The 1998 National Fusarium Head Blight Forum

CHAPTER 4 Part 2

Host Resistance and Variety Development

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U.S. Wheat & Barley
Scab Initiative

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CHAPTER 4 Part 1

Host Resistance and Variety Development

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O

DNA Markers for Fusarium Head Blight Resistance QTL in Two Wheat Populations.

James A. Anderson, Blair L. Waldron, Benjamin Moreno-Sevilla, Robert W. Stack, and Richard C. Frohberg.

Objectives:

The objectives of this research were to determine the number of genes and the magnitude of their effects in conditioning resistance to *Fusarium* head blight in wheat, identify DNA markers linked to them, and verify the markers in a second population.

Introduction:

Recent epidemics of *Fusarium* Head Blight (FHB) in the USA and Canada have caused severe yield and end-use quality losses in common (*Triticum aestivum* L.) and durum (*T. turgidum* L.) wheat. *Fusarium* head blight resistance is inherited as a quantitative trait and numerous chromosomal regions have been reported to influence resistance (reviewed by Bai and Shaner, 1994). Because of the difficulties in breeding wheat for resistance to this disease using conventional methods, the identification of DNA markers associated with resistance would be useful for wheat breeders and geneticists.

Materials and Methods:

A population of 112 F_5-derived recombinant inbred lines (RIL) from the cross Sumai 3 (resistant)/Stoa (mod. susceptible) grown in the greenhouse was evaluated for reaction to inoculation with conidia from *Fusarium graminearum* in two experiments. At anthesis, an average of nine spikes of the same size and maturity in each of three replications per RIL were inoculated with a 10µl droplet (50,000 conidia/ml) of conidial suspension placed directly into a single spikelet near the center of the spike following procedures described by Stack (1989). The conidial suspension contained either a single (Expt. 1) or a mixture of three (Expt. 2) *F. graminearum* isolates. This procedure bypasses primary infection and targets Type II resistance (Schroeder and Christensen, 1963; Mesterhazy, 1995). A gentle overhead mist was applied and plants were covered with a plastic humidity tent for three nights following inoculation. Three weeks after inoculation, spikes were scored individually for visual symptoms on a 0-100% FHB severity scale (Stack and McMullen, 1995). Because RILs reached anthesis at different times, four (Expt. 1) or five (Expt. 2) inoculation dates were necessary. Severity data from Expt. 2 was adjusted to account for differences in means of the inoculation dates. This adjustment improved the F-test for RILs. A subset of 27 lines were re-evaluated in a subsequent experiment. The FHB scores agreed with those obtained in the first two experiments ($r = 0.87$, $P < 0.001$), thus giving confidence in the repeatability of this data. Likewise, a population of 139 F_5-derived recombinant inbred lines (RIL) from the cross ND2603 (Sumai 3/Wheaton) (resistant)/Butte 86 (mod. susceptible) was evaluated for reaction to inoculation with conidia from *Fusarium graminearum* in two experiments (Mitchell Fetch et al., 1998). A subset of lines from this population also were re-evaluated in a subsequent experiment. The FHB scores agreed with those obtained in the first two experiments ($r = 0.82$, $P < 0.001$). For both populations, the entry means of the two experiments that included all RI lines were used in subsequent analyses.

RFLP analyses on the Sumai 3/Stoa population were performed as described by Riede and Anderson (1996). Initial screening was done with clones that mapped approximately 10 cM apart throughout the wheat genome according to previous wheat RFLP maps (Nelson et al., 1995a,b,c; Van Deynze et al., 1995; Marino et al., 1996). Polymorphic markers were hybridized onto membranes containing a subset of 59 or 72 RI lines. As linkage groups were formed, large gaps in the map were revealed and markers known to reside in these regions were selected for probings. Markers significantly associated with FHB resistance were hybridized onto membranes containing the remaining individuals of the population. Because of difficulties in obtaining linked markers for genomic regions significantly associated with FHB resistance (e.g. 3BS), AFLP analysis was initiated to target markers to these regions using a selective genotyping approach with RI lines selected based on FHB reaction of lines and/or RFLP genotype of markers in the target region. We used the AFLP kits from Gibco, BRL with slight modifications from manufacturer instructions. Primer pairs revealing segregation that suggested association with resistance or linkage to the intended RFLP marker target in the 10 RILs composing the selective genotyping array were used to genotype all members

of the population. All other polymorphic fragments revealed by such primer pairs were mapped on the entire population. Only one pair of AFLP primers (EagcMcta) and those RFLP markers significantly associated ($P < 0.05$) with FHB on the Sumai 3/Stoa population were screened for polymorphism and mapped in the ND2603/Butte 86 population.

Linkage maps were constructed using MAPMAKER Macintosh v2.0 (Lander et al., 1987) and aneuploid analysis was used to determine the chromosomal arm location of markers in linkage groups as described by Anderson et al. (1992). Markers were subjected to regression and interval analysis using the computer program Qgene (Nelson, 1997) to identify significant ($P < 0.01$) associations between individual DNA markers and FHB resistance.

Results and Discussion:

The Sumai 3/Stoa population displayed a normal distribution, transgressive segregants, and significant variation among RILs for FHB severity (data not shown). Six hundred and nineteen RFLP clones were screened for polymorphism between the parents. A total of 292 clones were polymorphic, yielding 360 loci. Sixteen pairs of AFLP primers were used on the entire population, revealing 151 mappable loci. Four genomic regions containing putative quantitative trait loci (QTL) were associated ($P < 0.01$) with FHB resistance from the combined analysis of two experiments, two from Sumai 3 and two from Stoa (Table 1).

Encouragingly, the two markers associated with FHB resistance from Sumai 3 in the Sumai 3/Stoa population were also associated with resistance in the ND2603/Butte 86 population (Table 1). This is one important step in verifying the effectiveness of these markers in other genetic backgrounds. In addition, another RFLP marker associated with resistance from ND2603 was serendipitously discovered in this population. Loci associated with FHB resistance from Stoa have not been mapped in ND2603/Butte 86 due to lack of polymorphism.

The most significant genomic region associated with FHB resistance in these populations is located on the short arm of chromosome 3B. Interval analysis revealed a peak LOD score of 6.3 for this region in the Sumai 3/Stoa population (Fig. 1). The leaf rust resistance gene *Lr27* also has been located to this region in a different population (Nelson et al., 1997).

These markers may have utility in indirectly selecting for FHB resistance, thereby accelerating the development of resistant varieties. Future research will focus on i) mapping additional markers on 3BS and obtaining PCRable markers for this region; and ii) verifying these and other new markers in the ND2603/Butte 86 population and others.

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Figure 1. Interval analysis of chromosome 3B for Fusarium head blight resistance in a Sumai 3/Stoa recombinant inbred population.

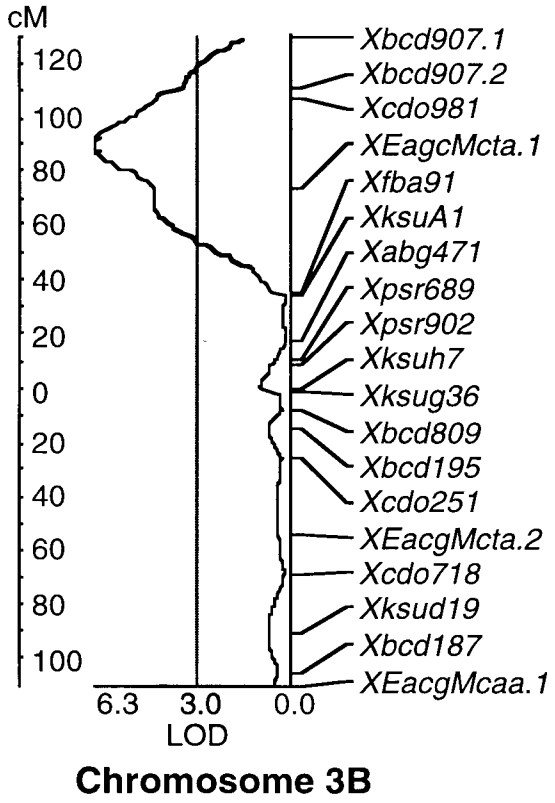

Table 1. Coefficients of determination and ***P*** values for DNA markers associated with ***Fusarium*** head blight resistance in the Sumai 3/Stoa and ND2603/Butte 86 recombinant inbred populations.

Marker	Chromosome	Resistant Source	Sumai 3/Stoa		ND2603/Butte 86	
			R^2 X 100	*P*	R^2 X 100	*P*
XEagcMcta.1	3BS	Sumai 3	17.6	<0.001	15.6	<0.001
XksuH16	2AL	Stoa	14.3	<0.001	–	–
XPaccMcga.1/ Xfbb82	6BL[1]	Sumai 3/ ND2603	9.0	0.004	6.3	0.004
Xwg909	4BL	Stoa	7.2	0.007	–	–
Xbcd941	3AL	ND2603	1.9	0.27	9.1	<0.001

[1] Both *XPaccMcga.1* and *Xfbb82* were mapped in the Sumai 3/Stoa population and are less than 10cM apart on chromosome 6BL, thus likely representing the same QTL. Only *Xfbb82* was mapped in the ND2603/Butte 86 population.

P

AFLP markers for QTL Controlling Scab Resistance in Wheat

[1]Gui-Hua Bai, [2]Frederic L. Kolb, [3]Gregory Shaner, and [2,4]Leslie L. Domier

INTRODUCTION

Wheat scab, also called wheat head blight, is caused mainly by *Fusarium graminearum* and is an important wheat disease worldwide. It significantly reduces wheat yield and grain quality. The mycotoxins produced by the fungus in infected grains are detrimental to humans and livestock. The use of resistant cultivars is the most effective way to control the disease (Bai and Shaner, 1994). Because scab resistance is a quantitative trait and evaluation of resistance is complicated and laborious, breeding for scab resistance with traditional methods requires substantial time and effort.

Molecular identification of quantitative trait loci (QTL) has been reported for many important traits in several crops. Once a QTL is tagged with molecular markers, it can be transferred selectively into different genetic backgrounds by marker-assisted selection. Restriction fragment length polymorphisms (RFLPs) have been used successfully to analyze single gene traits and QTLs (Sorrells and Wilson, 1997). Recently, the application of polymerase chain reaction (PCR)-based molecular markers, especially AFLPs, have provided powerful tools to detect a higher level of polymorphisms in plants (Thomas, et al., 1995). AFLPs have proven to be very important in the analysis of crop species such as wheat that have relatively low degrees of DNA polymorphisms.

OBJECTIVE

Our objective in this study was to identify AFLP markers associated with major QTL for scab resistance.

MATERIALS AND METHODS

Recombinant inbred lines (RILs) were derived by single seed descent from a cross made between Ning 7840 (resistant cultivars) and Clark (susceptible cultivar) in 1990 at Purdue University, Indiana, USA. F_7 RILs were evaluated for disease spread in spikes in the greenhouse at Purdue University from 1995 and F_{10} at the University of Illinois in 1998. Nine plants per family were evaluated by injecting 1000 conidiaspores (a mixture of *Fusarium graminearium* isolates from Purdue University) into a central floret of a spike at early anthesis with a hypodermic syringe. To initiate infection after inoculation, the plants were placed in a moist chamber for 3 days at 23°-25°C and 100% relative humidity. Spikelets showing symptoms were counted at 3, 9, 15 and 21 days after inoculation. Final disease severity was calculated as the percentage of scabbed spikelets per spike on the 21st day after inoculation (D21). Also, area under the disease progress curves (AUDPCs) was calculated according to Shaner and Finny (1974).

DNA was isolated from Ning 7840, Clark and the 133 F_9 RILs grown in a greenhouse at the University of Illinois using the CTAB procedure (Saghai-Maroof et al., 1984). For each sample, 500 ng genomic DNA was digested simultaneously with *Eco*RI and *Mse*I restriction enzymes. The genomic DNA fragments were ligated to *Eco*RI and *Mse*I adaptors to generate template DNAs for amplification. The *Eco*RI selective primers were labeled with $^{33}P\gamma$-ATP. PCR was performed in two consecutive reactions in a thermocycler (MJ Research, Inc.). Primers for preamplification and selective amplification of genomic DNAs were designed according to Thomas, et al (1995). Based on F_7 data, two DNA bulks were formed by pooling DNA from two groups of five plants with contrasting disease reactions. Primer combinations showing AFLP products present in one bulk, but absent from the other were used to evaluate all 133 RILs. Linkage analysis was performed with the Mapmaker program (V.2.0 for MacIntosh, Lander et al., 1987) with the Kosambi mapping function and using a maximum recombination fraction of 0.4 and a minimum LOD of 6. For QTL analysis, ANOVA and regression analysis for single markers and interval analysis were performed by using Microsoft Excel and the Qgene program (Nelson, 1997). To identify the putative chromosome location of QTL, primer combinations that produced markers closely linked to the QTL were used to amplify DNA of RILs from W7984/Opata

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M85 cross (ITMI mapping population). The ITMI map data were retrieved from GrainGenes at the following URL address: http://probe.nalusda.gov. If a marker linked to the QTL was mapped in both populations, the chromosome location of the marker in the ITMI map was tentatively considered as the chromosome location for the marker.

RESULTS AND DISCUSSIONS

Of the 208 AFLP primer combinations tested, about 85% amplified clearly separated band patterns from parental DNA. One primer combination can amplify 70-140 bands and 2 to 16 bands were polymorphic. Amplified fragments ranged from 50 to 900 base pairs, but most polymorphic bands ranged from 50 to 500 base pairs. About 10% of the primer combinations amplified at least one polymorphic band between bulked DNA samples. Primer combinations with three selective nucleotides amplified more bands than those with four selective nucleotides, but in the latter case, the amplified bands were separated better.

Twenty primer combinations were selected to screen 133 RILs based on bulk segregant analysis. After inoculation, significant differences were observed in AUDPC and D21 between the two allelic classes for 9 AFLP markers in two greenhouse evaluations. (Table 1.) These significant differences in scab severity between marker allelic classes indicate that these markers are closely associated with scab resistance genes.

Single marker regression analysis indicated that 9 markers have consistently high R^2 values in F_7 and F_{10} (Table 2). The 9 markers were grouped in one linkage group, which covered 25.9 cM (Figure 1). By interval analysis, we identified one major QTL for scab resistance with R^2 value from 0.52-0.56 and LOD values from 10.8 to 15.3 in two generations evaluated. One marker (GCTG.CGAC1) was mapped in the ITMI map and located in the long arm of chromosome 7B. Therefore, the major QTL in Ning 7840 can be tentatively mapped on chromosome 7B.

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Figure 1. AFLP linkage maps constructed from the cross Ning 7840/Clark (on the left) and from the cross W7984/Opata M85 (ITMI mapping population). The putative scab resistance QTL is shown as shaded bars. Left: the linkage map derived from Ning7840/Clark cross indicating QTL location (solid bar), AFLP markers (right), and genetic distance (cM) between markers (left). Right: the linkage map derived from ITMI mapping population indicating both RFLP and AFLP markers (right), and genetic distance (cM) between markers (left).

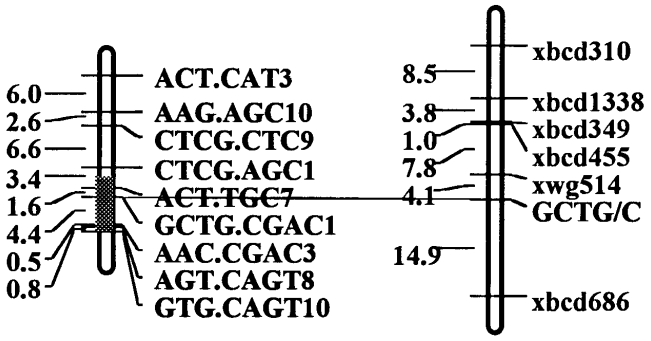

Table 1. Mean percentage of scabbed spikelets per spike on the 21st day after inoculation (D21) for RILs with Ning 7840 marker type (N), with Clark marker type (C), and differences between N and C (D). All differences (D) are significant at 0.001 probability level in the ANOVA analysis.

	F_7			F_{10}		
Marker[a]	N	C	D	N	C	D
GCTG/CGAC 1	0.42	0.85	0.43	0.38	0.82	0.44
ACT/TGC7	0.43	0.84	0.41	0.38	0.81	0.43
CTCG/AGC1	0.42	0.85	0.43	0.38	0. 79	0.41
AGT/CAGT8	0.44	0.82	0.38	0.39	0.79	0.40
GTG/CAGT10	0.44	0.82	0.38	0.38	0.79	0.41
CTCG/CTC9	0.46	0.83	0.37	0.42	0.79	0.37
AAC/CGAC3	0.44	0.81	0.37	0.39	0.78	0.39
ACT/CAT3	0.49	0.82	0.33	0.44	0.79	0.35
AAG/AGC10	0.43	0.81	0.38	0.42	0.75	0.33

[a] Marker names consist of abbreviations of selective nucleotides of *Eco*RI primer (left) and *Mse*I primers (right).

Table 2. R^2 values from simple regression analysis for 9 AFLP markers .

Marker[a]	D21(F_7)	AUDPC(F_7)	D21(F_{10})	AUDPC (F_{10})
GCTG/CGAC 1	0.53[b]	0.50	0.51	0.49
ACT/TGC7	0.50	0.47	0.48	0.48
GTG/CAGT10	0.45	0.40	0.43	0.42
AGT/CAGT8	0.43	0.37	0.42	0.42
AAC/CGAC3	0.40	0.36	0.40	0.39
CTCG/AGC1	0.52	0.47	0.39	0.38
CTCG/CTC9	0.39	0.39	0.35	0.37
ACT/CAT3	0.31	0.33	0.31	0.35
AAG/AGC10	0.40	0.41	0.28	0.30

[a] Marker names consist of abbreviations of selective nucleotides from *Eco*RI primer (left) and *Mse*I primers (right) separated by a slash.

[b] R^2 values based on percentage of scabbed spikelets rated at 21 days after inoculation and AUDPC from 2 generations.

Greenhouse Evaluation of Italian Wheat Accessions

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Objective

Greenhouse experiments were conducted to evaluate wheats from Italy for resistance to scab.

Introduction

There is some indication that the resistance to scab in certain Chinese wheat cultivars derives originally from Italian wheat cultivars. We therefore decided to screen wheat accessions in the National Small Grain Collection from Italy for reaction to scab.

Materials and Methods

We searched the GRIN database for wheats originally collected from Italy, both winter and spring habit. Forty-two accessions were evaluated for scab resistance. Seed of these accessions were sown in flats of soil in the spring of 1997. Once plants had emerged, they were vernalized for 65 days in a lighted cold room (3° C, 12 hr/day fluorescent lamp illumination) and then transplanted individually into 15-cm-diameter pots of soil. Plants were reared in a greenhouse that received supplemental illumination from high discharge lamps for 16 hr/day. When the main culm of each plant reached the beginning of anthesis (Feekes 10.5.1), the spike was inoculated by injecting a suspension of macroconidia into a central floret (point inoculation). The spore suspension contained 4 x 10^4 conidia/ml. After inoculation, plants were placed in a moist chamber for 3 successive nights. The chamber was open during the day to prevent buildup of heat, but closed during the night (1600 hours until 0900 hours the following day). Beginning 7 days after inoculation, and at 5-day intervals, the blighted spikelets on each inoculated spike were counted until 27 days after inoculation. Total spikelets were counted and used to calculate the percentage of blighted spikelets at each assessment date. For most lines, four plants were evaluated. A few plants of each line were not inoculated in order to assure a supply of seed for each line for future work.

Seed from individual plants in this preliminary test was saved and used for a second trial during fall of 1997. Vernalization and plant culture conditions were as described above. In addition to the point inoculation, half of the plants of each entry were inoculated by spraying the entire spike with an aqueous suspension of macroconidia (spray inoculation). The point inoculation method was intended to detect Type II resistance (resistance to spread of symptoms in the spike), whereas spray inoculation was intended to detect Type I resistance (resistance to initial infection). Five plants of each entry were inoculated by each method in this experiment. Disease assessment was as described above.

Results

In the initial test, we observed a considerable range in area under the disease progress curve (AUDPC) among lines. The more striking result was the considerable variation in amount of head blight among plants within accessions. For example, AUDPC values for the four tested plants of Funo (CItr 14349) were 0.68, 9.07, 12.77, 0.97. Wide variation was observed in several other accessions. We surmised that the original accessions were heterogeneous for reaction to scab, and therefore saved seed of tested individual plants for further evaluation.

Progeny of 19 accessions from the preliminary test were selected for further evaluation in the fall of 1997. Although the second test represented lines from plants that appeared to be resistant in the original test, there was still a wide range in AUDPC values, for both the point and spray inoculations (Table 1). The correlation between the AUDPC for the parent plant and the mean AUDPC for the 5 progeny plants subjected to spray inoculation was only 0.013. A similarly low correlation was noted when data for the point inoculation of progeny plants were analyzed. Nonetheless, several of the lines in the second test had low ratings for scab, and the effect of lines in the analysis of variance was highly significant. Mean AUDPC values for the point inoculation ranged from 0.88 to 15.66. The range of values for the spray inoculation was 0.31 to 20.01. Selections from each accession tended to have similar mean values for AUDPC, but there were a few exceptions. For example,

selection #3 of Funo had an AUDPC of 6.76 whereas selection #1 had an AUDPC of 16.08 for the spray inoculation. Mentana-1 had a value of 9.49 whereas Mentana-2 had a value of 0.88 for the AUDPC following point inoculation.

As we noted in the evaluation of the Uniform Fusarium Head Blight Winter Wheat Nursery, there was a poor correlation between AUDPC means for the spray and point inoculations, although it was significant (R=0.565, P<0.0001). Several lines had fairly low AUDPC values with point inoculation, but high values for spray inoculation, suggesting that they have Type II, but not Type I resistance. Only one line (Mentana-1) appeared to be the opposite (a high value for the point inoculation, but a low value for the spray inoculation), suggesting that it has Type I but not Type II resistance. Several lines had low values with both types of inoculation.

Whether these lines represent several different genes for resistance and whether there are genes represented that differ from those in Sumai 3 and Ning 7840 is not known. The range in intensity of scab among the lines suggests that several genes for resistance may be represented in this material. We have once again selected the more resistant plants and will grow and test their progeny in the greenhouse this fall. We also plan to make crosses between these lines and adapted lines with Chinese and other sources of resistance. Following the scab tests this fall, we will be able to distribute small quantities of seed to interested persons.

Table 1. Area under the disease progress curve for head blight development on Italian wheat selections following inoculation with *Fusarium graminearum* either by point inoculation or spraying the entire spike with inoculum.

Damino Chiesa	PI 132851	11.74	
Fubav D	CItr 15163	1.63	4.21
Funo-1	CItr 14349	2.66	16.08
Funo-2	CItr 14349	3.20	10.33
Funo-3	CItr 14349	5.33	6.76
Marhein 26-1	CItr 15164	4.34	6.75
Marhein 26-2		2.41	4.07
Marhein 26-3		2.44	5.33
Marimp 3	CItr 15165	5.81	7.99
Mentana-1	CItr 12448	9.49	3.35
Mentana-2		0.88	4.31
Oscar I-1	CItr 15125	7.55	16.34
Oscar I-2		9.72	17.76
Oscar I-3		5.06	17.07
Oscar III-1	CItr 15126	9.73	18.85
Oscar III-2		2.09	9.89
Oscar III-3		7.51	11.23
Oscar V-2	CItr 15128	1.61	1.76
Oscar V-3		3.43	3.54
Oscar V-4		4.49	0.31
Oscar V-6		3.92	16.66
Oscar V-1		5.54	10.34
Padre Gemelli-1	CItr 15268	3.93	9.25
Padre Gemelli-2		5.46	18.66
Paula VZ 434	CItr 15156	4.78	5.78
San Pastore-1	PI 157918	8.33	12.62
San Pastore-2		6.09	12.61
San Pastore-3		1.87	16.64
Sparta-1	CItr 15269	8.94	14.40
Sparta-2		5.90	13.59
Sparta-3		10.97	16.90
Sparta-4		10.20	16.49
Tilchifun 2 -1	CItr 15166	4.34	14.54
Tilchifun 2 -2		2.66	6.80
Tilchifun 2 -3		6.32	13.16
Victor II-1	CItr 15121	13.66	18.80
Victor II-2		15.66	15.04
Victor II-3		12.98	16.92
Victor II-4		13.19	17.15
Victor IV-1	CItr 15123	6.89	8.33
Victor V-1	CItr 15124	9.37	20.01
Victor V-2		5.29	8.45
Victor V-3		11.06	12.09
LSD (0.05)		2.87	3.00

General Issues in Wheat and Barley Transformation and Current Research at the ARS Barley Genetics Lab in Fargo

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Transformation is the direct insertion of new genes into plants. Using transformation techniques, we can insert genes into barley and wheat that can't be incorporated by traditional crossing, such as genes from other plant species, animals, bacteria, or fungi. These methods also let us change the expression patterns of genes that are naturally found in wheat and barley. For example, there may be genes for proteins that inhibit fusarium growth that are turned on only in leaves. With transformation, we can alter these genes so they are turned on in the spike tissues that fusarium attacks.

Transformation of wheat and barley has the potential to help in the fight against FHB, in reducing both disease and toxin levels. But, these techniques are relatively new to wheat and barley, and there still are several areas that need improvement. This report will discuss some of these areas and will briefly describe some of the current barley transformation work at the USDA, ARS barley genetics lab in Fargo.

Barley and wheat transformation techniques currently require the use of tissue culture systems, in which cells are grown as unorganized clumps called callus, on artificial media. These calli are then induced to form green plants by altering the hormone levels in the media and placing them in the light. Plant regeneration from these calli is one of the first limitations in the system. Current transformation programs use the cultivars 'Bobwhite' in wheat and 'Golden Promise' in barley because they regenerate many plants. Neither of these cultivars meet current agronomic and quality standards. Using these older cultivars means more work to breed the new genes into modern cultivars. The newer barley and wheat cultivars generally do not regenerate many plants using standard tissue culture systems. This may soon change, as altered culture systems and artificial media compositions are being developed to improve regeneration rates from newer cultivars and advanced breeding lines.

Another limitation in transformation is the efficiency. The usual targets for transformation are immature embryos. The embryos are induced to form calli from which the transformed cells are selected. In typical experiments, only four to eight percent of these embryo-derived calli regenerate transformed plants. This low efficiency means time and resources are wasted. Research projects investigating ways to improve transformation efficiency are underway in both wheat and barley.

A third area that causes problems in transformation is somaclonal variation, the mutations caused by tissue culture. Most regenerated plants have lower yields than the cultivar they are derived from, and many have other undesirable mutations affecting traits such as heading data, height, seed weight, etc. Again, research projects are underway to reduce somaclonal variation.

Additional areas of research being investigated include gene stability and gene expression. Sometimes, an inserted gene that is expressed in the original regenerated transgenic plant is not reliably transmitted to its progeny. In other cases, the inserted gene is transmitted to progeny but the gene is inactivated and does not make its protein product. Altering the DNA code that makes up the gene, or adding sequences to the gene to target where it inserts into the chromosomes, can help overcome these problems. Other issues important in transformation include the genes used and the promoters that control expression of the genes. Dr. Pat Okubara will be discussing these areas in more detail.

I'd like to finish by providing a short summary of some transformation research going on in my barley genetics lab in Fargo. We are currently working with two genes, TRIr and PDR5, which may help reduce DON levels in FHB-infected plants. We have inserted these genes into Golden Promise barley and are currently testing the regenerated plants and their progeny for the presence and expression of the new genes. We will then be selecting the best transgenic plants for FHB and DON testing in the greenhouse and field. These lines also will be crossed to advanced lines from the FHB resistance breeding programs, to get the transgenes into adapted, high quality barley lines. We also have been investigating plant regeneration from current cultivars and advanced breeding lines

using improved tissue culture systems and media. Several lines have been selected for use in future transformation efforts because they appear to have sufficient green plant regeneration from tissue cultures. Any of these lines would be an improvement over Golden Promise from a plant breeding perspective, and use of these lines for transformation will help speed the development of adapted, high quality, transgenic barley.

P

Evaluation of barley genotypes for repeatability of reaction to Fusarium head blight (FHB)

C.K. Evans(1), R. Dill-Macky(1), D.C. Rasmusson(2).

Fifteen barley genotypes were tested at St. Paul, Morris, and Crookston, Minnesota, for the repeatability of their reaction to *Fusarium graminearm* in 1997. Randomized complete split-block plots (3 replicates) were either inoculated (I) or non-inoculated (NI) with a macroconidial suspension. Cultivars Robust, Stander, and Karl provided FHB-susceptible reactions and Chevron provided an FHB-resistant reaction for comparisons. There were significant (P=0.05) differences of mean FHB severity among Morris (39%), Crookston (28%), and St. Paul (8%) locations. Over the three locations, there was a higher degree of concordance among genotype rank-correlations in I plots (0.83-0.87, P=0.01) compared to NI plots (0.53-0.76, P=0.05-0.01). This demonstrated that macroconidial inoculum was reliable for testing genotypes and cultivars of barley for resistance to FHB.

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P

COMPARISON OF INOCULATION METHODS FOR SCREENING TETRAPLOID WHEAT LINES FOR REACTION TO FUSARIUM HEAD BLIGHT

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NTRODUCTION AND OBJECTIVES

Fusarium head blight (FHB), ear blight, or scab, caused by *Fusarium graminearum* Schwabe., is endemic in the spring wheat growing area of the north central plains of North America. Since 1991, it is estimated that the cost of the disease to the cereal industry has exceeded US$2.6 billion [1]. In wheat, two types of resistance are generally recognised: Type I, resistance to initial infection, and Type II, resistance to spread within the head. The FHB-resistant Chinese hexaploid cultivars such as Sumai 3 have Type II resistance. Single floret injection (SFI) inoculation is thought to be a relatively reliable method for screening for this type of resistance [2].The durum wheat class is amongst the most susceptible to the disease. In Canada, the principal durum growing area of Saskatchewan so far has not been directly threatened by FHB, but there are concerns that global/environmental changes may be such that conditions may promote further spread of the disease. Attempts to cross resistance into durum lines from Chinese hexaploids have been unsuccessful, therefore the objectives of the study were to identify FHB resistant germplasm in a tetraploid background and to cross this resistance into an adapted durum wheat background. Spray inoculation was used for field screening and the results compared to reactions obtained from both spray and SFI inoculation methods under controlled conditions.

MATERIALS AND METHODS

Ninety-seven tetraploids, mostly *Triticum dicoccoides*, were screened for reaction to FHB using spray inoculation. Six *T. dicoccoides* accessions were rated moderately resistant, of which two, Td160 and Td161, were subsequently used in crosses with the susceptible durum cultivar AC Morse. A limited number of F_3 lines were planted in an artificially inoculated, irrigated nursery in 1996. The F_4-derived F_5 lines were subsequently screened under controlled conditions using both spray and SFI inoculation methods. The F_4-derived F_6 lines retested in 1997. All heads were inoculated at anthesis. In the field, initial inoculum was applied when 50% of the heads were in flower, and a second application was made 4 days later. For all experiments, a concentration of 50,000 *F. graminearum* conidia/mL was used. In field tests, 50 mL of inoculum was sprayed per 1.5 m row, and sprinkler irrigation was applied in the evening and the following morning of each inoculation. Under controlled conditions, 10 mL were injected into single florets or 3 mL was sprayed onto individual heads, and plants were kept at 100% humidity for 24 hours. Infected spikelets were counted after 18-21 days. For field tests, an FHB severity index was calculated from percent heads infected X percent infected spikelets / 100.

RESULTS AND DISCUSSION

In both years of field testing, a higher percentage of lines from the cross with Td161 were resistant (FHB index < 30), while more susceptible lines were observed in Td160 progeny (FHB index > 50) (Fig. 1). Under controlled conditions the average number of infected spikelets from progeny of Td161 crosses was lower than in progeny of Td160 crosses, i.e. 38% and 52% respectively. Following spray inoculation, average number of infected spikelets was higher, 70% and 61% in Td161 and Td160, respectively. SFI inoculation under controlled conditions was highly correlated ($P < .002$) with the FHB index obtained from field screening for progeny from the more resistant Td161 cross (Table 1). Under controlled conditions, ratings for SFI and spray inoculation also were correlated ($P<0.05$) for this cross. Field reactions for the cross with the Td160 parent for each year were correlated with the two year average (Table 1). Spray inoculation in field nurseries was correlated to SFI (Type II) resistance in controlled conditions and can be used for large-scale screening required by breeding programs.

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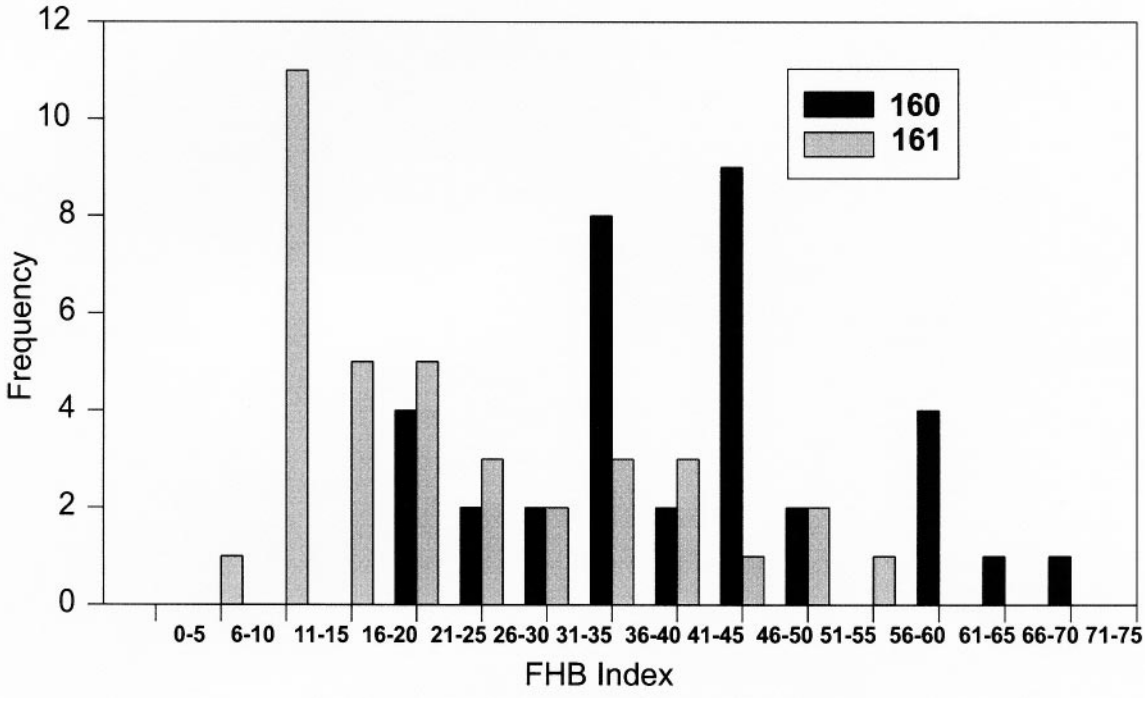
Table 1. Correlations between fusarium head blight field ratings and ratings for two inoculation methods (single floret injection, SFI, and spray) under controlled conditions, for progeny from crosses between durum wheat cultivar AC Morse and two *Triticum dicoccoides* lines.

Table 1. Correlations between fusarium head blight field ratings and ratings for two inoculation methods (single floret injection, SFI, and spray) under controlled conditions, for progeny from crosses between durum wheat cultivar AC Morse and two *Triticum dicoccoides* lines.

Cross	Field 1996	Field 1997	Field Average	Spray
AC MorseXTd160				
Field Average	0.0001	0.0001		NS
AC MorseXTd161				
Field Average	0.0001	0.0001		
SFI	NS	0.01	0.02	0.0421

Figure1. Fusarium Head Blight Index of lines from two crosses between durum x *Triticum dicoccoides*.



P

Assessment and selection for scab resistance in soft red winter wheat

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OBJECTIVES

1) to discern resistance levels in soft red winter wheat (SRW) compared to those of other reported resistance sources; 2) to determine the feasibility of identifying scab-resistant progeny from crosses between scab-resistant spring wheat and adapted soft winter wheat.

INTRODUCTION

Fusarium head blight causes great losses in wheat grain yield and quality worldwide. Development of adapted cultivars and germplasm with diverse resistance genes is currently the most feasible and economical means for controlling scab (Griffey et al., 1997). High levels of resistance have not been identified in soft red winter wheat and, therefore, the crop is at great risk as evident by significant losses observed in recent years. Most of the currently-used resistance resources are spring type, and many of them are derivatives of 'Sumai 3' (Liu and Wang, 1990) , which suggests a lack of genetic diversity for this trait. From a breeding perspective, it would be desirable to identify adapted SRW wheats possessing some resistance or tolerance to scab, which could be used as recipient parents in transferring resistance from unadapted spring types such as Sumai 3. A precise method for evaluation and classification of resistance type is needed to facilitate the transfer and combination of diverse types of resistance.

MATERIALS AND METHODS

A total of 182 wheat lines were evaluated in greenhouse tests, of which 78 F_4 lines were field-selected from 20 F_3 populations screened for scab resistance in 1997, 31 commercial cultivars or lines were from the Virginia State Test and a mid-Atlantic Joint Test, 40 scab resistant resources were from China, Italy, Brazil, Canada and France, and 33 advanced resistant lines were from the 1998 Uniform FHB Nursery. Three individuals of each line were tested by the floret inoculation method. Infection type for invasion resistance (Type II) was assessed once a week after inoculation (J.Chen, 1989). All materials evaluated in greenhouse tests were subsequently evaluated in field tests.

Twenty commercial cultivars or lines, included in yield loss tests, were grown in replicated 100 ft^2 (9.29 m^2) plots using a randomized complete field-plot design. Thirty-six advanced resistant lines from the Uniform FHB Nursery were planted in 20ft^2 (1.86 m^2) plots with three replicates. An additional 380 lines, of which 244 were F_4 families, were planted in similar-size plots without replicates. In a head-row selection-nursery, 1698 F_4 lines were planted in single 4ft (1.2 m) rows. Conidial suspensions (1L/100ft^2 at 50,000 spores/ml) were sprayed onto plots at heading and flowering stages. After inoculation, all field plots received overhead irrigation as a fine mist of water from 8-9 a.m. and again at 6-8 p.m. Scab incidence and severity were assessed once a week after inoculation. Grain yield, test weight, 1000 kernel weight and percentage of infected scabby seeds were determined after harvest. All data analyses were performed as for a Randomized Complete Block Design using Agrobase software.

RESULTS AND DISCUSSION

Differences between genotypes for type of resistance based on spike invasion (Type II), kernel infection (Type IV) and yield losses (Type V) were significant. Chinese resources mainly possess type II resistance, while some American cultivars have type IV and V resistance. Seven Chinese resources with type II resistance have been confirmed to be the best ones through 1997 and 1998 tests, seven other resources with type II resistance were verified in this year's test (Table 1). 'Roane' a Virginia variety was confirmed to possess type IV and V resistance through 1997 and 1998 tests(Table 2). Soft red winter wheat genotypes Ernie from Missouri, Freedom from Ohio and P92823A1-1-4-4-5 from Indiana also exhibited similar resistance in 1998 tests. In addition, 13 soft red winter wheats from other states have been found to possess type IV and V resistance. Some F_4 lines with either type II or another type of resistance were advanced for further testing.

Correlation analysis data suggest that scab severity and percentage of scabby seed may be more predictive than scab incidence for evaluating type IV and type V resis-

tance. The correlation value for grain yield with scab severity and percentage of scabby seeds was much higher than that of grain yield with scab incidence (Table 3). Correlation values between yield loss and mean treatment differences for scab severity and scabby seeds for non-inoculated versus inoculated plots also were significantly higher than that with scab incidence. This data suggests that increases in scab severity and percentage of scabby seeds are the main factors contributing to reduction of grain yield and test weight. The level of scab incidence may represent type I resistance, and may be affected by plant height, growth stage and climatic conditions under natural infection but not with artificial inoculation.

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Table1. Evaluation of type II scab resistance in greenhouse tests in 1998.

Genotype	Source	Infection Type(1-5)
Shaan85-2	China	2-3(R)
W14	China	2-3.4(R)
Futai 8944	China	3-3.4(R)
Futai 9002	China	2-3(R)
Ning 9016	China	3.4-3.8(MR-MS)
Sumai 3	China	3.4-3.8(MR-MS)
Ning 7840	China	3.4-3.8(MR-MS)
H821	Canada	2-3.2(R)
HC374	Canada	2-3(R)
H181	Canada	3-3.2(R)
H185	Canada	3-3.2(R)
H192	Canada	2-3.4(R)
VR95B717	France	2-3.2(R)
VR95B295	France	3-3.6(R-MR)
IL95-1549	Illinois	2-3.4(R)
Roane	Virginia	3-4(R-S)
ERNIE	Missorri	3.2-4(R-S)
FREEDOM	Ohio	3.4-4(MR-S)
P92823A1-1-4-4-5	Indiana	4-5(S)

Table2. Summary of scab resistance of 20 soft red winter wheat genotypes at Blackburg, VA in 1998.

FREEDOM	**4**	**21**	18	**18**	83	70
ERNIE	**6**	**18**	**8**	**19**	**42**	63
ROANE	**15**	**23**	18	**20**	79	74
P92823A1-1-4-4-5	**16**	**21**	**15**	**14**	67	**82**
AGRIPRO FOSTER	21	32	27	**19**	83	78
POCAHONTAS	21	42	38	29	93	**80**
PION 2552	21	30	25	23	84	77
VA96-54-234	22	27	22	25	83	78
COKER 9803	22	32	20	**19**	65	60
WAKEFIELD	23	32	23	23	71	74
MADISON	23	45	34	31	73	69
AGRIPRO MASON	25	37	27	26	**71**	**85**
PION 2580	26	29	20	37	69	77
PION2643	27	42	36	25	86	75
PION2684	28	42	36	32	87	71
JACKSON	29	32	25	39	77	68
VA96-54-216	**40**	35	31	25	89	72
FFR555W	**41**	**46**	**41**	27	89	78
COKER 9835	**47**	**52**	**50**	**42**	**95**	60
GA GORE	**48**	**85**	**83**	**47**	**97**	60
LSD(0.05)	8.2	10.3	12.5	7.5	15.5	7.6
AVERAGE	25.4	36.2	29.9	27	79.2	72.6

Table3. Linear correlation analysis between parameters for scab assessment in inoculated plots over 20 wheat genotypes.

	Scab Severity	Scab Index	Scabby Seeds	Scab Incidence	Test Weight
Grain Yield	-0.7082 0.0000	-0.7010 0.0000	-0.6636 0.0000	-0.4316 0.0007	0.7332 0.0000
Test Weight	-0.7310 0.0000	-0.7286 0.0000	-0.7402 0.0000	-0.4032 0.0017	
Scab Incidence	0.5640 0.0000	0.7046 0.0000	0.4043 0.0016		
Scabby Seeds	0.6702 0.0000	0.6571 0.0000			
Scab Index	0.9738 0.0000				