TRI8 IN *FUSARIUM* ENCODES A TRICHOTHECENE C-3 ESTERASE

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ABSTRACT

The elucidation of the biochemical pathway of trichothecene production by *Fusarium* species has been the focus of our laboratory for a number of years. The complex pathway begins with the sesquiterpene hydrocarbon trichodiene and consists of multiple oxygenation, cyclization, and esterification steps. As is found in a number of other organisms that produce toxic/antibiotic compounds, many of the genes involved in the pathway of trichothecene production in *Fusarium* are located within a gene cluster. Identified genes within this cluster are two genes encoding P450 oxygenases (*TRI11* and *TRI4*), a sesquiterpene cyclase (*TRI5*), two acetyltransferases (*TRI3* and *TRI7*), a pump (*TRI12*), and a gene for transcriptional regulation (*TRI6*). We have now identified the function of *TRI8*, a gene located adjacent to *TRI7*. To determine the function of *TRI8*, we disrupted the gene in both *F. graminearum* and *F. sporotrichioides*, transformed the parental strains, and analyzed transformants. Gene disruption was confirmed by PCR analysis as well as Southern hybridizations. The culture filtrate of *F. graminearum* mutants produced by genetic disruption of *TRI8* accumulated three C-3 acetylated compounds not normally seen in filtrates of the parent strain. We also conducted whole-cell and cell-free feeding experiments using acetylated trichothecenes. The wild-type parental converted the compounds into the deacetylated form whereas the disruptant mutants did not. Heterologous expression of *TRI8* and *TRI12* in yeast resulted in a strain that could remove the C-3 acetyl group from a number of trichothecenes. Based on these lines of evidence, we have identified that *TRI8* encodes an esterase that removes the C-3 acetyl group of *F. sporotrichioides* and *F. graminearum* trichothecenes.

TRANSGENIC WHEAT OVEREXPRESSING PR-PROTEINS SHOWS A DELAY IN FUSARIUM HEAD BLIGHT INFECTION

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OBJECTIVES

To identify and pyramid the best pathogenesis-related (PR-) protein and its combinations for enhancing resistance in wheat to Fusarium head blight.

INTRODUCTION

Genes for pathogenesis-related (PR-) proteins are useful tools in enhancing the resistance of plants to pathogen and pest infestations. They have been successfully employed in improving resistance of plants to several fungal pathogens such as *Rhizoctonia solani* (Lin et al., 1995) and *Fusarium graminearum* (Krishnaveni et al., 2001, Chen et al., 1999). We intend to utilize combinations of these genes for enhancing resistance to wheat scab. The PR-protein group includes biochemically diverse proteins including fungal cell wall hydrolyzing enzymes (e.g. chitinase and ß-1,3-glucanase), inhibitors, peroxidases, oxalate oxidase and membrane permeabilizing proteins (e.g. thaumatin-like protein) and lipid transfer proteins. Genes/cDNAs for several of these proteins have been isolated in our laboratories from fungus-infected rice and wheat plants (Li et al., 2001). We have utilized previously a *tlp* gene to obtain transgenic wheat plants with somewhat improved resistance to scab. Our proposed research is based on the hypothesis that combinations of PR-protein genes will prove to be more effective in controlling scab than single genes. We have generated additional transgenic wheat plants with various combinations of PR-protein genes by biolistic transformation. These combinations include chitinase/glucanase and chitinase*/tlp*. Homozygous transgenic plants and their progeny with high level expression of multiple PR-proteins will be tested for resistance to the scab pathogen using standard evaluation protocols. Once the specific combinations of PR-proteins that result in maximum protection against scab infection are identified, these transgenic plants will be evaluated in a scab nursery in Kansas for scab resistance. Promising lines will be made available to breeding programs at KSU and elsewhere to generate elite wheat varieties resistant to this devastating pathogen.

MATERIALS AND METHODS

Wheat Transformation- Immature embryos (10-12 days after anthesis) of spring wheat cultivar 'Bobwhite' were co-transformed with different PR-gene (transformation cassettes in pHAC20 vector) using the particle inflow gene gun.

Biochemical and molecular analysis of putative transgenic plants- The primary transformants were selected on glufosinate plates (5 mg l^{-1}) and the regenerated T_0 plants were subjected to PCR detection for *bar* gene and gene of interest. RT-PCR using gene

specific primers and western blot analyses with appropriate antisera was used for confirming the expression of the transgenes. The enzyme activity of the transgenes was assayed by incubating or overlaying the native PAGE gel with appropriate substrate. DNA blot and RNA blot analyses were carried out using the standard protocols.

Scab bioassay- GZ3639, a highly virulent isolate of *Fusarium graminearum*, was used at final concentration of 5x 10^5 spore ml^{-1} to inoculate the experimental materials. The experimental materials included a resistant check (MN99112, courtesy Dr. Jim Anderson), Bobwhite non-transgenic control and the transgenic lines (32A2#3 and 26E5#6). Single floret of adult plants with spikes at anthesis were inoculated and placed under high humidity conditions in the greenhouse. The experiments were repeated thrice at different days and scored using the scoring system of Xu and Chen (1993) on a weekly basis.

RESULTS AND DISCUSSION

Twenty-six independent transgenic T_0 plants were characterized and of this 10 Liberty (0.2%) resistant lines were identified for their stable expression, which are being propagated. The current status of these lines is summarized in Fig. 1. They are being tested for stable expression and inheritance of the transgenes. Some lines have been propagated to obtain T_4 generation plants and homozygous lines have been identified (Table 1). Preliminary bioassays with three of these lines showed a delay in the progression of scab infection (Fig. 3). Based on progeny analysis and fluorescent *in situ* hybridization (FISH) we have already identified some homozygous lines with stable expression of chitinase 383 and chitinase 383/glucanase 638 combinations. The homozygous line stably over expressing a chitinase 383/glucanase 638 combination was monitored for the expression of the transgenes periodically at weekly intervals starting from 3 weeks to 5 weeks (Fig. 2a & 2b) and the chitinase and glucanase activity in the spikes was analyzed before and after inoculation (72 hai). High expression of the transgene protein was detected about the heading stage and similarly higher activity of the enzymes was detected in the spikes before and after infection in this line. A moderate resistant reaction was observed in this line when compared with the control plants and the spread of infection was delayed for over 14 days. These lines are currently being grown for seed increase. Homozygous T_3 parents of 2 other lines with single (383) and combinations (289:383) of PR-protein genes were identified by progeny analyses based on Liberty painting assay.

Southern blot analysis of the 10 Liberty-resistant transgenic lines revealed that the number of copies of the transgene varied from 3 to 15 copies and they varied for each event. Fluorescent *in situ* hybridization (FISH) coupled with progeny testing (Liberty painting, and transgene PCR) was used to identify homozygous plants.

Crosses of the homozygous *tlp* transgenic wheat plants with a moderate improvement in resistance to scab and the homozygous wheat lines expressing the 383 chitinase and 383 chitinase/638glucanase gene combination have been completed. The homozygous F_2 LInes will be obtained and then evaluated for their resistance against *Fusarium graminearum*.

The moderately scab-resistant *tlp*-transgenic lines (Bobwhite background; Chen et al, 1999) has been crossed with Heyne. A backcross of the F_1 lines to Heyne, selection of Liberty-

resistant plants and production of BC_2 seeds was accomplished. An additional backcross, followed by selfing will be used for selecting for herbicide-resistant and herbicide-susceptible BC_3F_3 lines. Five lines homozygous for the transgene, and five non-transgenic lines in isogenic background will be isolated and evaluated for scab resistance. Crosses of the homozygous *tlp* transgenic wheat plants with a moderate improvement in resistance to scab and the homozygous wheat lines expressing the 383 chitinase and 383 chitinase/ 638glucanase gene combination have been completed. The homozygous F_2 lines will be obtained and then evaluated for their resistance against *Fusarium graminearum*.

It would thus be important to identify the specific gene combinations that are most effective against the scab pathogen. Our approach would be to pyramid the PR-protein genes and evaluate the synergistic effect (if any) of the different combinations towards scab.

ACKNOWLEDGEMENT

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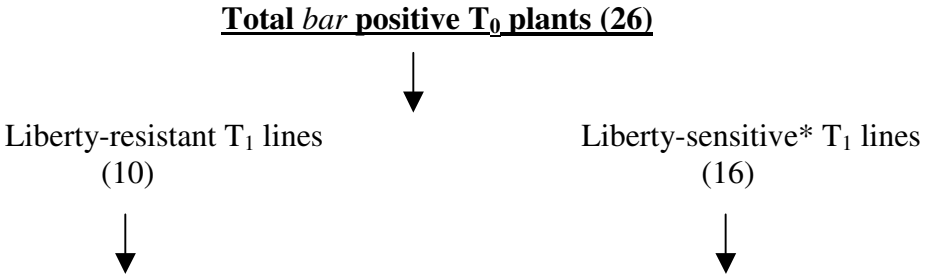


bar PCR		Transgene PCR		RT-PCR		Western blot			*bar* PCR		Transgene PCR		RT-PCR	
+	-	+	-	+	-	+	-		+	-	+	-	+	-
10	0	9	1	8	2	8	2		16	0	10	6	1	15

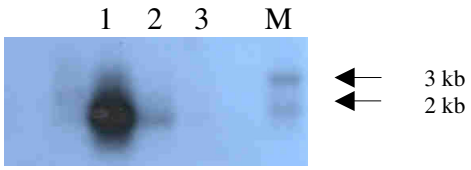
* Liberty-sensitivity is probably due to gene silencing. These lines are PCR positive for *bar*

Figure 1. Schematic diagram showing the total number of transgenic plants with PR-protein genes under current analysis

Table1. Status of the transgenic lines stably over-expressing different PR- protein transgenes

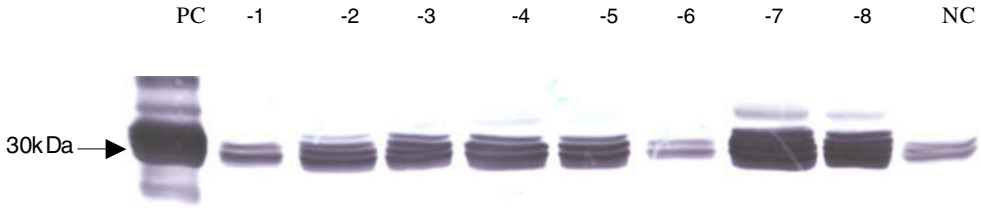
Gene (s)	No. of lines	Type of PR-protein	Status
383	2	Wheat chitinase	Homozygous line identified in both line (T_4 & T_3)
638	2	Wheat glucanase	Heterozygous (T_3)
383:638	2	Wheat chitinase/Wheat glucanase	Homozygous line identified in both line (T_4)
289:383	2	Wheat glucanase/Wheat chitinase	Homozygous line identified (T_3)
tlp	1	Rice TLP	T_2
tlp/chi11	1	Rice TLP/rice chitinase	T_2

a) Northern blot analyses



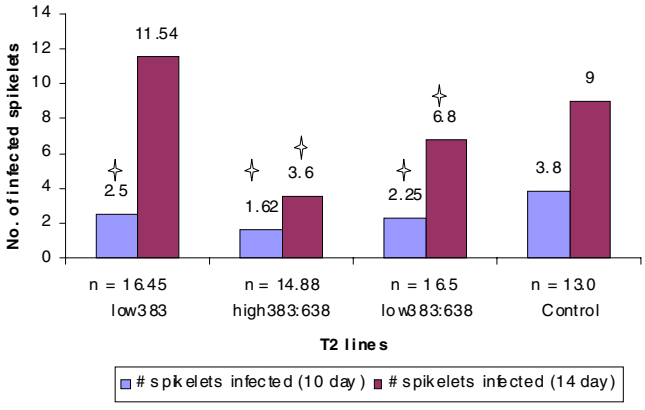
1: high expressing line; 2: low expressing line; 3: non-transgenic control and M) RNA marker. 12 µg of total RNA isolated from leaves was loaded in each lane. The expected 1.3 kb β-glucanase transcript was detected in the transgenic lines within 6 h of exposure to X-ray film.

b) Western blot analyses for glucanase expression in the homozygous line 32A2#3



PC: scab infected leaf, 32A2#3: homozygous line for 638 glucanase, NC: non-transformed Bobwhite control

Figure 2. β-glucanase expression in different transgenic lines



p=0.01; n: average number of spikelets (of 3 replicates).

Conclusion: Transgenic line expressing a combination of a wheat chitinase and a wheat glucanase at a high level showed a delay in the spread of the infection when compared to the non-transgenic line and transgenic lines with either the chitinase alone or a line with the same transgenes expressed at a lower level.

Figure 3. Preliminary scab bioassay data from 3 different transgenic lines

GENETIC DIVERSITY IN SCAB-RESISTANT WHEAT CULTIVARS BASED ON MOLECULAR MARKERS

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ABSTRACT

Wheat scab can dramatically reduce grain yield and quality. Breeding for resistance is an effective measure for disease control. Wheat cultivars with various levels of Type II resistance have been reported worldwide, however, the genetic relationships among the cultivars are not well characterized. Sixty-five wheat cultivars from eight countries varying in resistance levels were evaluated for Type II resistance and for genetic diversity based on 322 AFLP and 19 SSR marker alleles. Cluster analysis of AFLP and SSR markers linked to the major QTL on chromosome 3BS of Ning 7840 indicated that two Japanese landraces and most of the cultivars related to Ning 7840 carried the same major QTL. However, the QTL are different from those in Wangshuibai and other Chinese landraces as well as those that originated from countries other than China and Japan. Fingerprinting suggested that Taiwanxiaomai is the donor of the major QTL in Ning 7840 or Sumai 3. Combining major resistance QTL from Ning 7840 and Wangshuibai or other sources not related to Ning7840 may facilitate pyramiding different QTL. Cluster analysis based on AFLPs and SSRs provided the best estimate of genetic relationships among accessions studied. The result indicates that US cultivars are more closely related to cultivars from Europe and Argentina than cultivars from Asia; therefore, integrating scab resistance from Chinese sources may increase the genetic diversity of US wheat cultivars and combining Chinese and non-Chinese sources of scab resistance offers a way to enhance the level of scab resistance. (This poster was presented at the Annual Meeting of 2001 CSSA)

THE EFFECTS OF HOMOEOLOGOUS GROUP 3 CHROMOSOMES ON RESISTANCE TO FUSARIUM HEAD BLIGHT IN TETRAPLOID WHEAT

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most destructive diseases of wheat in areas where the weather is warm and humid after the heading. Previous studies indicate that level of resistance to FHB varies not only among wheat cultivars but also among some of their wild relatives. No accession, however, has yet been identified to be completely immune to FHB among the Gramineae. It is know that durum wheats (*Triticum turgidum* L. conv. durum) are consistently more susceptible to FHB than common wheat (*T. aestivum* L.). The importance of D genome in conferring resistance to FHB has been emphasized. Meanwhile, recent studies using molecular markers report effective QTLs on chromosome 3BS in hexaploid population and on 3A in tetraploid recombinant inbred chromosome lines. In this study, we performed to evaluate the effects of homoeologous group 3 chromosomes of *T. turgidum* ssp. *dicoccoides* on resistance to FHB using a set of chromosome substitution lines of a durum wheat cultivar 'Langdon'. The accession of *T. turgidum* ssp. *dicoccoides* examined in this study was higher susceptible for Type II resistance (resistance to spread of FHB in the head) than Langdon. Both of the chromosome substitution lines of 3A and 3B showed same level of resistance with Langdon, but bleaching of the heads was completely prevented in the substitution lines of chromosome 3A without relationship to rachis fragility. It is concluded that the chromosome 3A of *T. turgidum* ssp. *dicoccoides* carries resistance gene(s) to head bleaching caused by FHB. (This poster was presented at the 4th International Triticeae Symposium, Córdoba, Spain, September 10-12, 2001. The abstract will be in Hereditas 2002.)

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED SEQUENCE TAGS FOR SCAB RESISTANCE IN WHEAT USING BSA AND SSH

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ABSTRACT

Wheat scab is a destructive disease of wheat (*Triticum aestivum*). Genome-wide analysis of gene expression in response to infection by *Fusarium graminearum* may lead to discovery of novel genes for scab resistance and provide insight into further understanding of genetic mechanisms of wheat resistance to scab. To enrich differentially expressed sequence tags (ESTs) for scab resistance, cDNA subtraction libraries were generated from *Fusarium*-infected spikes of two bulked recombinant inbred lines (RILs) using the suppression subtractive hybridization (SSH) method. Two bulked RILs differing in scab resistance were formed by pooling infected spikes from five $F_{8:12}$ scab-resistant and five $F_{8:12}$ susceptible RILs, respectively, based on their Type II resistance from four greenhouse tests. The RILs derived from the cross between the resistant cultivar Ning 7840 and the susceptible cultivar Clark. The selected RILs were grown in the growth chamber and inoculated with a conidiospore suspension of *Fusarium graminearum* by single floret inoculation at early flowering stage. The inoculated spikes were enclosed in a moist plastic bag to maintain high humidity. The infected spikes were harvested for mRNA extraction at 36 and 72 hours after inoculation (HAI). To eliminate mRNA contamination from the fungus, the inoculated floret from each spike was removed before the spikes were harvested. About 550 clones were isolated from the two libraries constructed from infected spikes at 36 and 72 HAI. Eighty-five clones were randomly picked from the libraries and sequenced. Most of them (95%) were singletons. Sequence homology search using the NCBI BLAST program showed that some of them were stress and other defense-related genes or genes for PR-proteins and signal regulation. Construction of an additional SSH library at 6 HAI is underway. The clones from the libraries will be used for temporal gene expression analysis with macroarrays.

HEREDITY AND MOLECULAR MARKERS FOR WHEAT SCAB RESISTANCE

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RESEARCH OBJECTIVES

1) To determine the inheritance of scab resistance in identified resistance sources W14, Shaan 85 and Ernie; 2) To elucidate the genetic relationship between type II, III and IV resistance based on segregation in four F_2 populations for scab severity, DON content and scabby seeds; 3) To identify SSR molecular markers associated with type II, III and IV resistance in source W14 using an F_2 population Pioneer 2684 x W14.

INTRODUCTION

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* [*Gibberelle Zeae* (Schwabe) Petch], is a devastating disease of all classes of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) worldwide. Deployment of resistant varieties is an effective, economical and environmentally safe way to control FHB in wheat. However, success of this endeavor is greatly dependent on first obtaining knowledge of the amount of genetic diversity for resistance, identity of different mechanisms governing resistance, inheritance of resistance, and most importantly identifying selectable markers for incorporating and pyramiding resistance genes into wheat cultivars. DNA markers for FHB resistance QTLs have been identified in the primary resistance source Sumai 3 and its progeny Ning 7840 (Anderson, 2001; Bai et al., 1999). One major QTL was mapped on wheat chromosome 3BS, and explained 25 to 42% (Anderson et al., 2001) and 60% (Bai et al., 1999) of the phenotypic variation of disease severity in three recombinant inbred line populations. This QTL is associated with resistance to disease spread, but associations with resistance to DON production and seed colonization has not been determined. Identification of QTLs associated with resistance to disease spread (Type II), seed colonization (Type III) and DON production (Type IV) is imperative for the implementation of gene pyramiding to develop germplasm and cultivars possessing resistance levels approaching immunity and conferring near-zero losses.

MATERIALS AND METHODS

Plant Materials - Three resistance sources, W14, Shaan 85 and Ernie, identified in previous studies (Griffey et al., 1998), were crossed with susceptible soft red winter (SRW) wheat cultivar Madison and/or Pioneer 2684. W14 is an improved type II resistance source developed by recurrent selection (Jiang, 1997), which may include genes from Sumai 3 and other resistance sources. Shaan 85 is an improved type II resistance source derived from Sumai 3. Ernie is a scab resistant SRW wheat cultivar that lacks any of the known scab resistant sources in its ancestry. Four F_2 (Pioneer 2684 x W14, Madison x W14, Pioneer 2684 x Shaan 85, and Ernie x Pioneer 2684) and two $F_{2:3}$ (Pioneer 2684 x W14 and Madison x

W14) populations were used in genetic studies. F_2 population Pioneer2684 x W14 (156 DNA samples) was used in a mapping study. In order to elucidate the genetic diversity among currently deployed resistance sources, Funo, one of the parents of Sumai 3, Sumai 3, W14, Shaan85, VR95B717 (source from France), Ernie, and two susceptible parents Madison and Pioneer 2684 were used in the genotyping study.

Disease screening - One to three heads per individual in F_2 populations, 10 to 30 individuals per family in $F_{2:3}$ populations, and 28 to 51 individuals per parent were inoculated via floret inoculation procedures. A droplet (30ul) of macro conidia (5×10^4 spores/ml) was placed into a floret in the middle of spike at early anthesis using a SAMCO transfer pipette. Ratings of severity (percentage of infected florets) were assessed three times at 7, 14 and 21 days after inoculation. Severity rated on 21st day was used for analysis. Percentage of scabby seeds for each individual was determined based on the mean number of colonized seed per hand-threshed single spike. DON content was analyzed as ppm by a SIM Shimadzu QP5000 GC/MS system at the University of Minnesota. Severity, scabby seeds and DON content were evaluated in F_2 populations and only severity was evaluated in $F_{2:3}$ lines.

Microsatellite analysis – A total of three hundred SSRs were synthesized and included 172 published by Roder et al., (1998); 40 by Bryan et al., (1997); and 88 kindly provided by Dr. Cregan of USDA/ARS (2001). Based on previous genetic and molecular marker work, the following linkage groups are expected to contain QTLs for FHB reaction: 1B, 2B, 3B, 3A, 5A, 6A, 6B, 6D, and 7B. Therefore, SSRs known to be located on these chromosomes were selected and used to survey DNA polymorphism among four parents (Ernie, Pioneer 2684, Madison, and W14) and the four bulked DNA samples (R and S bulks from the cross of Pion2684 x W14; R and S bulks from the cross of Madison x W14). Bulked DNA samples were obtained by mixing an equal amount of DNA from six putative homozygous resistant and six homozygous susceptible F_2 individuals, respectively. DNA extraction, PCR amplification and SSR assays were conducted as described by Saghai Maroof et al. 1984,1994; Bryan et al. 1997; Roder et al. 1998.

Statistical analysis - Agrobase Software was used for statistical analyses. One-way ANOVA was conducted to confirm significant ($P < 0.05$) association between putative resistance-related markers and resistance to scab.

RESULTS AND DISCUSSION

Identification and characterization of scab resistance in four F_2 populations - Significant differences in type and level of resistance were found between parents and among individuals in F_2 populations (data not shown). W14 and Shaan 85 are highly resistant to disease spread, seed colonization and DON production. Ernie is moderately resistant to disease spread and highly resistant to seed colonization and DON production. Pioneer 2684 and Madison are highly susceptible to disease spread, seed colonization and DON production. Highly-resistant individuals with type II resistance were found to also possess type III and type IV resistance, having consistently lower severity, scabby seeds, and less than 10 ppm toxin accumulation. Highly susceptible individuals were found to have variable or consistently high ratings for scabby seeds and DON content. About 25 % of individuals

with type III and IV resistance were moderately resistant to moderately susceptible to disease spread. Individuals with type IV resistance were found to have type III resistance in most cases. Significant positive correlations were found between disease severity (type II resistance), toxin content (type III resistance), and scabby seeds (type IV resistance) based on analysis of segregation data from four F_2 populations. Correlation between scabby seeds and DON content ($r = 0.8646$) was much higher than those between severity and DON content ($r = 0.5388$), and severity and scabby seeds ($r = 0.5911$), which suggests that DON content could be predicted by percentage of scabby seeds in most cases. Therefore, assessing severity before harvest and assessing scabby seeds after harvest may be an effective and economical way to select for resistance as a whole.

Inheritance of resistance to disease spread, seed colonization and DON accumulation in four F_2 populations - Discrete classes were not observed within the segregating populations of any crosses or for any parameter evaluated. Different frequency distributions were observed among the four populations for severity, scabby seeds and DON production. A normal distribution with transgressive segregation for both susceptibility and resistance was observed in cross Pioneer 2684 x Ernie for all parameters. This indicates that resistance in Ernie is controlled by quantitative gene. Four genes were estimated for resistance in Ernie based on a quantitative approach (Wright, 1968).

Disease severity in the F_1 was greater than that of the resistant parent but less than that of the mid-parent in three crosses (Pioneer 2684 x W14, Madison x W14, Pioneer 2684 x Shaan 85), and indicates that resistance is controlled by partially dominant gene effects. A right skewed distribution with two peaks coinciding to the parents was observed in crosses with W14 and Shaan 85 as the resistant source, and suggests that resistance of W14 and Shaan 85 is controlled by major genes. In W14 and Shaan 85, two major genes with complementary effects were indicated by $?^2$ analysis, and two to three genes were estimated by quantitative approach (Wright, 1968). Transgressive segregants were also observed in these three crosses with increased susceptibility based on severity and both increased susceptibility and resistance based on scabby seeds and DON content, which suggests the presence of the minor gene.

***Identification and characterization of marker-QTLs for scab resistance in common* wheat -** DNA polymorphism among four parents (Ernie, Pioneer 2684, Madison, and W14) was significant, and was observed for seventy-six percent of primers (152 out of 200); however, DNA polymorphism among parents was not always observed among bulks (R and S bulks from the cross of Pion2684 x W14; R and S bulks from the cross of Madison x W14). Therefore, all polymorphic markers were used to map scab QTLs in the population Pion2684 x W14. A total of 45 loci were mapped to five chromosomal regions in this population and a major QTL, in addition to the 3BS QTL, was identified, potentially located on 2BS. Fifteen markers identified in three QTL regions were significantly ($p < 0.05$) associated with scab resistance, and explained 23, 28, 21, and 36 % of total variation in percentage of scabby seeds, DON content, and severity in 82 F_2 individuals and severity in 82 corresponding $F_{2:3}$ families, respectively.

Eight DNA markers from the five chromosome regions were used to genotype six diverse resistance sources. Differences and similarities in these markers among the six lines indicate that some lines may possess different resistance genes that could be useful in pyramiding resistance from these sources and, thereby improve the level of scab resistance. W14 may possess different gene or allele than Sumai 3 on 2BS QTL region, and Ernie may possess resistance genes different from other type II resistance sources in both 2BS and 3BS QTLs regions. DNA polymorphism was found among type II resistance sources W14, Shaan 85, Sumai 3, Funo, and Ernie for marker loci that are associated with postulated resistance genes. This indicates that genetic diversity exists; however, gene interactions may adversely effect pyramiding of different resistance genes.

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SCREENING OF WHEAT GERMPLASM FOR POLYMORPHISM OF SSR MARKERS LOCATED ON CHROMOSOME 3B

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ABSTRACT

The development of molecular genome analysis tools has created an interest in the use of marker-assisted selection (MAS) in applied plant breeding programs. MAS may become an important aid to plant breeders in selection of superior genotypes. Instead of using phenotypic selection for a trait, once a tight linkage has been established between the trait and the marker, the marker can be used for selection. MAS may become especially useful for traits that are difficult to assay by phenotype such as scab or head blight resistance. Bai et al. (1999) identified molecular markers linked to a major QTL controlling resistance to scab present in the Chinese cultivar Ning 7840. This QTL, located on chromosome 3B, explained up to 60% of the variation in scab resistance. Knowledge of the variation for markers linked with disease resistance within wheat germplasm, however, is essential before MAS can be applied widely. In this research, we examined the variation of three SSR (Simple Sequence Repeat) markers located on chromosome 3B (Roder et al. 1998) within a sample of wheat germplasm to determine their future usefulness for selection.

A STS MARKER FOR SCAB RESISTANCE QTL IN WHEAT DERIVED FROM PST I-AFLP

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ABSTRACT

Large-scale field screening for scab resistance in wheat is difficult because environments significantly affect expression of the resistance genes. Marker-assisted selection (MAS) may provide a powerful alternative. Amplified fragment length polymorphism (AFLP) is an efficient marker system for mapping and tagging of quantitative trait loci (QTL). However, the complexity of AFLP procedure makes it difficult to be used as routine in breeding programs. Conversion of AFLP to sequence-tagged site (STS) may produce breeder-friendly markers for MAS. One major QTL on chromosome 3BS was mapped by using AFLPs and the population from the cross of Ning 7840/Clark. Fine mapping of the QTL identified two dominant markers individually explained up to 50% of phenotypic variation in the same population. One 35bps DNA fragment linked to the QTL in coupling phase and another 222bps fragment linked to the QTL in repulsing phase. The larger DNA fragment was cloned and sequenced. Five different DNA fragments were recovered and one with five identical copies was selected to design STS primers. A co-dominant marker was amplified and explained about 50% of phenotypic variation for scab resistance in F7 population. The STS was also validated in several other cultivars having the major QTL on 3BS. This is the first codominant STS marker for the major scab resistance QTL converted from an AFLP marker. Application of this marker in breeding programs may speed up breeding process to enhance wheat scab resistance in wheat.

RAPID DNA EXTRACTION FROM WHEAT TISSUE FOR HIGH THROUGHPUT PCR MARKER-BASED ANALYSIS

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ABSTRACT

High throughput marker assisted selection in plant breeding programs is limited by the ability to extract DNA from large populations of plants. The primary objective of this project was to develop a high throughput DNA extraction procedure without the need for greenhouse space or growing wheat (*Triticum aestivum* L.) plants to maturity. A sodium hydroxide rapid DNA extraction was modified for 96-well format to reduce costs. Also, compared were stored versus fresh tissue. Extracts were done on 4-day old seedling tissue from seeds germinated in 8-well tissue culture plates. Approximately 2 µg of genomic DNA can be isolated per 0.02 grams of tissue at a cost of $0.20/sample. To test the robustnest of the extraction procedure, two well characterized PCR based microsatellite markers were analyzed for size and repeatability. Data for the microsatellite markers was the same for fresh and stored tissue extracts as well as recently extracted and stored DNA. This technique will allow one person, in a single workday, to extract nearly one thousand storage stable DNA samples that are immediately ready for PCR analysis.

PROGRESS TOWARDS SATURATION MAPPING AND BAC CONTIG DEVELOPMENT FOR *QFHS.NDSU-3AS*, A MAJOR FHB QTL IN DURUM WHEAT

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ABSTRACT

The research conducted by our group using Langdon-*dicoccoides* chromosome 3A recombinant inbred lines (LDN-Dic 3A RICLs), has allowed the targeting of molecular markers to the region of a single chromosome (Otto et al. 2001). A major 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 were identified (Otto et al. 2001). In this report we present our initial results towards development of a saturated linkage map for the region surrounding this QTL, and identification of bacterial artificial chromosome (BAC) clones from this region using a *Triticum monococcum* BAC library.

Three methods are planned for generating molecular markers in the region surrounding this QTL viz., EST (expressed sequence tag)-derived primers, synteny-derived primers and RNA fingerprinting-differential display. The first method involves design and amplification of target DNA using primers generated from publicly available wheat ESTs generated from the National Science Foundation-funded wheat EST project. As of date, 391 loci have been assigned to homeologous group 3. Our laboratory is part of this multi-institution consortium and we have been mapping wheat ESTs onto the cytogenetic deletion stocks. This valuable resource of mapped ESTs can be easily used to derive polymerase chain reaction (PCR)-based markers by designing primers from these ESTs and amplifying DNA of interest. A locus-specific marker derived from *Xgwm2* was used as a DNA-based probe on a set of 'Chinese Spring' cytogenetic deletion stocks and was assigned to the chromosomal bin location 3AS4-0.45-1.00. Primer pairs were designed from ESTs that map to this bin location, and used for polymerase chain reaction (PCR) amplification of the LDN-Dic 3A RICLs . Amplified products from four of these primer pairs have been analyzed by polyacylamide gel electrophoresis, and we report initial results for these experiments. We have also screened a *T. monococcum* BAC library with a DNA-based probe derived from the microsatellite locus *Xgwm2*, and have identified 13 BAC clones. We also report initial results from the characterization and progress towards subcloning these BAC clones.

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UNDERSTANDING FUSARIUM HEAD BLIGHT RESISTANCE IN TETRAPLOID AND HEXAPLOID WHEAT

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ABSTRACT

Fusarium head blight, more commonly known as scab, is a fungal disease of small-grain crops that causes yield loss and poor grain quality. The extensive damage caused by Fusarium head blight (FHB) has made it necessary to develop resistant lines of durum (AABB; 2n=4x=28) and hexaploid (AABBDD; 2n=6x=42) wheat. One species that shows promise as a source for FHB resistance in tetraploid cultivars is an accession of *Triticum turgidum* L. var. *dicoccoides* (AABB, 2n=4x=28). The Langdon-dicoccoides chromosome 3A disomic substitution line was shown to have Type II resistance to FHB. A Recombinant Inbred Chromosome Line (RICL) population of 83 individuals derived from LDN(Dic-3A) has been analyzed over multiple seasons. Phenotypic screening data, molecular marker mapping data (RFLP, AFLP, RGA, and microsatellite), and QTL analysis results have delineated the location of FHB resistance loci on the 3A chromosome. At the present time, 14 markers have been placed on the molecular map of chromosome 3A covering 133.9 cM. Quantitative trait analysis suggests 37% of the phenotypic variance, or 55% of the genetic variation for FHB resistance is explained by the locus *Xgwm2*. Effectiveness of this marker in selection of durum cultivars with improved FHB resistance is being tested.

A study was conducted in North Dakota adapted hexaploid cultivars deriving their resistance from Sumai 3 to identify markers linked to quantitative trait loci (QTL) for FHB resistance. The advantages of using ND wheat lines are three-fold. First, fewer individuals need to be genotyped for marker loci as compared with mapping populations. For in this type of analysis a priori selection has been placed on the trait of interest as opposed to mapping populations where segregation is needed. Second, the power of detecting QTL regions associated with FHB resistance is much greater than that with mapping populations. Third, the materials used in this project have been extensively screened (both field and greenhouse) over a 5-year period for their reaction to FHB, precluding the need for additional screening for resistance. Analysis of these lines for presence of markers coming from Sumai 3 indicates two significant regions (a region on chromosomes3B and 7B each). Probability of linkage between markers and introgressed resistance gene was calculated using a binomial probability formula. Probability of the 3B region being present by chance is less than 3×10^{-15} and that on 7B is 5×10^{-7}. Markers are being utilized to select durum and hexaploid wheat lines carrying these FHB resistance loci. Populations carrying sources of FHB resistance different from those describe above are being investigated for their value in identifying different loci and pyramiding useful genes in a single genetic background.

MAPPING GENES CONFERRING FUSARIUM HEAD BLIGHT RESISTANCE IN A MIDWEST BARLEY ACCESSION HIETPAS 5

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INTRODUCTION

Fusarium head blight (FHB), incited primarily by *Fusarium graminearum*, adversely affected the quality of barley grown in eastern North Dakota and northwestern Minnesota the last nine years. Quality of harvested grain was reduced because of blighted kernels and the presence of deoxynivalenol (DON), a mycotoxin produced by the pathogen. A line currently used by Midwestern barley breeding programs as sources of genes for FHB resistance is C93-3230-24. This six-rowed line from the cross B2912/Hietpas 5 was identified by researches at Busch Agricultural Resources, Inc. (BARI) to have FHB resistance similar to Chevron, and better FHB resistance than either of its parent in a greenhouse test. Field tests conducted the last three summers in mist-irrigated FHB nurseries in North Dakota confirmed that C93-3230-24 has FHB resistance approaching Chevron. The genetic background of C93-3230-24 appears to be completely different than that of any of the FBH resistant accessions identified by Prom et al. (1996). Thus, this line may have alleles for FHB resistance and DON accumulation not currently identified.

METHODS

An F_1-derived doubled-haploid (DH) population consisting of 300 lines was created from the cross 'Foster'/C93-3230-24. Foster is susceptible to FHB. One hundred eighteen lines were randomly chosen from the population and are being used for construction of a molecular marker map. Phenotypic data for FHB resistance, DON accumulation, heading and maturity dates, plant height, and spike angle are being collected so their respective QTL can be placed on the linkage map. Heading and maturity dates, plant height, and spike angle have been identified as traits associated with FHB resistance and DON accumulation in previous studies.

RESULTS

Field experiments were conducted in three mist-irrigated FHB nurseries in 2000 using the 118 DH lines and parents. Two nurseries were located in North Dakota and the third was located at Zhejiang University in Hangzhou, China. Construction of a linkage map consisting of RFLP and SSR markers is in progress. Single locus analysis using available marker data identified three chromosomal regions associated with FHB resistance (Table 1). The regions are located in chromosomes 2H, 5H, and 7H. Results in this study are similar to those obtained in studies using the resistant six-rowed cultivar 'Chevron' (de la Pena et al., 1999; Ma et al., 2000). Thus, preliminary results suggest that the Midwest accession C93-3230 and the Swiss cultivar Chevron may have similar alleles for FHB resistance.

Research is continuing to complete the molecular marker linkage map. The mapping population and parents will be grown at Zhejiang University during winter 2001-2002 and at three North Dakota locations in 2002. Once the linkage map is completed, QTL analysis will be performed to obtain better estimates of the chromosomal location of QTL conferring FHB resistance and reduced DON accumulation. QTL for heading and maturity dates, plant height, and spike angle also will be mapped.

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Table 1. Coefficient of determination (r^2) values for markers significantly associated with Fusarium head blight (FHB) and other traits at three environments in 2001.

		Environment						
		Langdon, ND		Osnabrock, ND				Hangzhou, China
SSR marker	Chr.†	FHB severity	Plant height	FHB severity	Plant height	Physiological maturity	Spike angle	FHB severity
Hvm07		0.03		0.05				
Hvlue								
Bmag0345	1H		0.04		0.04			
Bmac0090	1H		0.04					
EBmac501	1H		0.06					
Bmac0134	2H							
Bmag0140	2H	0.14	0.18	0.09	0.09	0.21		0.09
Ebmac521	2H	0.16	0.23	0.16	0.08			0.17
Bmag0378	2H	0.13	0.14	0.09	0.07	0.26		0.07
Ebmac557	2H	0.17	0.22	0.13	0.08	0.28		0.15
HVBKASI	2H	0.17	0.22	0.14	0.08	0.27		0.15
Bmac0126	2H	0.17	0.22	0.13	0.08	0.28		0.15
Bmac0310	4H	0.06						
Bmac0181	4H							
Hvm67	4H				0.05			
Hvm06	5H							
Bmac0113	5H			0.05				
Bmag0337	5H							
Bmac0163	5H			0.05				
HVM65	6H							0.03
Hvm22	6H				0.09			
Bmag0009	6H							
HVM04	7H	0.04		0.04	0.12	0.06		
Bmag0341	7H							

TRANSFORMATION OF A COMMERCIAL BARLEY CULTIVAR WITH GENES FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight, incited primarily by *Fusarium graminearum*, has caused devastating losses to barley since the 1990's. Production of the mycotoxin deoxynivalenol (DON) by *F. graminearum* is harmful to humans and livestock. Expressing certain anti-toxin genes such as *TRI101* and *PDR*5 could improve resistance to fungal infection and reduce DON levels. *TRI101* encodes a 3-OH trichothecene acetyltransferase that converts DON to a less toxic acetylated form. *PDR5*, an ATP-binding cassette, acts as an efflux transporter, shunting DON across the plasma membrane from the interior of the cell. We have transformed the commercial malting barley cultivar Conlon with these genes to reduce DON levels in infected grain. Ten-day old calli derived from immature embryos were co-bombarded with the herbicide-resistance gene bar as the selectable marker. Putative transgenic plants were confirmed by Southern analysis. A total of seven independent events with *TRI101* and six with *PDR5* were recovered. Northern analysis indicated the expression of *PDR5*. Expression of *TRI101* was confirmed by detecting acetyltransferase activity in seeds of the transgenic plants. T_2 lines of three events with *TRI101* and two events with *PDR5* were field tested for disease and toxin level. Both genes appeared to reduce FHB infection and *PDR5* also may reduce DON accumulation.

USING MOLECULAR GENETICS TO ENHANCE SCAB RESISTANCE IN WHEAT AND BARLEY

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* is a major disease problem on the wheat and barley crops in the United States and around the world. Our goals are to understand the biology of this plant-pathogen interaction in order to develop biotechnology strategies for FHB resistance. Histological studies showed that *F. graminaerum* can infect wheat through multiple pathways. Examination of gene expression in wheat spikes within 48 hours after infection by *F. graminearum* showed that the classical defense response genes are upregulated at the site of infection and in a systemic fashion. Application of benzothiadiazole (BTH) to wheat spikes resulted in upregulation of the BTH-induced genes but did not result in Type I or Type II FHB resistance. Expressed sequence tags (ESTs) have been generated from cDNA libraries prepared from wheat and barley spikes infected with *F. graminearum*. Bioinformatic comparisons of these ESTs revealed four sets of genes: (1) classical biotic and abiotic stress response genes; (2) fungal genes associated with pathogenicity; (3) library specific genes; and (4) genes in common with other plant-pathogen interactions. To further characterize this plant-pathogen interaction, additional bioinformatic comparisons are ongoing and large-scale expression studies are underway.

Biotechnology approaches to developing resistance are also in progress. Our approach is to overexpress a set of antifungal protein genes in wheat and barley. We have used particle acceleration to generate transgenic wheat and barley with a set of antifungal protein genes. To date, we have generated 25, 25 and 31 wheat plants overexpressing a wheat α-1-purothionin, a barley thaumatin-like protein-1 (tlp-1), and a barley ß-1,3-glucanase, respectively. We have also developed wheat and barley plants carrying an overexpressed barley ribosome inactivating protein gene. Two FHB disease screens have been conducted on the transgenic wheat plants carrying the ß-1,3-glucanase, tlp-1 and α-1-thionin transgenes. Our results from the FHB disease screens indicate that there are lines carrying each transgene that reduce FHB severity. Transgenic plants carrying other AFP genes or AFP genes in combination will be tested in the future.

EXPRESSION OF TWO DIFFERENT CANDIDATE ANTI-FUSARIUM PROTEIN GENES AFFORDS PARTIAL PROTECTION AGAINST THE SPREAD OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

Host plant resistance is an efficient, cost-effective and environmentally friendly way to fight plant disease and would be particularly helpful in decreasing grower losses due to Fusarium head blight. More sources of resistance, preferably tagged with molecular markers, are needed. We are using genetic transformation in a biotechnology approach to introduce novel candidate anti-*Fusarium* genes into hexaploid wheat. The genes encode proteins targeted against either the fungus itself or the mycotoxins it produces during infection. Six different candidate anti-*Fusarium* coding regions were fused to the maize *Ubi1* promoter, first intron and exon for widespread expression. Several transgenic plants have been obtained for each construct. The transgenics show a range of expression levels in semi-quantitative RT-PCR assays. On average, expression from a construct with a wheat coding region is higher than from those with fungal coding regions. Homozygous plants have been identified for many of the transgenic events. Ten of these have been tested in greenhouse trials for Type II resistance. Two transgenic lines exhibited small increases in resistance, compared to their non-transformed parent, in three independent tests. One of these lines accumulates DON acetyltransferase enzyme activity encoded by a transgene containing the *Fusarium sporotrichioides TRI101* gene. The other line contains a transgene that encodes a *Fusarium venenatum* glucanase (FvGlu). In an effort to boost the expression levels of the two most promising transgenes, we have modified sections of the FvGlu and *TRI101* coding regions to make them more wheat-like in sequence. These improved constructs are currently being introduced into wheat.

EVALUATION OF TRANSGENIC WHEAT LINES EXPRESSING THE BACULOVIRUS OP-IAP FOR TOLERANCE TO SCAB INDUCED BY *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB) induced by the fungal agent *F. graminearum* is a significant problem in many wheat production areas worldwide. Chemical control strategies are currently not cost-effective and limited resistance has been observed with in the cultivated wheat germplasm. Therefore, an alternative approach is required to manage this devastating disease. A negative regulator of programmed cell death from baculovirus, Op-IAP was introduced into wheat via Agrobacterium-mediated transformation. Expression of the Op-IAP gene was regulated by the maize ubiquitin promoter coupled with its first intron. This Op-IAP cassette was subcloned to the binary vector pPZP212 which harbors a npt II cassette under the control of the CaMV 35S promoter. The resultant binary plasmid is referred to as pPTN226. The binary vector was mobilized in to Agrobacterium strain C58C1 (pPM90) and the resultant transconjugant was used for wheat transformations. Immature embryos of wheat (cv Bobwhite) were inoculated following a 4-d preculture period. The wheat transformants were selected on 10 mg/L G418 for 2 weeks followed by an additional 8-12 weeks on 25 mg/L G418 selection pressure. Primary transformants were characterized via an ELISA assay for npt II expression and subsequently by Southern blot. A total of 20 independent events were generated. A transcript corresponding to the Op-IAP transgene was detected in a subset of the wheat lines via northern blot analysis in progeny of the transgenic wheat lines. Resistance to scab was monitored in the greenhouse by inoculating immature spikes with *F. graminearum* with a conidia spore suspension (70 X 10^3 conidia/ml) using a needle to inject the conidial suspension between the palea and lemma in the center of the spike. Included in each of the scab inoculation tests were a susceptible and resistant check, Bobwhite and ND2710, respectively. Disease severity ratings were scored 19 days post inoculation and were tabulated as percentage of infected florets per head. Over three independent inoculations the susceptible check (Bobwhite) and resistant check (ND2710) scored an average disease severity ratings of 89.4% and 14.8%, respectively. From the 20 Bobwhite transgenic lines carrying the Op-IAP transgenes we have identified four events that have consistently displayed a reduction in disease severity ratings (app. 32 to 36%). Theses events are currently at the T4 or T5 generation and homozygous lines have been identified. Field trials are being planned for this summer and the lead lines are currently being crossed to winter wheat germplasm.

APPLICATION OF A HIGH THROUGHPUT, LOW COST, NON-DENATURING POLYACRYLAMIDE GEL SYSTEM FOR WHEAT MICROSATELLITE MAPPING

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INTRODUCTION

Microsatellite markers, one kind of tandem repetitive DNA sequence containing a 2-5 nucleotide motif, also referred to as simple sequence repeats (SSR), have turned out to be a valuable source of highly polymorphic DNA markers. Based on differences in the length of simple sequence repeats at loci defined by locus-specific PCR primers flanking the microsatellite, microsatellite markers have begun to supercede the RFLP or RAPD to construct linkage map for many different species, including human (Dib et al. 1996), mouse (Dietrich et al. 1996), rat (Serikawa et al. 1992; Jacob et al. 1995), dog (Mellersh et al. 1997), chicken (Groenen et al. 1998), and plants such as rice (Temnykh et al 2000), maize (Chin et al. 1996; Taramino and Tingey 1996), potato (Milbourne et al. 1998), wheat (Bryan et al. 1997, Röder et al,1998) and soybean (Akkaya et al. 1995; Cregan et al. 1999). For individual scientists, one of the largest barriers to applying microsatelites to any genetic problem is the cost and technical challenges presented by traditional means of electrophoresis. We anticipate that automated and ultra-high throughput capillary electrophoretic systems will be the primary means of genotyping in the future. Meanwhile, recent innovations inspired by P. Cregan, B. Diers, and D. Wang, have led to development of a low-technology, inexpensive, and relatively high-throughput gel system. Here, we report our experience to date with this system, which is produced by C.B.S Scientific Co. (619/ 755-4959) as Model # C-DASG-400-50 (Fig 1).

MATERIALS AND METHODS

Primers: Wheat microsatellite primer pairs from P. Cregan at the Beltsville Agriculture Research Center of USDA-ARS are applied for linkage mapping using the ITMI (International Triticeae Mapping Initiative) population. The allele sizes of the parents of the population, M6 and Opata are shown for six primer pairs in Table 1.

Table 1. Length in base pairs of allele of six primer pairs for M6 and Opata, the parents of ITMI population.

	Barc218	Barc124	Barc222	Barc137	Barc219	Barc196
Size at M6	210	216/206	182	250	208	145
Size at Opata	212	219/208	185	246	220	163

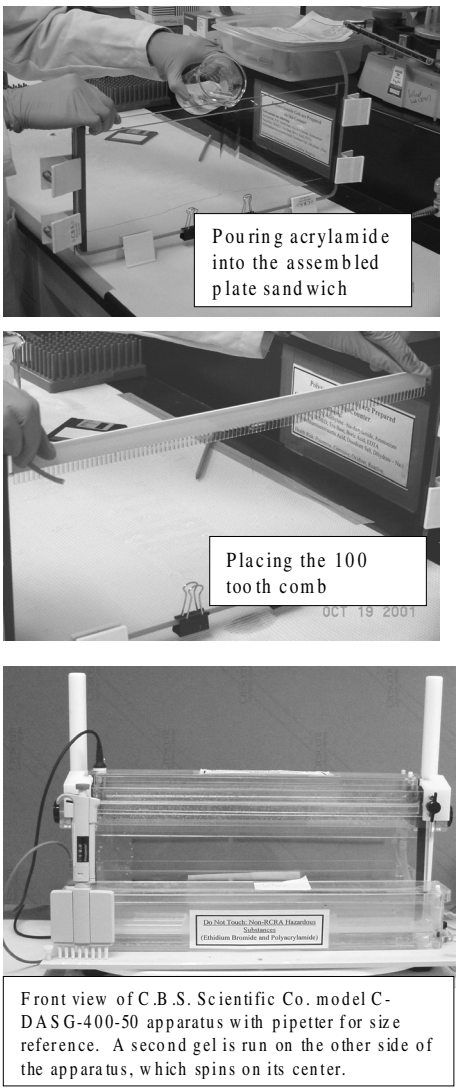

Figure 1. Images of the C.B.S. Scientific Co. two gel system.

PCR reaction: The amplification is done in a PTC-0220 DNA Engine Dyad Peltier Thermal Cycler, MJ research, using 15 ul reaction mixture, each containing 37.5 ng template DNA, 0.15 uM Primer, 1.5 mM MgCl, 0.15 mM each of dNTP and 1X PCR buffer and 2.0 U Taq polymerase. The PCR profile is followed: initial denaturation at 95 °C for 2 min; followed by 38 cycles of 94 °C denaturation for 25 sec, specified annealing temperature for 25 sec of annealing, 45 sec extension at 72 °C; after the final cycle there is a 10 min extension step at 72 °C, after which the temperature is held at 4 °C until removal from the thermal cycler.

Plate sandwich: The solidified non-denaturing polyacrylamide gel is created in a 'Plate Sandwich' consisting of two glass 49.0 cm wide. The back plate is UV transparent and rectangular in shape with a height of 21.5 cm. The outside edges of the front plate are the same height as the back plate. The front plate is notched so that the height between the edges is several centimeters less than that at the edges. A gel-wrap gasket is applied to the edges of the back plate. Side spacers (1.5 mm thick) are placed on both sides of the plate and then the notched front plate is set on top of the spacers (inside down) and aligned with

the back plate on each side of the plate. Two spring clamps (one on each round corner) and two medium binder clips (evenly spaced) are applied at the bottom of the plate sandwich so that the gasket forms a waterproof seal. All clamps are placed over the spacers and close to the gasket. The plate sandwich is then stood upright using the two spring clamps at the bottom for support.

Gel solution: 190 ml gel solution for 100-well gel is prepared by mixing the following solutions:

28.5ml	40 % acrylamide solution (Final concentration in gel 6%)
160.0ml	0.5931x TBE buffer (Final concentration in gel 0.5 x)
1.35ml	10 % APS
0.15ml	TEMED

TEMED should be added right before the gel is poured.

Gel pouring: The gel solution is poured directly into the plate sandwich. If necessary, a thin (1 mm or thinner) plastic ruler or spacer can be used to remove any air bubbles. A 100 well comb is placed at the top of the plate sandwich between the two plates and two spring clamps (evenly spaced) are applied to hold the comb tightly against the back plate.

Gel system setting: After the gel has solidified (about one hour after pouring), the plate sandwich is set on a plate holder and all the clamps, binder clips and the gel-wrap gasket are removed. The plate sandwich is then placed in a vertical apparatus for eletrophoresis. The electrophoretic apparatus holds two gels, one on each side, and has a rotating base for easy access to both gels. The running buffer is (0.5x TBE) is added to both the upper and lower reservoirs. Prior to loading the gel, 50µl of Ethidium Bromide (10 mg/ml) is added to the lower reservoir, and the gel is warmed for one hour at 350 volts.

Loading, Electrophoresis, and Visualization: The comb is removed carefully and the samples are loaded with a multi-channel pipette with 8 or 12 tips. Loading volume can be up to 25ul. One gel can be used sequentially up to 6 times. Electrophoresis is carried out at 350 volts for 1- 2 hours. If necessary, a small fan (12 inch) may be used to cool the system. The plate sandwich is taken out and put horizontally on a UV box with the back plate (which is UV transparent) down. Note that the gel is NOT removed from the plate sandwich. Size limitations on our UV light box requires two photographs per 100-lane gel, one for the left half and the other for the right half of the gel. Digitized images of the photographs are stored on a computer.

RESULTS

Figure 2 illustrates the resolution obtained with Barcs 196 and 222 after 1.5h electrophoresis. This result clearly demonstrates that allele size differences of 6 bp are readily distinguished with fragments in the 150 - 190bp size range. The same duration of electrophoresis (1.5h) did not, however, enable good resolution of allele sizes for Barc 218, where the ITMI parent alleles differ by only two bp (Fig. 3, top). That problem is overcome, though, with additional electrophoresis (Fig 3, bottom).

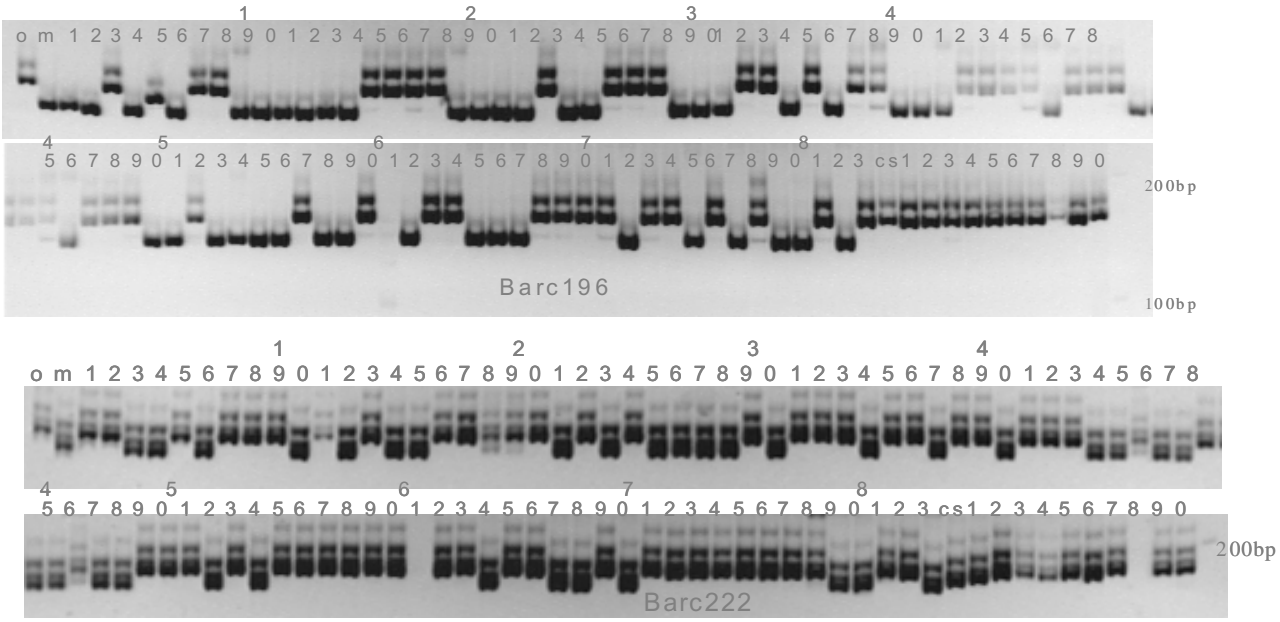

Figure 2. Negative images of two 100 lane gels with PCR products of Barc 196 (top), and Barc 222 (bottom) for the ITMI mapping population (o=Opata, m=M6, lanes 1-83 are progeny 1-60, and 61-84), and and 11 U.S. Wheat genotypes using the non-denaturing polyacrylamide gel system with UV exposure. Products from Barcs 196 and 222 were subjected to 1.5 hours of electrophoresis. (The allele sizes of ITMI population were 145 bp and 163 bp for Barc 196, and 174 bp and 180 bp for Barc 222)

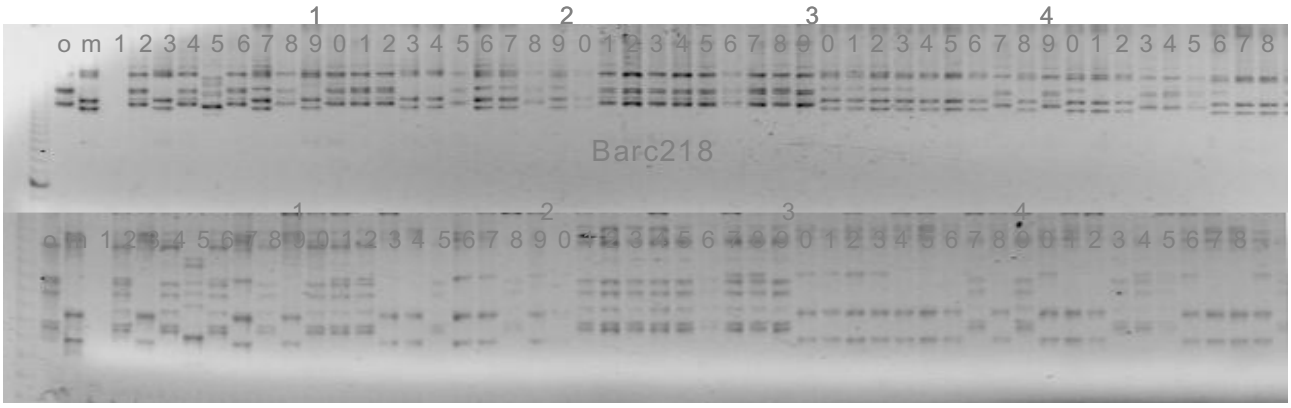

Figure 3. Negative mages of Barc 218 products electrophoresed for 1.5 (above) and 3.0 hours (below). The allles are 210 and 212 bp in length. Template genotypes are from from the ITMI mapping population (o=Opata, m=M6, lanes 1-48 are progeny 1-48).

Visualization with the UV light box does not involve disassembly of the plate sandwich, which permits the user to check resolution during a single run. Portability of the plate sandwich also enables re-loading of a completely new set of PCR products. We have successfully re-loaded the same plate sandwich with 6 distinct sets of PCR products, each time adding Ethidium Bromide to the lower reservoir. It is also possible to load a second set of PCR products either simultaneous to the first set (assuming no overlap in allele sizes), or after the first set is loaded but before it has moved off the gel. Figure 4 shows an image of the first 50 lanes of a single gel loaded at 1.5 hr intervals with PCR products amplified from wheat microsatellites Barc124 and Barc137. Barc124 amplifies two loci (124-1, alleles 206 bp and 208 bp; and 124-2, alleles 216 bp and 219 bp). The allele sizes of Barc137 from

Opata and M6 were 246bp and 250bp, respectively. The amplification products of Barc 124 were loaded first and Barc137 was loaded 1.5 hr later.

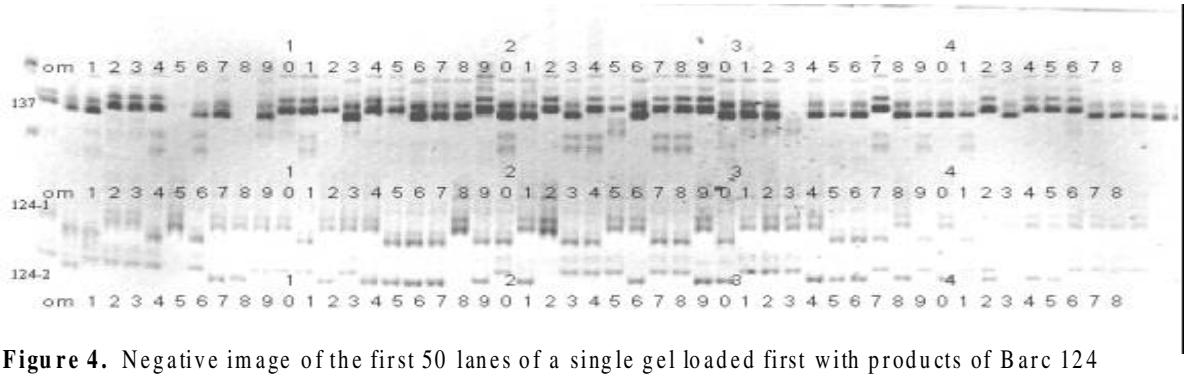

Figure 4. Negative image of the first 50 lanes of a single gel loaded first with products of Barc 124 followed 1.5h later with products of Barc 137. Lane layout of genotypes is identical to that for Fig.s 1 and 2.

DISCUSSION

We have found the system described here to be extremely effective for microsatellite mapping work. At a minimum, we are able to run two sets of 100 PCR products on each gel, two times per day. Currently, we are mapping new BARC microsatellites. Since each apparatus mounts two gels, one lab worker can acquire microsatellite genotype information on eight (2 gels x 2 runs/day x 2 loadings per run) BARC primer pairs (usually 1 locus each) per day with each C.B.S. apparatus. In our experience, a skilled worker can manage two systems and therefore generate data on at least 16 microsatellite loci per day (note that PCR becomes the limiting step). Assuming only 96 lanes are used (because PCR plates have 96 wells), that amounts to at least 16*96= 1536 data points per day. Gels are never removed from the glass plate sandwich, eliminating one of the more challenging steps involved with manual polyacrylamide gels which require staining of some sort. We estimate the cost of a single gel (excluding PCR costs) to be $2.60, which compares very favorably with the costs associated with automated non-capillary sequencing gel costs. We do not currently have an automated system for data capture and rely on manual input of data acquired from the digital images.

In summary, we feel the system described here is a revolutionary development warranting serious consideration by any lab currently employing traditional polyacrylamide gels for microsatellite work. In the future automated capillary systems are likely to be the most cost effective approach to genotyping, but today, the system described here is very attractive.

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DEVELOPMENT AND MAPPING OF MICROSATELLITE (SSR) MARKERS IN WHEAT

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ABSTRACT

A total of 410 markers were developed from random genomic libraries, and of these, 279 were polymorphic among Chinese Spring, Opata and M6. One hundred and sixty-seven of these markers were polymorphic in the ITMI mapping population and 143 were positioned on the ITMI map, 156 were positioned using nulli-tetrasomic lines of Chinese Spring. A total of 67, 75 and 61 markers were mapped on A, B, and D genomes respectively. New microsatellites were integrated into a framework map consisting of previously published RFLP and microsatellite markers. A total of 43 of the new markers were positioned in gaps larger than 10cM.

INTRODUCTION

Due to large genome size, high homoeology among A, B, and D genomes and high level of repetitive sequences in the genome of wheat, development of informative microsatellite markers in wheat is difficult and time consuming. Currently, approximately 350 publicly available wheat microsatellite primer sequences have been reported. This is a small number of markers relative to the huge genome size of wheat. The current ITMI map (http://wheat.pw.usda.gov/ggpages/ maps.html**)** contains a total of 1201 markers including RFLPs and SSRs. The total map length is approximately 3332 cM. There are 121 gaps of greater than 10cM. Although, there is on average one marker every 2.8cM, the likelihood that only one in three marker loci will be polymorphic in any given single cross dictates the need for much greater marker density. Clearly, development and mapping of more microsatellite markers in wheat, whose genome size is 37 times larger than that of rice, six times larger than that of corn and 15 times than that of soybean, should be important objectives of wheat geneticists.

The present paper reports the development of 269 microsatellite markers and their mapping position and also attempts to evaluate the consistency of mapping using segregation analysis versus physical mapping with cytogenetic stocks (nullitetrasomic, ditelosomic and deletion lines).

MATERIALS AND METHODS

Wheat library construction, screening, DNA sequencing and primer design:

For microsatellite isolation, various libraries were constructed. (1) Genomic DNA of Chinese Spring was either digested with combination of enzymes, or sheared using a nebulizer and then size selected on a 1% agarose gel. Sheared DNA was treated with mungbean exonuclease or T4 DNA polymerase to create blunt ends. DNA fragments in the 400 to 750 bp range were isolated from the gel using Geneclean II. Purified DNA fragments were ligated into the *SmaI* site of pBluescript. (2) The library was enriched based on the procedure described by (SONG *et al.* 2000; PULIDO *et al*. 1994). (3) The library was enriched for microsatellite by GIS.

Clone selection, rescreening, sequencing and primer design followed as described by Cregan et al (1994), Song et al (2001). To determine the level of polymorphism, primer sets were tested using genomic DNA of Chinese Spring; Opata 85 and M6. Opata 85 and M6 are the parents of the ITMI (International Triticae Mapping Initiative) mapping population.

Mapping of markers using Opata 85x M6 IBL population:

The first 83 recombinant inbred lines of ITMI population were used for the segregating analysis. All published microsatellites and RFLP segregation, including 940 RFLP (NELSON *et al* 1995, VAN DEYNZE *et al* 1995) and 281 microsatellite markers (RODER *et al,*1998a), were collected, out of which 534 loci, which have an exact chromosome position, were constructed as a framework and retested for linkage (LOD = 3.0 or greater). All the new microsatellites were integrated into this framework.

Physical mapping of markers:

Nulli-tetrasomic, and ditelosomic lines were used to assign microsatellite markers to their respective chromosome arms. Various numbers of single-break deletion lines on each chromosome were used for subarm localization of the markers (ENDO and GILL 1996).

RESULTS

SSR marker development and marker information

Effect of different motifs (especially di- vs tri- and tetra-nucleotide motifs) on the level of polymorphism of markers. Primers were designed to the sequences containing dinucleotide [CT/GA]n or [CA/GT]n, the tri-nucleotide motif [ATT/TAA], and the tetra-nucleotide [TAGA/ATCT]. The rate of polymorphic markers was 28%, 24%, 36% and 28% for [CT/GA]n, [CA/GT]n, [ATT/TAA]n and [TAGA/ATCT]n, respectively. Although the success rates of [CA/GT]n and [CT/GA]n were similar, [CT/GA]n is preferable to [CA/GT]n due to its higher frequency in the genome and higher average number of repeats in the repeat-containing sequences. Our previous work indicated that the [ATT/TAA]n motif was superior to all other trinucleotide repeats for the successful development of polymorphic microsatellite markers (Song *et al* 2001). Our current data strongly suggest that the [ATT/TAA]n motif is also superior to the two

most commonly used dinucleotide motifs and one tetra-nucleotide motif in terms of the rate with which polymorphic marker loci can be developed.

Effect of length of repeat on the level of polymorphism of markers As shown in Table 1, primers flanking higher numbers of repeats showed a higher probability of providing polymorphic markers.

Functionality of primers The percentages of primer sets that amplified the expected size product was 53%, 53%, 55%, and 56% for [CA/GT]n-, [CT/GA]n-, [ATT/TAA]n- and [TAGA/ATCT]n-containing fragments, respectively. A total of 410 markers was developed, among them, 11 were based on [CA/GT]n, 135 on [CT/GA]n, 204 on [ATT/TAA]n, 24 on [TAGA/ATCT]n and 36 contained other motifs. One hundred and sixty-seven of those amplified polymorphic products between Opata 85 and M6. The primer information for each marker can be accessible at: http://www.scabusa.org.

Genetic mapping of SSR markers

A total of 209 markers was mapped either using tetra-nullisomic and deletion stocks or using the 84 ITMI RILs (Table 2). Among them, ninety-eight markers were positioned on both maps, 66 and 53 were only positioned on the physical map or the ITMI map, respectively. Among the markers which had been placed on both maps, ninety were mapped on the same chromosomes by both methods, the order of 82 markers on both maps was consistent. There were 11 instances where inconsistencies wsere observed between positioning based upon the two mapping approaches. The inconsistencies were mainly confined to chromosome 5A (3 instances) and 5B (5 instances).

Forty-three of the newly developed markers were mapped in gaps greater than 10cM, nine were mapped to the ends of linkage maps. These markers extended current map length.

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Table 1. Effect of length of repeat on the level of marker polymorphism.

	[CT/GA]n		[CA/GT]n		[ATT/TAA]n		[TAGA/ATCT]n	
	Monomorphic	Polymorphic	Monomorphic	Polymorphic	Monomorphic	Polymorphic	Monomorphic	Polymorphic
1-5					2		3	
6-10	16	5	4	2	35	33	6	9
11-15	21	24	1	3	20	34	2	3
16-20	9	21			6	29		
21-25	6	9		1	9	14	1	
26-30	2	7			2	8		
31-35	2	1				3		
40-45		1				2		
Ave. repeat length	15.0	17.4	9.4	14	12.8	15.1	8.3	10

Table 2. Number of markers mapped on each chromosome

Chromosome	Number of markers mapped on the same chromosome based on ITMI and cytological lines	Number of markers mapped based on cytological lines	Number of markers mapped based on ITMI lines	Total
1A	4	5	2	11
1B	5	1	4	10
1D	4		5	9
2A	3	1	0	4
2B	6	5	3	14
2D	1	1	4	6
3A	5	5	2	12
3B	4	5	3	12
3D	2	2	1	5
4A	6	2	3	10
4B	2	3		5
4D	1	2	3	6
5A	6	4	3	13
5B	6	9	2	15
5D	1	2	6	8
6A	4	2	3	9
6B	4	1	2	7
6D	7	2	3	12
7A	5	3		8
7B	6	4	2	12
7D	8	3	2	15
Total	90	66	53	209

PHYSICAL MAPPING OF MICROSATELLITE MARKERS ON WHEAT CHROMOSOMES

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ABSTRACT

Microsatellite or simple sequence repeats (SSRs) consist of short DNA motifs, usually 1-6 nucleotide core elements that are repeated from two to several thousands times, have emerged as an important source of markers for molecular plant breeding. In wheat, SSRs are the marker of the choice due to their locus specificity, and co-dominant inheritance. Efforts are being made among three Institutes (ARS-USDA, Beltsville, KSU Manhattan, MSU Michigan,) through the U. S. Wheat and Barley Scab Initiative to develop a new set of microsatellite markers and map them on the ITMI mapping population and deletion stocks of wheat. In this study we analyzed 243 SSR markers on a set of deletion stocks of wheat chromosomes. Forty-four primer pairs, each amplified products of the same size from all the 21 stocks, suggesting their presence on more than one chromosome and no polymorphism among loci on homoeologous chromosomes. Two primer pairs did not amplify a PCR product and the remaining 197 primer pairs amplified a total 199-microsatellite loci, which could be assigned to all chromosome arms of seven groups (Table 1). Fifty-seven fragments were mapped to the A-genome, seventy-six to the B-genome and fifty-six to the D-genome. Of 197 primer pairs for which the corresponding loci could be assigned to a specific chromosome, 195 primer pairs each amplified only a single locus and two primer pairs amplified two loci on two different chromosomes. Thus, the primer pairs, amplifying a single locus showed high locus specificity and could be useful when chromosome specificity is desired. The two loci amplified by Barc204 and assigned to 6A and 6D chromosomes could be homoeologous loci. On other hand, the two loci amplified by primer pair Barcm70 and assigned to 2D and 4A may represent a case of interchromosomal duplication involving non-homoeologous chromosomes or a case of homoeologous segments transferred to non-homoeologous chromosomes. The markers assigned to specific chromosome bins at KSU along with their mapping in ITMI mapping population at MSU, will be useful for intra-chromosomal mapping, chromosome identification in aneuploid stocks, and targeted mapping of useful genes in wheat breeding. In addition to the microsatellite markers developed from genomic library at Beltsville, we have designed and synthesized 250 primer pairs from the sequences of wheat EST database containing SSRs. Standardizing the conditions for amplification of EST-SSRs and mapping them in wheat will be our focus in the future.

Table 1. Mapping of BARC and BARCM primers on wheat chromosomes

Chromosome	Primer	
	Short arm	Long arm
1A(10)	BARC09, BARC25, BARC28, BARC148, BARC176, BARCM48	BARC17, BARC83, BARC158, BARCM22
1B(10)	BARC08, BARC137, BARC181	BARC61, BARC80, BARC81, BARC174, BARC187, BARC188, BARCM15
1D(8)	BARC149, BARC152	BARC62, BARC66, BARC99, BARC119, BARC169, BARCM42
2A(5)	BARC112, BARC124, BARC212	BARC05, BARC15
2B(16)	BARC07, BARC13, BARC18, BARC35, BARC55, BARC91, BARC160, BARC200, BARCM24, BARCM72	BARC16, BARC101, BARC128, BARC167, BARCM27, BARCM64
2D(5)	BARC95, BARC168, **BARCM70**	BARC159, BARC228
3A(11)	BARC12, BARC19, BARC45, BARC54, BARC67, BARC86	BARC51, BARC57, BARC197, BARCM21, BARCM60
3B(13)	BARC68, BARC73, BARC75, BARC87, BARC102, BARC147, BARC156	BARC77, BARC84, BARC139, BARC164, BARCM44, BARCM77
3D(4)	BARCM40	BARC06, BARC42, BARC71
4A(9)	BARC206, BARCM52, **BARCM70**	BARC52, BARC78, BARC106, BARC153, BARC170, BARCM47
4B(5)	BARC20, BARCM01, BARCM45	BARC60, BARC163
4D(5)	BARC98	BARC27, BARC93, BARC225, BARCM69
5A(12)	BARC56, BARC117	BARC01, BARC40, BARC92, BARC94, BARC100, BARC122, BARC141, BARC151, BARC165, BARC186
5B(13)	BARC04	BARC11, BARC58, BARC59, BARC69, BARC74, BARC88, BARC89, BARC109, BARC140, BARC142, BARCM32, BARCM61
5D(9)	BARC130, BARC143, BARC205	BARC44, BARC110, BARC133, BARC144, BARCM02, BARCM18
6A(9)	BARC03, BARC195	BARC37, BARC107, BARC113, BARC171, **BARC204**, BARC104, BARCM55
6B(9)	BARC14, BARC48, BARC134, BARC198	BARC24, BARC48, BARC79, BARC178, BARC180
6D(12)	BARC123, BARC173, BARC183, BARC196	BARC21, BARC23, BARC96, BARC146, BARC175, BARC202, **BARC204**, BARCM30
7A(11)	BARC64, BARC70, BARC127, BARCM04, BARCM05, BARCM25, BARCM34	BARC29, BARC49, BARC108, BARC121
7B(10)	BARC63, BARC72	BARC32, BARC50, BARC65, BARC82, BARC85, BARC90, BARC182, BARCM73
7D(13)	BARC125, BARC126, BARC154, BARC214, BARCM33	BARC26, BARC53, BARC76, BARC97, BARC111, BARC172, BARCM46, BARCM75

*Number given in parentheses after the chromosome indicate the total SSR markers mapped on particular chromosome and the primers mapped more than one chromosome is bolded.

EXPRESSED SEQUENCE TAGS FROM DEVELOPMENTAL STAGES OF *GIBBERELLA ZEAE*

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ABSTRACT

Gibberella zeae (anamorph: *Fusarium graminearum*) causes head scab, or head blight of wheat, barley, and oats, and foot and crown rot of corn. Recent scab outbreaks in Asia, Canada, Europe, South America and the United States highlight the increased threat this disease poses to food supplies worldwide. A better understanding of the biology of the scab organism is warranted to develop new control strategies. Our longterm goals are: (1) to understand the genetic basis for inoculum development, mycotoxin production and pathogenicity; (2) to use genomics to develop a biology-based control program for scab, using the genomics programs of wheat and corn to enhance this program. We have sequenced and analyzed a cohort of over 12,000 ESTs from 3 cDNA libraries representing different culture conditions and developmental stages. We will present the functional categorization of the genes in these libraries and a comparative analysis of gene expression.

ASSESSING THE GENETIC DIVERSITY OF FUSARIUM HEAD BLIGHT RESISTANT SOURCES IN BARLEY

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ABSTRACT

A number of barley varieties from around the world have been identified as potential sources of Fusarium head blight (FHB) resistance genes. All of these varieties exhibit partial resistance and, based on several mapping studies, it is known that resistance to FHB is controlled by multiple genes. To better exploit these resistant sources, we have initiated a study using simple sequence repeat (SSR) markers to determine the genetic diversity among important FHB resistant barley varieties that are being used in breeding programs and genetic mapping studies. To date, we have screened 10 resistant sources that are currently being used in mapping studies or breeding and six varieties with 59 SSR markers spanning the barley genome. SAHN cluster analysis using NTSYS-pc shows the resistant sources clustering into two groups that are 16% similar to one another. The first group consists of Atahualpa, Kitchin, Ac Oxbow, Chevron, Gobernadora, Frederickson, and Zhedar #1. Within this group Frederickson and Zhedar #1 are the most alike at 95% similarity while the others are less than 56% similarity. The second group consists of five susceptible varieties, and four resistant sources (MNBrite, CIho 4169, PFC88209, and Hor211). Hor211 is the most dissimilar line within this group with 21% similarity to the rest of the cluster. The four susceptible varieties developed in the Minnesota breeding program are greater than 80% similarity. These results show considerable differences in the relatedness of FHB resistant sources. These differences should be considered when choosing FHB-resistant sources for breeding efforts or new genetic studies. We are working to expand this SSR allele database to include more lines, both resistant and susceptible, by integrating several existing databases. In addition, we are currently incorporating 14 newly identified FHB resistant (B. Steffenson pers. comm.) accessions into the above data set.

ESTS POSSIBLY RELATED TO VIRULENT OR AVIRULENT GENES OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

To understand molecular interaction of wheat (*Triticum aestivum*) and *F. graminearum* during the FHB development, FHB was induced on Sumai 3 (FHB-resistance) and Wheaton (FHB-susceptible) with spikelet-inoculation of isolate Fg4 or water (as control) in greenhouse. The inoculated florets were sampled in 0, 1, 16, 32 and 64 hours after inoculation. With DDRT-PCR technique, several ESTs were revealed to be expressed only in Fg4-inoculated Sumai 3, or expressed in both Sumai 3 and Wheaton but with different level of expression. We further confirmed that two of these ESTs are corresponding to the genes of *F. graminerum* with Southern and Northern analysis. These genes might be related to virulence or avirulence of the fungal. These ESTs were cloned and sequenced. (This poster was presented at 2001 ASA annual meeting.).

QTLS MAPPING OF TYPE I AND TYPE II RESISTANCE TO FHB IN WHEAT

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OBJECTIVES

To identify the number, the position, and the magnitude of QTLs for the resistance to initial infection (Type I) and the resistance to fungal spread within plant tissues (Type II) of Fusarium head blight in wheat.

INTRODUCTION

Several types of the host resistance and tolerance to Fusarium head blight (FHB) have been described in wheat (Ban, 2000a, b): resistance to initial infection (Type I), resistance to fungal spread within plant tissues (Type II), resistance based on the ability to degrade the mycotoxin (Type III), tolerance to high mycotoxin concentrations (Type IV), and resistance to kernel infection (Type V). Of them, the Type I and Type II resistance are most important to screen the resistant varieties in breeding programs. The recently developed molecular techniques provide opportunities for a well understanding of the genetic mechanism of host resistance to FHB. However, previous studies for molecular mapping of resistance to FHB were mainly focused on Type II resistance since it is relatively easy to evaluate in greenhouse under controlled conditions (for a review, see Kolb et al., 2001). Only Type II resistance is not enough to prevent the FHB damages under sever epidemics. In order to increase the comprehensive resistance level to FHB, different resistance types to FHB should be pyramided in the improved varieties. In this study, we evaluated both Type I and Type II resistance for a population of double haploid lines (DHLs) and analyzed the QTLs for the two resistance types. The obtained result may give us a better understanding for the mechanism of resistance to FHB and help us to make a reasonable breeding strategy for resistance to FHB.

MATERIALS AND METHODS

Plant materials - a segregating population of 118 DHLs developed from the F_1 cross of Sumai 3 (Chinese resistant culrivar) and Gamenya (highly susceptible cultivar from Australia) with the wheat x maize system (Suenaga and Nakajima, 1989).

FHB resistance evaluation - the phenotype of Type I resistance were evaluated in the field condition with a sprinkler system in 1995 and 1996 (Ban and Suenaga, 2000). After the spraying of the macroconida suspension of *F. graminearum* 'G87-36B' in the flowering stage, the severity of disease on spikes for each DHL was scored. The phenotype of Type II resistance was evaluated in green house in 2000 with two replications. The inoculum, which concentration of macroconidia was adjusted to about 5×10^5/ml, was injected into a central

spikelet of spikes when they were just beginning to flowering. Inoculated plants were placed in plastic chamber, which were maintained at 22-25 °C with 100% relative humidity for three days. After 21 days of inoculation, the disease severity of each line was scored based on the average value of diseased spikletes percentage over 5 spikes.

Molecular marker analysis – Ninety nine of WMS markers developed by Röder et al. (1998) that showed polymorphism between Sumai 3 and Gamenya separated on 4% Metophor agarose gel were applied to construct the linkage map with AFLP, RFLP and RAPD markers. For AFLP analysis, genomic DNA was digested with *Eco*RI and *Mse*I. A total of 30 primer combinations were used for selective amplification. The *Eco*RI side primers were labeled with florescent dyes (6-FAM, HEX, or NED), then PCR products were analyzed by an ABI 373 sequencer with Genescan 3.1 software (Perkin Elmer/Applied Biosystems). The name of each polymorphic band was assigned by the selective bases of primer combination used to amply it followed by the fragment size.

QTL analysis - Linkage map construction and QTL analysis were performed by using Map Manager QTX (http://mapmgr.roswellpark.org) software. Chromosomes assignment of the linkage maps were determined based on the SSR maps of wheat (Röder et al., 1998; Pestsova et al., 1999).

RESULTS AND DISSCUSSION

Two hundred and thirty nine AFLP markers, together with 99 SSR, 21 RFLP, 21 RAPD markers, and one morphological marker (awnedness controlled by *B1* locus), formed 34 linkage groups covering a total genetic distance of 3739.3 cM (P=0.0001). The SSR markers assigned their chromosomes excepting chromosome 3A, 6A, and 4D.

Three genomic regions including five, one and two markers on chromosome 5AL, 5BS, and 2DS, respectively, were significantly associated with Type I resistance (Table 1). The QTLs on 5AL for Type I resistance further support our previous result that one resistance gene in Sumai 3 that was linked to *B1* locus located on 5AL (Ban and Suenaga, 2000). Two genomic regions on 3BS and 2DS assigned by three markers, respectively, were significantly associated with Type II resistance. The QTLs on 3BS were consistently detected in the populations including Sumai 3 or their derivatives in several studies by using RFLP, AFLP, and SSR markers (Kolb et al., 2001). The QTL on 2DS were associated with both Type I and Type II resistance. These results suggested that the genetic constitution for Type I and Type II resistance types is not identical. A strategy by pyramiding of resistance genes for different resistance types into an adapted background may be an effective way for FHB resistance breeding in wheat.

The QTL on chromosome 2DS showed negative effect on both Type I and Type II resistance to FHB and this negative effect was contributed by Sumai 3. Yao et al. (1997) reported that one susceptible gene in Sumai 3 located on chromosome 2D. Our study further mapped this susceptible locus originated from Sumai 3 on the chromosome 2DS. Sumai 3 is the most used resistance resource for FHB resistance in wheat breeding around the world. However, it usefulness has been hindered by its too many bad agronomic characters. The present study indicated that the Sumai 3 not only contain resistance genes but also include suscep-

tible genes for FHB. When Sumai 3 was used as a resistance resource in a breeding program, the susceptible gene should be excluded.

Table 1. Putative QTLs associated with Type I and Type II resistance to FHB in a doubled haploid population developed from the F_1 cross of Sumai 3 and Gamenya.

Type of resistance	Putative QTLs	Marker[a]	Chr.	*P* value	LOD	V (%)[b]	Add[c]	Resistant source
Type I	QTL-1	Xgwm410-5A	5AL	< 0.001	2.9	10	8.22	Sumai #3
	QTL-1	AAC/CATA221	5AL	< 0.001	3.4	12	8.78	Sumai #3
	QTL-1	AAC/CATA416	5AL	< 0.001	4.5	15	10.01	Sumai #3
	QTL-1	AAG/CGAC500	5AL	< 0.001	3.5	17	10.89	Sumai #3
	QTL-1	*B1* (awn suppressor)	5AL	< 0.001	5.9	20	11.24	Sumai #3
	QTL-2	ACG/CAAC103	5BS	< 0.001	3.1	11	8.31	Sumai #3
	QTL-3	Xgwm261	2DS	< 0.001	4.3	15	-9.69	Gamenya
	QTL-3	AAG/CAGT368	2DS	< 0.001	3.8	14	-9.33	Gamenya
Type II	QTL-4	Xgwm533a	3BS	< 0.001	2.3	9	2.65	Sumai #3
	QTL-4	AAG/CGAC186	3BS	< 0.001	3.1	12	8.40	Sumai #3
	QTL-4	AGC/CACT88	3BS	< 0.001	2.5	10	7.53	Sumai #3
	QTL-3	Xgwm296-2D	2DS	< 0.001	2.5	10	-7.60	Gamenya
	QTL-3	Xgwm261	2DS	< 0.001	4.2	17	-9.48	Gamenya
	QTL-3	AAG/CAGT368	2DS	< 0.001	3.4	14	-8.76	Gamenya

[a]: SSR markers are indicated by Xgwm code and *B1* is a morphorogical marker.
[b]: The amount of the total trait variance which would be explained by a QTL at this locus.
[c]: The additive regression coefficient for the association.

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USING THE MAIZE *AC-DS* SYSTEM TO OBTAIN MARKER-FREE TRANSGENIC BARLEY PLANTS THAT STABLY EXPRESS PUTATIVE ANTIFUNGAL PROTEINS

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ABSTRACT

Fusarium head blight (FHB) has been reported worldwide, especially in the upper Midwestern US. Infected barlely grain, contaminated by deoxynivalenol (DON), is not acceptable for malting and brewing. Introduction of recombinant antifungal proteins into barley offers the potential to suppress pathogen infection and growth. Transformation technologies were developed for the highly regenerable cultivar Golden Promise (Wan and Lemaux, 1994) and subsequently improved upon (Cho et al., 1998; Bregitzer et al., 2000). Despite these successes several hurdles exist to the use of these technologies to produce commercially acceptable germplasm. Existing methods result in the presence of selectable marker genes and plasmid sequences in the transgenic plants and introduced transgenes frequently become silenced during generation advance. To exploit potentially useful aspects of the maize *Ac/Ds* system, including the propensity of transposed sequences to reinsert into transcriptionally active chromatin, Koprek *et al.* (2001) transferred the essential parts of this system via transformation into barley. They discovered that transposase-mediated transposition of *Ds-bar* resulted in stablized expression of *bar*-mediated herbicide resistance. For example, transgene expression in F_2 plants with a single copy of a transposed *Ds-bar* was stable in 100% of the plants, whereas only 23% of F_2 plants carrying a single nontransposed copy of *Ds*-bar had stable expression of the transgene product (Koprek *et al*., 2001). We are using the *Ac-Ds* system to produce large numbers of transgenic plants containing independent insertions of genes encoding putative antifungal proteins, which will be unlinked to selectable marker genes and other plasmid sequences and which are stably expressed. The putative antifungal genes, *tlp1* (thaumatin-like protein) and *tlp4* from oat and two of the trichothecene pathway genes, *TRI101* and *TRI12*, isolated from *Fusarium sporotrichioides*, were put into a *Ds*-bordered, maize *ubiquitin*- or rice *actin* promoter-driven expression cassette. The resultant *tlp* constructs, together with pAHC20 (*ubiquitin* promoter-*bar-nos*), were introduced via bombardment into scutellar cells of immature embryos of two spring cultivars of barley, Golden Promise, a 2-rowed variety, and Drummond, an elite 6-rowed variety. The selection of putative transgenic lines is ongoing. To assist in characterizing the level of transgene expression, antibodies to the *tlp* proteins are being developed. Genes for TLP 1 and TLP 4 were inserted in vector pGEX and proteins were purified for antibody preparation. Bombardments with *TRI101* and *TRI12* genes will be initiated in the near future. In addition, *Ac*-transposase under control of maize *ubiquitin* or its own putative *Ac* promoter are being introduced into Drummond, both by direct transformation and by backcrossing from Golden Promise lines.

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