estimate the copy number.

CONCLUSION

To standardize the *in vitro* regeneration protocol, we studied the effect of three different growth regulators on callus induction and subsequent plant regeneration in four commercial durum wheat cultivars. Overall, the results showed dicamba to be most suitable for callus formation and plant regeneration across the four cultivars. However, Maier offered the best choice for use in genetic transformation experiments. Using microprojection, we have incorporated antifungal genes into Maier and inheritance of the transgene in the progeny is being studied. We are also standardizing an *Agrobacterium*-mediated transformation system for durum cultivars. We believe that chromosome-mediated gene transfers (involving sexual hybridization with potential gene donors in the primary and secondary gene pools) as well as transgenic approaches should be adopted to combat Fusarium head blight, a ravaging disease of cereal crops.

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SATURATION MAPPING OF A MAJOR FUSARIUM HEAD BLIGHT QTL ON BARLEY CHROMOSOME 2H

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OBJECTIVES

To dissect the barley chromosome 2(2H) major Fusarium Head Blight (FHB) Quantitative Trait Locus (QTL) by genetic and physical mapping and development of substitution lines containing small CI 4196 chromosome 2H fragments in a susceptible cultivar background.

INTRODUCTION

In every cross investigated in barley, chromosome 2(2H) has been identified to harbor a strong FHB resistance QTL. De la Penna et al.. (DE LA PENA *et al.* 1999) working with the Chevron x M69 cross, reported 3 QTL which we have designated FHBqtl1, 2, an3. Chr. 2(2H) FHBqtl1 extends from ABC311 to MWG858 (Bins 3 and 4). FHBqtl2 extends from ABG459 to MWG520A (Bin 5). FHBqtl3 extends from MWG887 to ABG619 (Bins 6 to 9). This region is just above and may include the 2 vs. 6-rowed gene *Vrs1*. Another study involving Chevron crossed with Stander (Ma *et al.* 2000) identified 2 chromosome 2(2H) QTL, one from BCD307D to CDO1407 (Bins 6 to 9). This QTL seems to correspond to the FHBqtl3 reported by de la Pena. Another QTL was reported between the markers BCD307B and CDO684B (Bins 12 to 15). This QTL was not previously reported and I have designated it FHBqtl4. In the above cases Chevron, a 6-rowed barley, contributed the resistant alleles. Zhu et al. (Zhu *et al.* 1999) working with 2-rowed genotypes Gobernadora x CMB643 reported a single major FHB QTL on chromosome 2(2H) for Type I and II resistance centered on marker MWG503 (Bin 11). This QTL overlaps with FHBqtl3 and may be the same. This work is reviewed in (Kolb *et al.* 2001). More recently, two other studies have identified FHB QTL on chromosome 2H (Mesfin *et al.* 2003 and Dahleen *et al.* 2003). Although the exact borders of the QTL vary slightly and it is sometimes difficult to make exact comparisons due to the use of different markers, these studies indicate that there are important FHB resistance QTL located on barley chromosome 2H. A FHB QTL mapping study in wheat reported a QTL centered near ksuH16 (Bin 15) (Waldron *et al.* 1999). This QTL may correspond to the barley FHBqtl4. All of the barley QTL were associated with DON accumulation, heading date, and plant height.

In the Foster x CI4196 (FosCI) cross, a very strong FHB resistance QTL was detected on chromosome 2(2H) extending from ABG005 (Bin5) to MWG882 (Bin12). This QTL was identified in all 3 locations and all 4 years that it was tested in North Dakota. The highly significant QTL appearing in all locations and all years extended from ABC306 (Bin8) to MWG503 (Bin11). Thus it appears that this chromosome 2(2H) region is extremely important for FHB resistance and the variation in the size and exact location of this QTL depends on the experimental design, the quality of the maps used, and environmental conditions. To more precisely identify smaller chromosome regions for their role in FHB resistance we need to develop isolines of this chromosome fragment from CI4196 into a uniform, susceptible genomic background. The FHB resistance QTL region on chromosome 2H is also involved in DON accumulation, heading date, and height.

Rice synteny with barley in this region is known. Rice chromosomes 4 and 7 appear to be involved (Van Deynze *et al.* 1995). The barley marker ABG716 (Bin 7) was mapped in rice on chromosome 7 at 83.8 cM, while the marker ABG356 (Bin 8) was mapped on rice chromosome 4 at 65.6 cM. A more detailed synteny is presented in Fig. 1.

MATERIALS AND METHODS

Saturate the target region with molecular markers - Rice PAC or BAC clone sequences are blasted (blastn) against the Triticeae EST database. One barley EST with the highest S-value from each contig within a PAC/BAC was selected. Rice PAC/BAC sequences were obtained from the Rice Genome Research Program website (http://rgp.dna.affrc.go.jp/) and/or the International Rice Genome Sequencing Project website (http://rgp.dna.affrc.go.jp/IRGSP/). Additionally, ESTs mapped to wheat group 2 deletion lines were used to identify homologous barley ESTs by blastn analysis. The identified ESTs are mapped in the Foster x CI4196 recombinant inbred population or, if not polymorphic, in one of the many other populations available to us.

The mapped EST clones are hybridized against the barley cv. Morex BAC library and the positive clones identified. Once the BAC addresses are identified, the BAC clones are picked, grown in 96-well plates, and colony blotted on filters using a 96-pin hand replicator.

Isoline development - Two selected recombinant inbreds from the FosCI population were crossed to Morex to initiate the isoline development. Morex is being used as the recurrent parent because we have a Morex male sterile line, thus facilitating backcrossing. The F_1 lines will be backcrossed to Morex, progeny with the chromosome 2(2H) FHB QTL region fragments selected and backcrossed to Morex again.

Develop a physical map of the chromosome 2(2H) FHB QTL region - BAC clones from the chromosome 2H FHB resistance QTL identified from the Morex BAC library are sent to Dr. Mingcheng Luo who is conducting the fingerprinting of the barley BAC clones under an NSF grant to Tim Close.

RESULTS AND DISCUSSION

The 2H FHB QTL region spans ~40cM and ~30cM on the FosCI and the Steptoe/Morex (SM) genetic maps, respectively. A total of 28 rice BAC clones, comprising ~2.5Mbp, span the rice chromosome 4 region with synteny to the barley chromosome 2H QTL region. The rice chromosome 4 BACs assemble into two contigs, BAC clones OSJNBb0091E11 to OSJNBa0029H02 (~365kb) and OSJNBa0014K14 to OSJNBa0010H02 (~2.2Mbp). There is a gap of unknown distance between OSJNBa0029H02 and OSJNBa0014K14. Ninety-seven unique barley ESTs were identified from the 28 rice BACs. Fourty-seven have been tested for polymorphism against several barley cultivars. Twenty-six of these 47 have been mapped to the 2H FHB QTL region. The rest either mapped elsewhere in the genome or were non-polymorphic. These results give a 55.3% efficiency of barley ESTs identified with homology to rice that map in syntennous positions. One EST mapped to the 2H QTL region that has homology to rice chromosome 7 PAC clone P0022E03.

Wheat ESTs mapped to group 2 deletion lines were used to identify 39 homologous barley ESTs that were screened for polymorphisms. Only four of these mapped to the 2H FHB QTL region. Due to the relatively large distances between deletion line breakpoints that delimit each wheat chromosome bin, several ESTs

were identified that mapped outside the FHB QTL region on 2H. Other probes were either non-polymorphic or mapped to other chromosomes.

A total of 34 probes (including genomic clones) have been mapped to the QTL region to date (Fig.1). Nineteen have been mapped in the FosCI population, three in SM DHLs, 10 in both FosCI and SM, one in Harrington x Morex DHLs, one in Harrington x TR306 DHLs, and one in a Chebec x Harrington population. All 34 probes have been used to screen the 6x cv. Morex barley BAC library, 17 of which have been confirmed as identifying 98 individual BAC clones.

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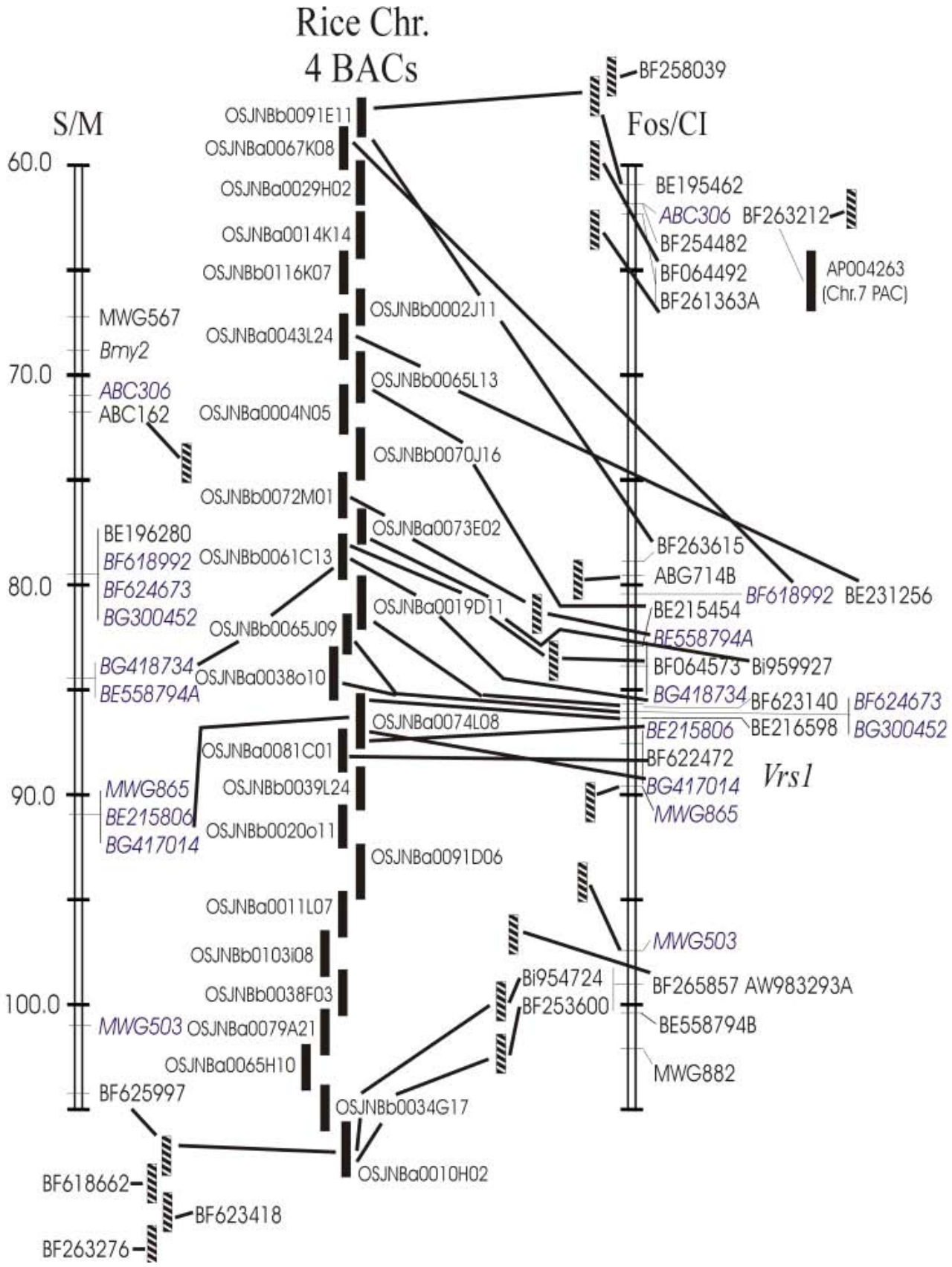
Fig.1 (Right). The Fusarium head blight (FHB) chromosome 2H major QTL region is depicted from the Steptoe x Morex (SM) 150 DHL map, the Foster x CI4196 (FosCI) 144 RIL map, and the syntennous rice chromosome 4 BACs. The SM map is shown on the left and the FosCI map on the right. Approximate centiMorgan values are given to the left of the SM map and correspond to the FosCI map. The rice chr. 4 BACs are between the two barley genetic maps. Rice BACs are represented by a black box, while barley BACs are represented by a striped box. Lines connecting ESTs to BAC clones show which ESTs have confirmed barley BACs and which rice BACs they are homologous to. The approximate position of the *Vrs1* locus is shown to the right of the FosCI map.


Rice Chr.
4 BACs
S/M
Fos/CI
60.0
70.0
80.0
90.0
100.0
MWG567
Bmy2
ABC306
ABC162
BE196280
BF618992
BF624673
BG300452
BG418734
BE558794A
MWG865
BE215806
BG417014
MWG503
BF625997
BF618662
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OSJNBb0091E11
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OSJNBa0029H02
OSJNBa0014K14
OSJNBb0116K07
OSJNBb0002J11
OSJNBa0043L24
OSJNBb0065L13
OSJNBa0004N05
OSJNBb0070J16
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OSJNBa0073E02
OSJNBb0061C13
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OSJNBb0039L24
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OSJNBa0091D06
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OSJNBb0103i08
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OSJNBa0079A21
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OSJNBb0034G17
OSJNBa0010H02
BF258039
BE195462
ABC306
BF263212
BF254482
BF064492
BF261363A
AP004263
(Chr.7 PAC)
BF263615
ABG714B
BF618992
BE231256
BE215454
BE558794A
BF064573
Bi959927
BG418734
BF623140
BF624673
BE215806
BE216598
BG300452
BF622472
Vrs1
BG417014
MWG865
MWG503
Bi954724
BF253600
BF265857
AW983293A
BE558794B
MWG882

CONTROLLING SCAB WITH PUROINDOLINE-EXPRESSING WHEAT AND BARLEY

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ABSTRACT

The wheat puroindolines (PINA and PINB) are small (ca. 120 a.a.), basic proteins that are normally expressed only in the endosperm. They have been shown to contribute to grain texture, with the presence of PIN leading to softer grain. The puroindolines have also been shown to have *in vitro* and *in vivo* anti-fungal properties. Analyses of the anti-fungal activity have been extended to wheat Fusarium scab. The growth of *F. graminearum* and *F. culmorum* was inhibited by PIN in *in vitro* bioassays. Control and transgenic HiLine wheat varieties that over-express the *pinB* gene driven by the constitutive maize ubiquitin promoter or by the endosperm-specific glutenin-promoter, were inoculated with *F. graminearum* or *F. culmorum* in field and greenhouse studies that have been replicated numerous times. Generally, Hi-Line and transgenic (only contain the selectable marker) control plants showed between 20-50% severely infected spikelets/head (over 40% infected spikelets). *PinB*-transgenic lines often showed a dramatic reduction in plants with severe infections, with a concomitant increase in heads with lesser infection. The transgenic plants showed a decrease of the percentage of tombstones, when compared to the control. There were no significant differences in toxin levels of heads with similar levels of infection, regardless of the plant. Thus, *pin*-expressing plants would have a deceased total level of toxin, since they have lower levels of infection overall. These data suggest that PIN proteins may provide protection to wheat and barley, which we are currently transforming with *pinA*, against *Fusarium* scab.

ACKNOWLEDGEMENT

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GENETIC AND PHYSICAL MAPS OF *XBARC* SSR LOCI IN WHEAT

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INTRODUCTION

Genetic maps saturated with informative markers are of great importance for localizing and manipulating important genes and 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 *et al.* 2000; Salina *et al.* 2000). Here we display our latest version of a genetic/physical map containing over 1400 total loci including 367 new SSR loci. Curated, interactive maps and primer (probe) details are available with GrainGenes Web site (http://wheat.pw.usda.gov).

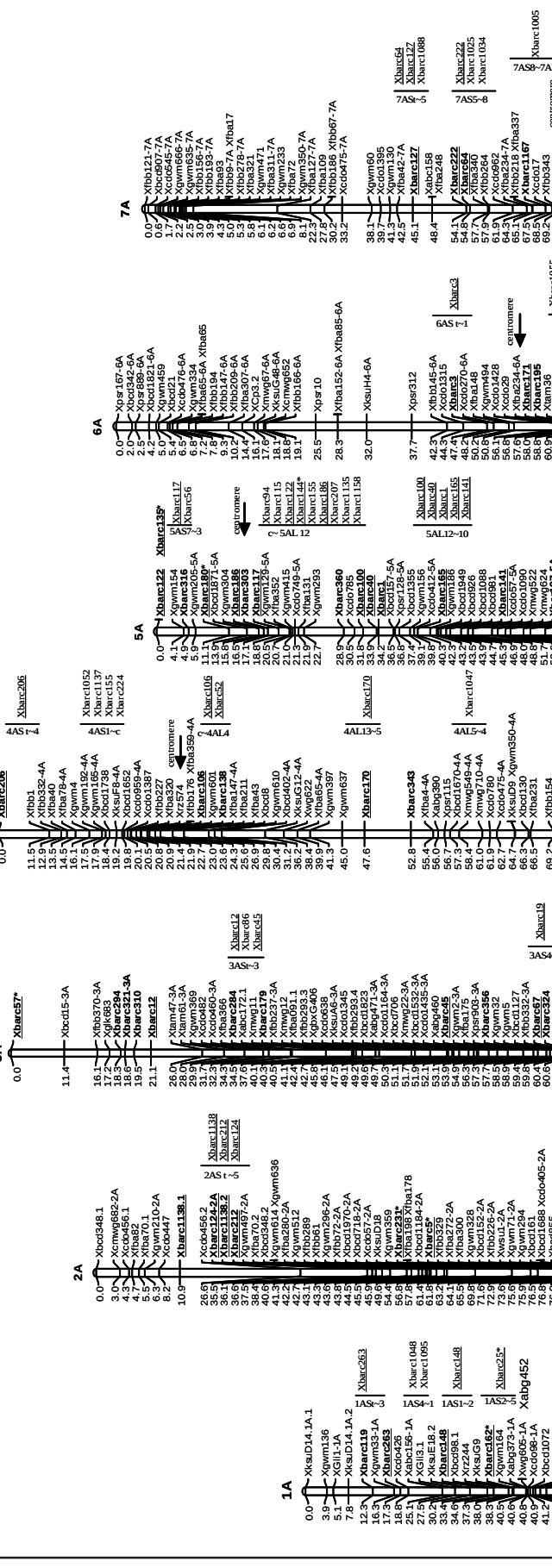
METHODS

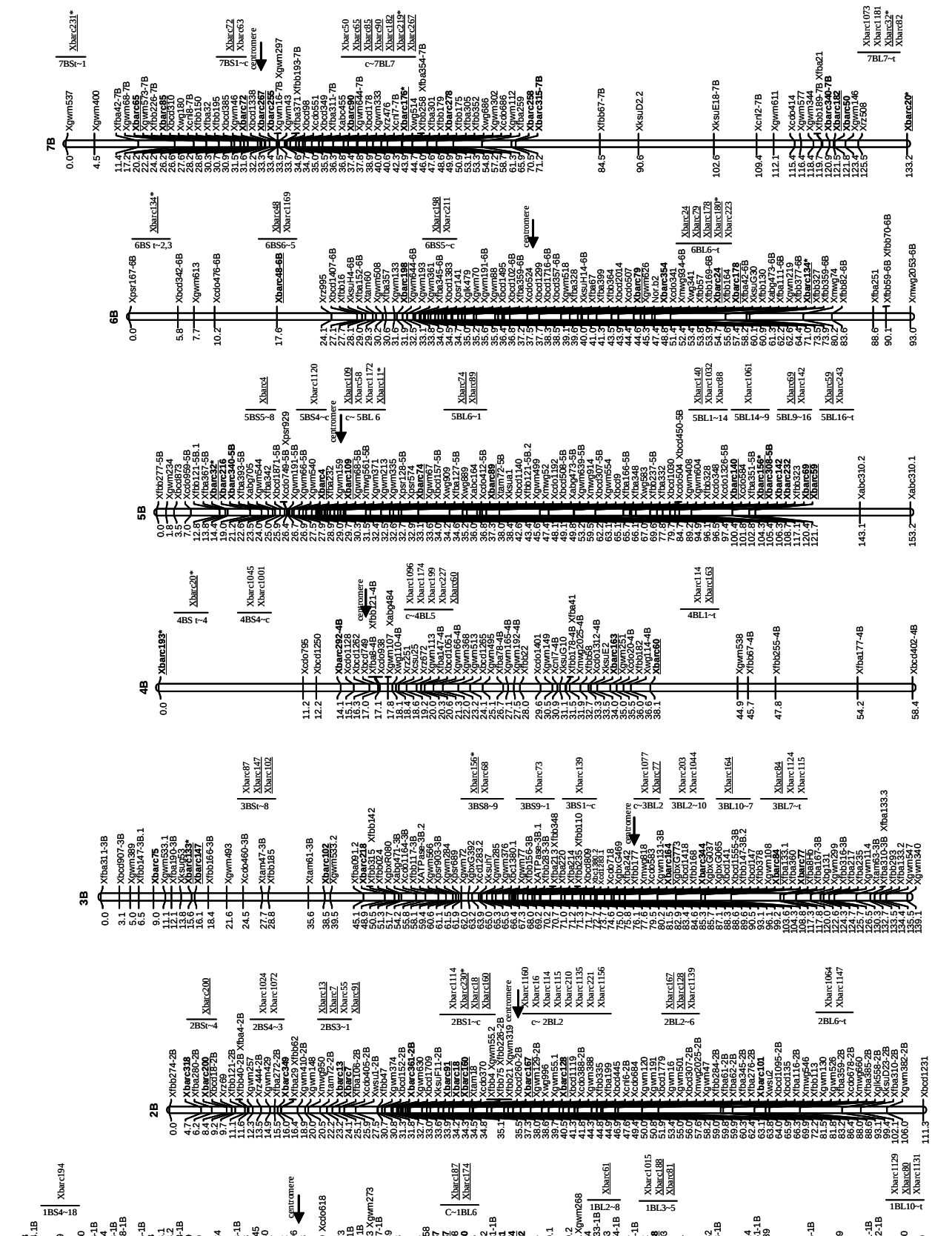
Over 700 "BARC" primer sets that amplify SSR loci were developed in P. Cregan's lab at USDA-ARS's Beltsville Agricultural Research Center (BARC) (Song *et al.*, 2000). The general genetic segregation analysis was conducted at MSU and physical (aneuploid and deletion) mapping was conducted at KSU and MSU. For primer pairs that were polymorphic between the ITMI population parents W7984 and Opata 85, the first 83 or 94 recombinant inbred lines (RILS) of the population were used for segregation analysis. PCR reactions and gel system were described by Shi *et al.* (2001). Linkage analysis and map construction were performed using MAPMAKER 3.0b (Lander *et al.* 1987) and Joinmap 3.0 (Van Ooijen *et al.* 2001). For primers which were not polymorphic in the ITMI population, we used 48 nullitetrasomic and ditelosomic lines to assign the markers to chromosome arms. Different numbers of single-break deletion stocks on each chromosome were then used for sub-arm localization of SSR loci.

RESULTS AND DISCUSSION

A data set containing 1469 markers, including newly developed microsatellites and 1232 markers previously mapped across 21 chromosomes downloaded from GrainGenes website, and 115 progenies of the ITMI population was used for analysis. Two hundred twenty-five Xbarc loci were assigned to linkage map. Another 142 Xbarc SSR loci (amplified by 137 primer pairs) were localized to chromosome arms with deletion mapping (Endo and Gill, 1996).

The final map combined genetic/physical line maps of all 21 chromosomes of bread wheat. Correspondence of physical and genetic positions of Xbarc loci is not perfect. Out of 102 Xbarcs which are mapped both genetically and physically, 82 Xbarcs are confirmed on both maps, 4 Xbarcs on same chromosome but different region, and 16 Xbarcs on totally different chromosomes.





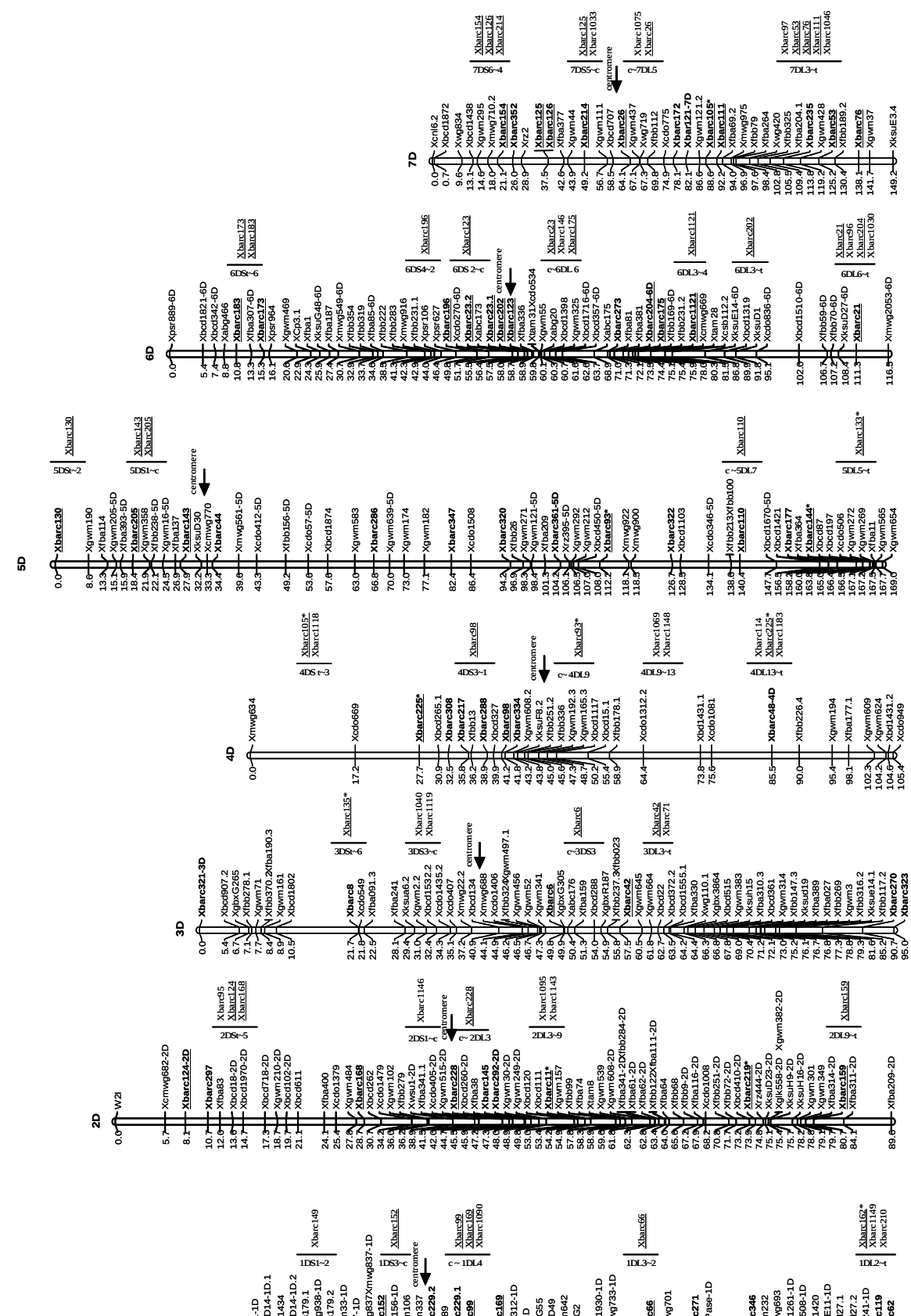


Figure 1. Genetic and Physical Maps of Wheat Xbarc SSR Loci. Genetic line maps were produced by JoinMap3.0. Cumulative Centimorgan (cM) distances are indicated on the left side of each chromosome. The short arm of each chromosome is on top. Loci genetically mapped are listed immediately to the right of the chromosome. Loci derived from GrainGenes data are in normal font. Xbarc loci are in Bold. Physical locations are indicated in BIN order. All loci mapped physically are listed according to the region in which a Xbarc marker is localized – i.e. 1AS-2~1 is the region between deletion breakpoints 2 and 1. For physical locations, "t" and "c" refer to the telomere and centomere, respectively. Centromeres are indicated with arrows. For the Xbarcs mapped both physically and genetically, the marker's name is underlined. Xbarc loci which mapped to different locations on the physical and genetic maps are denoted with asterisks.

ACKNOWLEDGMENTS

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Data for ITMI RILs for 1232 loci were obtained from the USDA-ARS GrainGenes website. The generosity of the various authors contributing that data is gratefully acknowledged.

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ASSESSING GENETIC DIVERSITY OF FHB RESISTANCE IN BARLEY USING MOLECULAR MARKERS

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ABSTRACT

Developing sufficient genetic resistance to FHB to keep barley a sustainable crop in the Upper Midwest is a substantial challenge. Research to date indicates that significant levels of resistance can be obtained only through the accumulation of resistance alleles at multiple loci derived from diverse sources of resistance. We are using molecular markers to characterize the diversity of FHB resistance and identify useful alleles for breeding. We are in various stages of mapping, validating, and fine mapping QTL for FHB in four different sources of resistance. Most studies conducted to date indicate that chromosome 2 carries at least 2 important QTL for FHB. Unfortunately, each of these QTL is coincident with other confounding traits (heading date and 2-rowed/6-rowed spike morphology). Another important QTL has been validated on chromosome 6 and the FHB resistance allele is linked to resistance to kernel discoloration and high grain protein concentration. We have used selective genotyping to identify new sources of FHB that will likely carry different genes for resistance. Preliminary evidence suggests that both sources Atahualpa and Hor211 carry FHB QTL not located on chromosome 2. In an effort to identify diverse genes for resistance, we have studied resistance to DON accumulation after point inoculation in the mapping population Frederickson x Stander. A single QTL on chromosome 3 accounts for significant variation for this trait and is not associated with FHB severity suggesting a resistance mechanism for accumulation of DON that is independent of resistance to infection. It is our hope that the development of genetic stocks that carry useful alleles for FHB resistance and the accompanying molecular marker information will provide substantial assistance to efforts to breed FHB resistant varieties.

CHARACTERIZATION OF ORGAN-SPECIFIC PROMOTERS FROM MAIZE AND BARLEY IN TRANSGENIC WHEAT

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ABSTRACT

Genetic engineering is a promising approach to increase plant resistance to fungal pathogens, including *Fusarium*. At present, constitutive promoters are widely used to achieve high expression levels of antifungal genes throughout most tissues of the plant. If only some tissues need to be protected, the use of specific promoters is desirable. Because the glume and lemma comprise the protective barrier encasing the reproductive organs, expression of anti-*Fusarium* genes in these outer floral structures is required to make either wheat or barley resistant to FHB. In order to identify promoters suitable for targeting anti-*Fusarium* gene expression to wheat tissues surrounding the developing seed, we have characterized the organ- and developmental specificity of the promoters from a maize glutamine synthase gene, $GS_{1\text{-}2}$, and from a barley floret-expressed gene, *Lem1*, in stable hexaploid wheat transformants. The plasmids pGS176 and pGS177 (GS:GUS) and pBSD5sGFP (Lem1:GFP) were introduced into immature embryos of *cv*. Bobwhite by particle bombardment. The expression of the reporter genes was monitored in the T_0 and T_1 plants. We found that the maize $GS_{1\text{-}2}$ promoter is expressed in the pericarp and in the scutellum of mature embryos. Thus, this promoter is not suitable for use in anti-*Fusarium* constructs. Monitoring GFP fluorescence in primary transformants revealed that *Lem1* promoter drove the highest *gfp* expression in the lemma, palea, glume, awns, and rachis at anthesis when the anthers first become visible outside the glumes. *Lem1* did not function in developing wheat florets and the surrounding tissues before anthesis when the young ovary and anthers are completely covered by the outer floral organs. After pollination, GFP fluorescence was restricted to a few cells of the lemma and palea and was not detectable in these organs at later stages of seed development. No *gfp* expression was detected in any vegetative organs. Thus, *Lem1* activity in transgenic wheat is identical to its pattern in its native context in barley. Transient assays indicate that *Lem1* is about 4-5 times less active than UBI, one of the strongest of cereal promoters characterized to date. The tissue specificity and moderate strength of the barley *Lem1* promoter suggest that it would be an excellent choice to target anti-*Fusarium* gene expression to wheat tissues surrounding the developing seed. Two cloning vectors have been constructed: pBGS9Lem1 carrying the *Lem1* promoter and the NOS 3' terminator sequence and pBGS9Lem1ADHi1, in which the first intron of the maize *ADH1* gene was added after the *Lem1* promoter, where it may serve as a quantitative element to raise expression levels. Cloning of candidate anti-*Fusarium* genes into these vectors is in progress.

DETERMINATION OF *FUSARIUM GRAMINERAUM* CHEMOTYPE BASED ON UPSTREAM SEQUENCES OF THE *TRI5* GENE

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ABSTRACT

The *Tri5* gene which encodes trichodiene synthase, the first step in the trichothecene biosynthetic pathway, is reported to co-segregate with the locus governing the type of trichothecene produced. Sequence analysis of 26 isolates with known chemotype, representative of the global lineages of *F. graminearum*, revealed that all deoxynivalenol (DON) chemotypes displayed characteristic deletions in a region in the upstream sequences of the *Tri5* gene. The distinct length polymorphisms in this region between the DON and nivalenol (NIV) chemotypes allowed a PCR assay to be developed in this study to distinguish between these chemotypes. Six *F. graminearum* isolates from southern NSW in Australia and twenty overseas isolates were analysed using this technique and compared with published assays utilising polymorphisms in the *Tri7* and *Tri13* genes to distinguish DON and NIV chemotypes. Results demonstrated the potential for reliable use of the molecular tool targeting the upstream sequences of the *Tri5* gene to differentiate NIV and DON chemotypes. Two of the isolates from southern NSW were of the DON chemotype while the other four were of the NIV chemotype. Further research is required to establish the relative distribution of DON and NIV chemotypes in the NSW and Australian grain-belt.

MOLECULAR, PATHOLOGICAL AND TOXICOLOGICAL EXAMINATION OF THE HUNGARIAN *FUSARIUM GRAMINEARUM* POPULATION COMPARED TO MOLECULAR LINEAGES OF THE WORLD-WIDE POPULATION

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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, aggressiveness and molecular variability of *Fusarium graminearum* isolates using different techniques. Altogether 27 Hungarian, 3 Austrian isolates and representatives of the 8 lineages identified by O'Donnell et al. (2000) were involved in this study. Mycotoxin producing abilities of the isolates were tested by thin layer chromatography. The mycotoxins tested included deoxynivalenol and its acetylated derivatives, nivalenol, zearalenone and fusarenone X. Most of the isolates produced zearalenone. All Central-European isolates were found to belong to chemotype I (producing deoxynivalenol). Most *F. graminearum* isolates were found to be highly pathogenic in *in vitro* aggressiveness tests. In our studies, the aggressiveness of *F. graminearum* isolates belonging to chemotype I was in general higher than that of isolates belonging to chemotype II, in accordance with previous observations. Phylogenetic analysis of random amplified polymorphic DNA (RAPD) profiles of the isolates obtained by using 40 different random decamers let us cluster the Central-European isolates into 10 haplotypes. The three Austrian isolates formed a distinct clade on the tree. We also examined the variability of the intergenic spacer region (IGS) of the ribosomal RNA gene cluster using IGS-RFLP. The Central-European isolates belonged into 9 haplotypes on the tree based on IGS-RFLP data. Representatives of the *F. graminearum* lineages formed distinct branches on both trees. When RAPD and IGS-RFLP data were combined, almost every single Central-European *F. graminearum* isolate could be differentiated from each other (27/30 haplotypes). Such a lack of strict correlation between trees based on different data sets indicates that recombination took place in the examined population due to frequent outcrossing. Based on RAPD and IGS-RFLP data, most Central-European isolates most probably belong to lineage 7 characteristic to the Northern hemisphere, with the exception of two Hungarian isolates. One of these was most closely related to lineage 6 originated from Asia, while the other isolate was not closely related to any of the other examined *F. graminearum* isolates. Correlation was not observed between mycotoxin producing abilities of the isolates and their position on either trees. Double-stranded RNA elements were observed in a single isolate came from South-Africa (lineage 3), but in none of the Hungarian or Austrian isolates. Sequence analysis of a putative reductase gene fragment and a translation elongation factor gene fragment of some of the isolates is in progress to clarify the assignment of them to lineages. Further work is also in progress to examine the role and organization of the dsRNA elements, and to compare the pathogenicity of the isolates belonging to different lineages in field tests.

ALTERNATIVE TRANSCRIPTION OF A PUTATIVE LONG-CHAIN ACYL-COA BINDING PROTEIN GENE POSSIBLY REGULATES THE PATHOGENIC STRENGTH OF *FUSARIUM GRAMINEARUM* IN RESPONSE TO CHANGING PATHOGENETIC-ENVIRONMENT

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ABSTRACT

Expression of a host resistance responder gene (designed as *HRR-2*) of *Fusarium graminearum* was found up-regulated during the Fusarium head blight (FHB) pathogenesis in FHB-resistant wheat. Two alternative *HRR-2* transcripts of different size (601 nt and 630 nt, respectively, without the poly A tail) were discovered. The two transcripts are basically identical except that the 630-nt transcript has an additional 29-nt fragment at its 5' end containing a 25-nt CT track. Analysis of the upstream regulatory region of the cloned *HRR-2* gene revealed two alternative promoter sites: TATA/CAAT motifs and a CT motif. It seems that the CT motif would initiate the high-level transcription of the 630-nt transcript in response to the encountered host resistance. The TATA/CAAT motifs should be responsible for the basal-level expression, producing the 601-nt transcript. Sequence analysis suggested that both the 601-nt and the 630-nt transcripts code for a putative long-chain acyl-CoA binding protein of 105 amino acid residues with a calculated molecular mass of 11.5 kD. It is possible that *HRR-2* regulates the pathogenicity of *F. graminearum* by participating in the acyl-CoA-mediated gene expression regulation and/or in trichothecene biosynthesis.

CONVERSION OF AFLP MARKERS ASSOCIATED WITH FHB RESISTANCE IN WHEAT INTO STS MARKERS WITH AN EXTENSION-AFLP METHOD

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ABSTRACT

Amplified fragment length polymorphism (AFLP) has been proven to be a powerful tool for tagging genes or QTLs of interest in plants. However, conversion of AFLP markers into sequence-tagged site (STS) markers is technically challenging in wheat due to the complicated nature of its genome. In this study, we developed an 'extension-AFLP' method to convert AFLP markers associated with Fusarium head blight (FHB) resistance into STS markers. When an AFLP marker of interest was detected with an *Eco*RI+3/*Mse*I+4-selective primer combination, the PCR product was used as a template for an additional selective amplification with four primer pairs in which one additional selective base (either A, C, G, or T) was added to the 3'-end of one of the two primers. The extended primer-pair that produced the targeted band was further extended by adding each of the four selective nucleotide bases for the next round of selective amplification. Extension selective amplification was performed until the target bands became clear enough for subsequent cloning and sequencing. By using the extension-AFLP method, we successfully converted two AFLP markers, which are located in chromosome 3BS and were associated with FHB resistance, into STS markers. Our results indicated that the extension-AFLP method is an efficient approach to converting AFLP markers into STS markers in wheat. The developed STS markers might be used for marker-assistant selection (MAS) for FHB resistance in wheat breeding programs.

HIGH THROUGHPUT GENOTYPING FACILITY FOR MARKER-ASSISTED BREEDING AND MOLECULAR MARKER DEVELOPMENT

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ABSTRACT

The USDA Genotyping Center in Manhattan, Kansas is one of the three newly established USDA regional genotyping centers. Its mission includes developing high throughput molecular markers for agronomically important traits of cereal crops and application of molecular markers in marker-assisted selection for breeding programs. A genotyping protocol has been optimized to improve throughput and automation of marker analysis. By grinding plant tissue in a Mixer Mill from Rebtosh, handling liquid with a robotic system and isolating DNA using NaOH method, more than one thousand samples of DNA can be isolated from fresh tissue in a single day. The quality of DNA isolated with this method is good enough for PCR to be analyzed in a Li-Cor DNA Sequencer. With this method, about 500mg of fresh leaf tissue can provide DNA for more than 50 reactions without damage of original plants. To improve resolution of PCR analysis, PCR products are analyzed in either Li-cor 4200 or ABI 3100 DNA Analyzer. Both systems can detect single nucleotide polymorphism and score data automatically. To reduce cost and time for PCR analysis, multiplex PCR is used to analyze several markers simultaneously. This method has been successfully used in MAS of 3BS major QTL for scab resistance. To construct a new map to identify new QTL, AFLPs and SSRs in coupling with bulk segregant analysis strategy are used. More than 100,000 marker data points can be collected in less than six months. In addition, an Odyssey Image System from Li-Cor is used for quick cloning of AFLP markers to develop breeder-friendly STS markers. With the high throughput protocol, it is feasible for the center to do marker-assisted genotyping for multiple breeding programs. Analysis of FHB resistant breeding materials from USWBSI with SSR markers linked to 3BS QTL is the first service provided by the center.

GENETIC RELATIONSHIP AMONG ASIAN WHEAT GERMPLASM RESISTANT TO FUSARIUM HEAD BLIGHT ASSESSED ON THE BASE OF MOLECULAR MARKERS

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ABSTRACT

The major QTL (quantitative trait locus) on 3BS from 'Sumai 3' and its derivatives has been used as a major Fusarium head blight (FHB) resistant source worldwide, but more resistant genes are needed to avoid complete dependence on single source. In Japan and China, many wheat cultivars and landraces were reported to have a high level of resistance to FHB. But their genetic relationships have not been documented because pedigree information is not available for many landraces. The objectives of this study were to evaluate Type II FHB resistance of 59 wheat landraces and cultivars from Asia and genetic relationships among these accessions based on amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSRs) from chromosome 3BS. The cluster and principal coordinate analysis (PCA) demonstrate that maker data are consistent with the existing geographic distribution and/or available pedigree information of these wheat accessions. Genetic diversity within Chinese resistant landraces is broader than that of accessions from different Asian countries. The haplotypes on 3BS were determined based on five SSR markers (Xgwm389, Xgwm493, Xgwm533, Xbarc133, and Xbarc102) associated with the 3BS QTL. Among the 59 accessions, five of them have at least four of the five Sumai 3 SSR alleles, and hence, are assumed to carry the 3BS major QTL. Twenty-two accessions didn't carry any Sumai 3 alleles on the five SSR loci and additional 25 accessions carried no more than two Sumai 3 alleles, suggesting that these lines may carry different QTL for FHB resistance, and are worth further study.

INTERPRETIVE SUMMARY

One major gene for scab resistance has been identified in the Chinese cultivar Sumai 3 and used in breeding programs worldwide. However, more genes for scab resistance are needed to enhance genetic diversity of the resistance genes. 59 old wheat cultivars with resistance to scab were collected from China and Japan and tested for scab resistance. They were also characterized with molecular markers to evaluate their genetic relationship. Result indicated that most of these accessions have resistance to scab in greenhouse experiment and may not have the major resistance gene from Sumai 3. Molecular data coincided with their geographic distribution or pedigree information.

INTRODUCTION OF PUTATIVE ANTIFUNGAL GENES INTO TWO-ROW AND SIX-ROW BARLEY THROUGH GENETIC ENGINEERING

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ABSTRACT

Fusarium head blight is a major fungal disease for barley and wheat throughout the world; its incidence in the upper midwestern regions of the U.S has reached epidemic proportions. Yield and quality losses have caused economic hardships for producers and users. Introduction of antifungal proteins into barley by genetic engineering offers the potential to suppress pathogen infection and growth. We are using the *Ac/Ds* gene delivery system to produce marker- and plasmid-free transgenic plants containing single insertions that will lead to stable expression of genes encoding putative antifungal proteins to improve Fusarium head blight resistance. Two pathogenesis-related genes from oat, *tlp1* and *tlp4*, and two trichothecene pathway genes from *F. sporotrichioides, TRI101* and *TRI12*, were put into expression cassettes driven by the maize *ubiquitin*- or rice *actin*-promoter-driven genes flanked by ~200 bp of *Ds* inverted repeat DNA that serves as the recognition site for transposition. An expression cassette with *Ac*-driven *Ac* transposase, which is necessary for the movement of *Ds*, was also used. The *Ds*-AFP and *AcTPase* expression cassettes were introduced separately via particle bombardment into scutellar tissues of immature embryos of Golden Promise (GP, a 2-rowed malting variety), and into highly regenerative, green tissues of Drummond (an elite 6-rowed malting variety). Also introduced with the expression cassettes were plasmids encoding either bialaphos (GP) or hygromycin (Drummond) resistance, to enable selection of transformed tissues. Plants derived from 3 DsUbiTlp1 and 3 DsUbiTlp4 transgenic GP lines were *bar*-positive and *tlp* positive. Plants were regenerated from bialaphos-containing medium from four putative DsActTri101 and one DsActTri12 GP lines. Two DsActTlp1, 2 DsActTlp4 and 4 DsActTri101 lines were generated from hygromycin-resistant green tissues of Drummond; seeds have been obtained from 2 DsActTri101 and 1 DsActTlp1 Drummond plants. PCR tests confirmed the presence of the AFP transgene for 1 DsActTlp1, 1 DsActTlp4 and 1 DsActTri101 line.This is the first confirmed report of the introduction of an AFP into a six-rowed barley variety. In addition, Drummond plants have been recovered which contain the *Ac*-driven *Ac*-transposase (*AcTPase*) gene, as confirmed by hygromycin resistance and PCR assays for *AcTPase*. The *AcTPase* gene is also being introduced into the Drummond background via backcrossing from *AcTPase*-positive transgenic GP lines. To assist in characterizing the level of transgene expression, antibodies to the TLP1, TLP4 and TRI101 proteins were developed. Further characterization of the transgene insertions, expression and inheritance of transgenic progeny is ongoing.

MOLECULAR MAPPING OF SCAB RESISTANCE QTL IN WANGSHUIBAI

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ABSTRACT

Wheat scab, or Fusarium head blight, is a destructive disease that can reduce both yield and quality in many regions of the world. Growing scab resistant varieties is an effective, economical, and environmentally sound way to reduce economic losses caused by this fungal disease. Evaluation of scab resistance is costly and laborious. Identification of scab resistance QTL and marker-assisted selection of identified scab resistance QTL will aid in the development of scab resistant cultivars by increasing selection efficiency and reducing the amount of phenotypic evaluation required. However, most reported scab resistance QTL are from a single source: Sumai 3 and its derived lines. To broaden the genetic base of scab resistance, it is important to identify new scab resistance QTL. Wangshuibai is a scab resistant landrace that originated from the Jiangsu province of China. It was selected and planted by farmers many years before Sumai 3 was bred and released in Jiangsu. To identify new scab resistance QTL from sources other than Sumai 3, F_5 derived recombinant inbred lines (RILs) were developed from a cross between Wangshuibai and Wheaton, a susceptible variety. This set of RILs has been evaluated for Type II scab resistance three times (in 2002 and 2003). SSR markers and AFLP markers associated with QTL for scab resistance in Wangshubai were mapped in this population. A major QTL is located on chromosome 3BS in Wangshuibai. It is most likely that Wangshuibai and Sumai 3 have the same major FHB resistance QTL on 3BS. Besides the 3BS major QTL, three minor QTL for Type II FHB resistance were located on chromosomes 7AL, 1BL, and 3BS (near the centromere). These QTL may be novel FHB resistant QTL because none of the resistance QTL in these chromosome regions has been reported from Sumai 3 and its derived lines. One of the objectives of mapping FHB resistance QTL with PCR based molecular markers is to improve the efficiency of selecting FHB resistant varieties. Novel FHB resistance QTL identified from this study may be useful for stacking these QTL with FHB resistance QTL from other sources to develop breeding lines with transgressive scab resistance.

SEGREGATION OF AN SSR ASSOCIATED WITH A QTL FOR FHB RESISTANCE ON CHROMOSOME 7A IN HEXAPLOID WHEAT

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OBJECTIVE

Our objective was to map SSR markers associated with a putative scab resistance QTL on chromosome 7A from Sumai 3.

INTRODUCTION

Wheat scab is a destructive disease that can reduce both yield and quality in many regions of the world (Bai and Shaner, 1994). Growing scab resistant varieties is an effective, economical, and environmentally sound way to reduce economic losses caused by this fungal disease (McMullen et al., 1997). Evaluation of scab resistance is laborious, costly, and time consuming. Identification of scab resistance QTL and marker-assisted selection of identified scab resistance QTL will aid in development of scab resistant cultivars by increasing selection efficiency and reducing the amount of phenotypic evaluation required (Zhou et al. 2002a, 2003). Molecular mapping has been used to tag scab resistance QTL since Bai first tagged scab resistance QTL with RAPD markers in 1995 (Bai, 1995).

A study on marker-assisted selection for the 3BS QTL showed that phenotypic selection among plants carrying the 3BS QTL was still necessary to identify lines with resistance similar to Sumai 3 and Ning7840 (Zhou et al., 2003). Identification of other scab resistance QTL should increase selection efficiency and enhance the implementation of marker-assisted selection.

In a recent study on the chromosome effect of Sumai 3 on Type II resistance and reduced DON accumulation, chromosomes 2B, 3B, 6B, and 7A from Sumai 3 were shown to carry scab resistance (Zhou et al., 2002b). In chromosome substitution lines where Sumai 3 chromosomes were substituted into a Chinese Spring background, chromosome 7A had the largest effect on both Type II FHB resistance and reduced DON levels (Zhou et al., 2002b). In that study, two sets of substitution lines were developed by crossing individual monosomic lines of Chinese Spring (recipient) with scab resistant cultivar Sumai 3 (donor). The monosomics were then used as the recurrent male parent for four backcrosses. Chromosome specific SSR markers were analyzed for polymorphism between Sumai 3 and Chinese Spring. Polymorphic markers were used to verify chromosome substitution of individual Sumai 3 chromosomes in all substitution lines. SSR markers on chromosome 7A verified that the chromosome substitution in the most resistant substitution line was authentic (Zhou, 2002b).

MATERIALS AND METHODS

Plant materials: Chinese Spring was crossed with a Chinese Spring (Sumai 3) 7A substitution line, F_1 plants were selfed, and F_2 seeds were harvested. About 300 F_2 seeds were germinated, and the seedlings were vernalized at 4^{o}C in a cold chamber for two months before transplanting into the greenhouse. The pots

were arranged randomly on benches in the greenhouse. Plant tissue for DNA isolation was harvested from individual plants three weeks after transplanting. At least one head from each plant was inoculated for evaluation of Type II scab resistance using the single floret inoculation method (Bai et al. 1999). Percentage of scabbed spikelets (PSS) on inoculated heads was determined 21 days after inoculation. PSS values recorded from inoculation of more than one head on the same plant were analyzed as sub-samples.

Quick DNA isolation: To isolate DNA, a 2-cm long piece of leaf tissue was inserted into a 1.5 ml centrifuge tube containing 100 µl 0.5 mol NaOH solution. The tissue was ground with a small plastic pestle. Ten µl of the liquid were pipetted into a new centrifuge tube containing 90 µl 0.1 mol Tris-HCL solution. Two µl of the DNA solution were used as the DNA template for polymerase chain reaction (PCR) with a total reaction volume of 20 µl.

PCR reaction and data analysis: PCR reactions were performed as described by Röder et al. (1998) in a Genius Thermal Cycler (Techne Ltd.) starting with 3 min at 94°C, then 40 cycles of 30 sec at 94°C, 30 sec for annealing, and 30 sec at 72°C, with a final extension of 5 min at 72°C. PCR products were separated on 2.5-3.0% agarose gel at 180 V for 1-2 hours. Gels were stained with ethidium bromide, and visualized and photographed under UV light. Linkage of the SSR markers was analyzed by using Mapmaker, version 3.0 for the PC (Lander et al. 1987). SAS V8.0 (SAS Institute Inc, NC 27513, USA. 2000) was used for variance and regression analysis.

RESULTS AND DISCUSSION

1) The frequency distribution of PSS values for the 196 F_2 plants is shown in Figure 1.

2) Preliminary mapping of the scab resistant gene(s) on Chromosome 7A of Sumai 3:
We genotyped 196 F_2 plants from the cross Chinese Spring/ Chinese Spring (Sumai 3, 7A) with *Xbarc49*, which is polymorphic between Sumai 3 and Chinese Spring. This marker separated the196 F_2 plants into three genotypic groups: 48 plants were homozygous for the allele from Sumai 3, 100 plants were heterozygotes, and 48 plants were homozygous for the allele from Chinese Spring. There were significant differences among average PSS values of the three groups as shown in Tables 1, 2 and 3. Based on the preliminary mapping data the F_2 plants derived from a cross between Chinese Spring and Chinese Spring (Sumai 3, 7A) segregated in a typical ratio of 1:2:1. Significant differences among the three genotypes indicated a possible scab resistance gene on chromosome 7A from Sumai 3. We are developing a set of recombinant inbred chromosome lines from the described F_2 populations and mapping more 7A SSR markers for a better molecular characterization of the scab resistance gene.

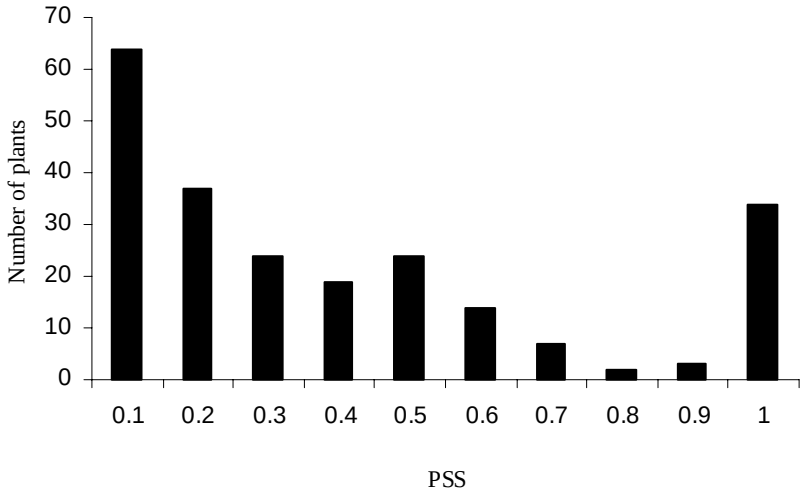

Figure 1. Frequency distribution of PSS values of 196 individual F_2 plants derived from Chinese Spring and Chinese Spring (Sumai 3, 7A).

Table 1. Analysis of variance table for average PSS values of three genotype groups based on marker Xbarc49.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.22374464	0.61187232	6.07	0.0028
Error	193	19.45271631	0.10079128		
Corrected Total	195	20.67646094			

Table 2. Means and standard deviation of PSS values of three genotypes.

barc49 genotype[§]	N	Mean	Std Dev
AA	48	0.3142	0.2808
BB	48	0.5088	0.3819
AB	100	0.3314	0.2997

§ A: allele from Sumai 3; B: allele from Chinese Spring

Table 3. Comparisons of means of PSS values of three genotypes

barc49 Comparison	Difference Between Means	95% Confidence Limits		
BB - AB	0.17745	0.06749	0.28740	*
BB - AA	0.19465	0.06683	0.32247	*
AB - BB	-0.17745	-0.28740	-0.06749	*
AB - AA	0.01720	-0.09275	0.12716	
AA - BB	-0.19465	-0.32247	-0.06683	*
AA - AB	-0.01720	-0.12716	0.09275	

Comparisons significant at the 0.05 level are indicated by *.

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