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

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ABSTRACT

Many Chinese wheat lines have been introduced into wheat breeding programs because of their resistance to Fusarium head blight (FHB). It is anticipated that these lines will provide diversity for resistance genes. Sumai 3, a popular resistance source from China, has been well characterized for scab resistance QTL. Fujian 5114, a Chinese wheat derived from the cross Longxi18/Ning8017, has resistance to FHB spread (Type II) as good as Sumai 3, and appears to differ from Sumai 3 in some resistance loci. The purpose of this study is to map the resistance QTL in Fujian 5114. A population of 78 F_7 derived recombinant inbred lines (RIL) from the cross Fujian5114/Norm was evaluated for FHB severity in mist-irrigated, inoculated field trials in the summer of 2000 and 2001. The population was also evaluated for spread within the spikelet from point inoculations in two greenhouse trials in 2001. Results from field and greenhouse trials are correlated and allow separation of lines for severity. Marker analysis will consist of simple sequence repeat (SSR) amplifications in regions containing QTL reported in Anderson et al., 2001. Preliminary analysis based on greenhouse and field FHB data shows that Fujian 5114 contains the QTL on 3BS and it explains up to 25% of the variation in this population. We hope to explain 60% or more of the variation in FHB using these or other markers.

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GERMPLASM CONTRIBUTION OF THE CIMMYT WHEAT PROGRAM TO THE U.S. WHEAT AND BARLEY SCAB INITIATIVE

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OBJECTIVES

Provide a genetically diverse spectrum of bread wheat and barley germplasm resistant to Fusarium head blight (FHB), for use in US breeding programs and for basic research.

INTRODUCTION

The agreement between the CIMMYT Bread Wheat Program and the USA Scab Wheat and Barley Initiative is nearing the end of its third year. The first seed shipment consisted of 27 advanced bread wheat lines, 20 synthetic hexaploid derivatives and 10 advanced barley lines with resistance to fusarium head blight types I and II. These resistance sources were shipped to Missouri and North Dakota respectively (McKendry, 2000). In addition to the above, selected germplasm originating from Romania (7) and China (15) was confirmed to be resistant under a severe scab epidemic in Toluca (central Mexican highlands) and also sent. These preliminary results confirmed the resistance in the Romanian germplasm as noted in the USA (Ittu, 2000, personal communications). A second shipment of new and outstanding germplasm will be sent as soon as we receive the pending APHIS permit. Data on the response of these lines to FHB and other diseases are presented in below.

MATERIAL AND METHODS

Advanced bread wheat lines were evaluated under natural conditions in Sierra de Jalisco, Guadalajara and Patzcuaro, and under of artificial inoculation in Atizapan, Toluca. Advanced barley lines were also evaluated in the last two locations. Evaluations were carried out during 1998-2000.

Stripe rust (*Puccinia striiformis*) is endemic in this location but we still conduct artificial inoculation with selected, virulent races, as determined by our rust pathologists. FHB due to *Fusarium graminearum* can also appear naturally but we also inoculate artificially to ensure good screening conditions.

The FHB inoculum is increased on mungo bean medium and its concentration adjusted to 50,000 spores/ml after growing for five days. Twenty wheat spikes per plot were inoculated at flowering, applying a liquid spore solution for type I resistance. The cotton method was used to determine type II resistance (Gilchrist et al. 1997). We counted infected spikelets per spike 25 days after inoculation for type I resistance and 35 days after inoculation for type II resistance, thus obtaining a percentage of resistant spikelets.

nvironmental conditions were optimal the past cycle for an excellent epidemic of FHB, and only rarely was additional irrigation required.

RESULTS AND DISCUSSION

After three years of evaluation 32 outstanding advanced bread wheat lines and 12 barley lines were selected for inclusion in the second shipment. The advanced lines were selected based on their resistance over the time and the consistency of low disease responses representative group of such lines is presented in Tables 1 and 2.

The shipment will also include new lines from Argentina (107), Brazil (19), and Japan (12) that showed high levels of resistance to FHB types I and II. Brazilian germplasm also expressed good agronomic type and resistance to leaf rust. However, only 50% of the latter group carry resistance to the local stripe rust races. The Japanese germplasm was relatively poorly adapted to the Mexican conditions and was highly susceptible to stripe rust. Some of the latter lines were also susceptible to leaf or stem rust. The Argentinean collection included lines of both good and poor agronomic types, but in general expressed good resistance to stripe, leaf, and stem rust.

Table 1. Advanced bread wheat lines with Fusarium head blight resistance types I and II evaluated in Atizapan, Mexico, during 1998-2000.

Crosses	Selection history	% type I	% type II	TGW loss %	Stripe rust	Grain (1 5)
PSN/BOW/4/MAYA/NAC/3/RPB14.6 8/PVN//PHO/5/MUNIA	CMBW91M03563T-0TOPY-13M-010Y-015M-010Y-8Y-0M-3SJ-0Y-0FGR	6.94	12.75	5.41	5 MR	2
SABUF	CM95073-3Y-0M-0Y-3M-0RES-4PZ-0Y-10PZ-0Y-9PZ-0Y-0FGR	7.47	8.15	4.58	0	1*
IAS64/ALDAN//URES/3/TNMU/4/TN MU	CMBW90M4487-0TOPY-14M-16AL-0AL-07Y-4M-0Y-3PZ-0Y-0FGR	2.92	18.18	12.81	0	2
SHA3/SERI//PSN/BOW	CMBW90M2470-7M-010M-010Y-015M-9Y-0M-2PZ-0Y-0FGR	5.05	5.05	4.34	0	1
PF83144/3/F3.71/TRM//VORONA	CO8687-7P-2P-0P-010Y-0M-0FGR	10.83	14.97	19.28	20MR	2
RL6043/6*NAC//TNMU/3/BAU	CMSS92Y02211T-27Y-015M-010Y-010Y-10M-0Y-1SJ-0Y-0FGR	11.14	7.18	16.87	30MR	2
BAU/MILAN	CM103873-2M-030Y-020Y-010M-2Y-0M-2PZ-0Y-8SJ-0Y-0FGR	2.3	18.26	16.19	0	1
SHA7/KAUZ	CM95113-8Y-0M-3FC-0FC-0FC-4FUS-0Y-6SJ-0Y-0FGR	3.98	8.33	3.49	0	2
KAUZ//PRL/VEE#6	CM94747-27Y-0H-0SY-4M-0RES-0SY-0ECU-010Y-0M-0FGR	8.7	11.2	19.78	5MR	3
ALD/PVN//YMI #6	CM91065-2M-0Y-0M-2Y-0B-12PZ-0Y-0FGR	4.21	10.38	3.14	0	1*
TINAMOU	CM81812-12Y-06PZ-3Y-1M-0Y-7AL-0Y-4AL-0AL-0M-0ECU-010Y-0M-0FGR	4.52	10.77	2.46	0	1
ALUCAN/DUCULA	CMBW89M3764-36M-0AL-2AL-2B-0Y-3SJ-0Y-0FGR	9.12	17.48	9.52	60MR-MS	2
CLC89/MILAN	CMSS92Y00573S-030Y-015M-0Y-0Y-18M-0Y-0FGR	3.23	13.24	4.99	0	1
JUP73R/PVN	CMBW91M04467S-5Y-2M-2Y-10M-0Y-0FGR	8.9	18.79	6.55	0	2
LIRA//AU/UP301/3/2*KAUZ	CMBW91Y02983M-030TOPM-19Y-010M-010Y-015M-7Y-0M-3SJ-0Y-0FGR	3.87	8.05	8.3	0	1
XIANG82.2661/2*KAUZ	CMBW91Y02917M-030TOPM-24Y-010M-010Y-015M-2Y-0M-6SJ-0Y-0FGR	3.01	12.43	12.45	0	1
TUI/MILAN	CMSS92Y00540S-030Y-015M-0Y-0Y-2M-0Y-1PZ-0Y-0FGR	8.75	19.41	11.72	0	2
WUH1/VEE#5//CBRD	CMSS92M01863S-015M-0Y-050M-0Y-13M-0Y-1SJ-0Y-0FGR	1.1	8.13	1.55		1*
OCEP14/BAU	CMBW90M1656-48M-1AL-0AL-07Y-3M-0Y-1PZ-0Y-0FGR	5.18	16.34	3.36	0	1*
BAU/DUCULA//BAU	CMBW91M03579T-0TOPY-23M-010Y-015M-010Y-7Y-0M-2PZ-0Y-0FGR	13.13	15.96	27.59	60MR-MS	3
WUH1/VEE#5//CBRD	CMSS92M01863S-015M-0Y-050M-0Y-18M-0Y-0FGR	6.87	8.05	4.01	0	1
GUAM92//PSN/BOW	CMSS92M01860S-015M-0Y-050M-0Y-11M-0Y-0FGR	4.9	13.16	6.62	0	1
R37/GHL121//KL/BB/3/JUP/MUS/4/2 *YMI#6/5/CBRD	CMBW91Y01575S-4Y-010M-010Y-015M-9Y-0M-0FGR	1.49	10.53	7.68	0	1
DESC/3/ALD/PVN//YMI #6	CMBW90M2417-9M-010M-010Y-015M-8Y-0M-0FGR	6.29	12.93	6.53	0	1*
CBRD/KAUZ	CMBW90M2494-8M-010M-010Y-015M-10Y-0M-0FGR	3.21	6.43	2.36	0	1*
WUH1/VEE#5//MUNIA	CMSS92M01862S-015 M-0Y-050M-0Y-15M-0Y-0FGR	3.19	5.08	6.61	0	1
WUH1/VEE#5//CBRD	CMSS92M01863S-015M-0Y-050M-0Y-13M-0Y-0FGR	0	12.15	9.08	0	1
BCN//DOY1/Ae SQUAROSA	Check S-S	24.07	32.93		0	5
FLYCATCHER (MS)	Check S-MS	29.8	21.04		0	4
SUMAI #3 (MR)	Check R-MR	1.84	9.2		10MS	2-Jan

Table 2. Advanced barley lines with resistance to Fusarium head blight types I and II evaluated in Atizapan, Mexico, during 1998-2000.

Crosses	Selection history	% type I	% type II	% TGW	Grain (1-5)
AZAF/KYOTO NAKATE//ALELI	CBSS96WM00287T-D-3M-4Y-2M-0Y	6.3	29.03	7.08	2
GOB/HUMAI10//ALELI/3/AZAF	CBSS95M00623T-C-1M-3Y-17M-1Y-2M-0Y	7.91	27.23	5.68	1
GOB/HUMAI10//ALELI/3/AZAF	CBSS95M00623T-C-1M-3Y-18M-1Y-1M-0Y	5.32	6.13	3.36	1*
AZAF/KYOTO NAKATE//ALELI	CBSS96WM00287T-D-3M-3Y-2M-0Y	9.07	32.32	6.25	2
GOB/HUMAI10//ALELI/3/AZAF	CBSS95M00623T-C-3M-3Y-3M-3Y-1M-0Y	4.83	34.93	1.8	2
GOB/HUMAI10//ALELI/3/AZAF	CBSS95M00623T-C-1M-3Y-20M-1Y-1M-0Y	9.69	16.1	2.15	1*
AZAF/KYOTO NAKATE//ALELI	CBSS96WM00287T-D-2M-1Y-1M-0Y	8.91	34.02	9.01	1
FRANKLIN-BAR//GOB/HUMAI10/3/AZAF	CBSS96M00672T-C-2M-1Y-1M-0Y	4.91	36.9	4.88	1
NE175-B/GOB96DH//AZAF	CBSS96M00675T-H-2M-1Y-1M-0Y	7.74	8.83	3.55	2
ALELI/KANTO NIJO 2//MSEL	CBSS96WM00438T-F-7M-1Y-1M-	7.2	10.36	6.27	2
ATACO/ACHIRA//HIGO/3/KANTO NIJO 2/4/SHYRI	CBSS96WM00270T-D-1M-3Y-1M-0Y	6.68	5.54	2.44	3
GOB96DH/3/ND10277/SHYRI//ND11231/SHYRI/4/AZAF	CBSS96M00681T-Y-2M-1Y-2M-0Y	2.74	6.87	3.8	2
ATAHUALPA	CHECK (MR-MR)	10.2	4.26		2
AZAFRAN	CHECK (MR-R)	8.52	6.44		2

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IDENTIFICATION OF QTL ASSOCIATED WITH RESISTANCE TO FHB IN NING 7840 AND FREEDOM

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ABSTRACT

Our objectives were 1) to map QTL for FHB resistance from Ning 7840 and Freedom and 2) to determine molecular basis for transgressive segregation. Two populations of $F_{2:4}$ families were developed from the crosses Ning 7840/OH542 and Ning 7840/Freedom. OH542 is a highly susceptible line while Freedom and Ning 7840 are resistant. FHB severity was rated in two years of greenhouse tests using hypodermic syringe inoculation technique and one year of field tests using corn kernel inoculum and mist irrigation. Families were genotypes with SSR markers and QTL analysis was performed using single point marker analysis.

A QTL for FHB severity was identified at 3BS in both populations with resistance conferred by the Ning 7840 allele in both cases. The Ning allele at 3BS appeared to be recessive to the susceptibility allele from either Freedom or OH542. This was observed in both greenhouse and field trials. The 3BS QTL accounted for 13 to 15% of the phenotypic variation. A QTL at 3AL was also detected in both populations, with resistance being conferred by Ning 7840. Resistance at this locus appeared to be additive and it accounted for 7 to 12% of the variation.

A QTL was identified on 2AS locus in the Ning 7840/Freedom population. This QTL explained 20% of the phenotypic variation for severity (field). Resistance was conferred by the Freedom allele and appeared to be additive. This QTL was also detected using greenhouse data. The R^2 value for this QTL was greater than what we found for the 3BS QTL in either population. This suggests that the 2AS region from Freedom is of equal or greater importance than the 3BS region from Ning 7840 in explaining resistance. This conclusion needs to be confirmed.

The importance of the 2AS QTL allele from Freedom is supported by genetic analysis of resistant transgessive segregants for severity from the Ning 7840/Freedom population. Nearly 80% of these resistant (field or greenhouse) lines were homozygous for Freedom alleles at 2AS. Only 50% of the resistant lines based on field data were homozygous for Ning 7840 alleles at, while 80% of such lines based on greenhouse data were homozygous for Ning7840 alleles at 3BS.

A major QTL for resistance from Ning 7840 was identified at 3BS. The QTL was not population specific. A major QTL for resistance from Freedom was identified at 2AS. The magnitude of the QTL at 2AS from Freedom appeared to have equal or greater effect on resistance than 3BS from Ning 7840. A combination of the Ning 7840 allele from 3BS and the Freedom allele at 2AS seemed important in obtaining resistant transgressive segregants.

(A PDF file of this poster will be available from the Ohio State University wheat breeding program web site.)

MOLECULAR AND PEDIGREE ANALYSIS OF SOURCES OF RESISTANCE TO FHB IN WHEAT

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ABSTRACT

There are multiple sources of FHB resistance, with 30+ sources being used in various prebreeding and variety development breeding programs. Many breeders plan to pyramid different FHB resistance genes to maximize resistance. This will require that we know the allelic nature of resistance in the many sources. It is difficult to ascertain this information when there are so many sources and phenotyping is cumbersome. Our objective was to determine if markers can be used to separate sources of resistance that are likely to have unique FHB resistance genes. Twenty-three lines were selected based on pedigree relationship and resistance to FHB, including Ning 7840, Sumai 3, Freedom, Mentana, Frontana, and Bezostaja. Each was genotyped with SSR markers from regions known to be associated with FHB resistance as well as other regions. Principal component analysis was performed to produce biplots of the data for each region of the genome.

Markers from the 3BS region clearly separated Ning 7840 and Sumai 3 from all other lines. This separation agrees with mapping reports that Sumai 3, Freedom, and Frontana all appear to have different FHB genes at the 3BS region. The unique nature of these sources of resistance is supported by pedigree analysis. Markers on chromosome 2AS, which has been associated with resistance from Freedom, also separate the resistant sources. Mapping studies also show that Freedom and Ning 7840 have different FHB genes in this region. The 2AS analysis suggests that Freedom and Bezostaja may possess similar FHB genes at 2AS, a notion supported by pedigree analysis. Thus, the marker analyses of 3BS and 2AS conform to what we know from other studies on allelism of FHB resistance genes. Other analysis suggests that Bezostaja may possess different alleles at 5A than other sources of resistance and that Ning 7840 and Sumai 3 are quite distinct from other sources of resistance at 7A. Only markers at 7B differentiated Ning 7840 and Sumai 3, suggesting that they could have different FHB gene at 7B. This may also be inferred from the literature where 7B has been found to be significantly associated with resistance to FHB from Ning 7840, but not Sumai 3.

The analysis of marker diversity for at key chromosome segments grouped sources of resistance. In some cases the differences between groups corresponded to known genetic differences for FHB resistance between some members of different groups. This indicates that SSR genotyping may be useful as a prescreening tool for new sources of FHB resistance. This presecreeing could be used to reduce the number of new lines that would be tested for uniqueness relative to our current sources of resistance.

(A PDF file of this poster will be available from the Ohio State University wheat breeding program web site.)

DEVELOPMENT OF SYNTHETIC HEXAPLOIDS WITH FUSARIUM HEAD BLIGHT RESISTANCE FROM *TRITICUM TURGIDUM* L. VAR. *DICOCCOIDES*

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ABSTRACT

Fusarium head blight (FHB) continues to significantly impact grain production of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) throughout the world. Incorporating host plant resistance from secondary gene pool species such as *Triticum turgidum* L. var. *dicoccoides* (2n=4x=28, AABB) may result in the transfer of unique sources of FHB Type II resistance to cultivars and consequently provide genetic diversity for resistance. Molecular mapping of FHB Type II resistance in a recombinant inbred chromosome line (RICL) population derived from a Langdon-*dicoccoides* chromosome 3A disomic substitution line [LDN(Dic-3A)], identified a quantitative trait locus (QTL) on *T. dicoccoides* chromosome 3A. The QTL explained approximately 55% of the genotypic variance for resistance. Incorporation and expression of the (Dic-3A) QTL in synthetic hexaploids and utilization of the tightly linked microsatellite marker *Xgwm2* for future marker-assisted selection may expedite the integration of this new source of resistance into hexaploid cultivars. Crosses were made between seven individuals of the LDN(Dic-3A) RICL and eight accessions of *Triticum tauschii* (2n=2x=14, DD). Hybrids (n=3x=21, ABD) were subsequently embryo rescued and colchicine treated for chromosome doubling. Accessions of *T. tauschii* were selected for hybridization based on previous studies demonstrating their resistance to other wheat pathogens. Currently, we have 45 synthetic hexaploids (2n=6x=42, AABBDD) for screening of Type II resistance in 2002 spring greenhouse and field experiments.

PROGRESS IN BREEDING FOR SCAB RESISTANCE IN ROMANIA ON WHEAT

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OBJECTIVES

In this report are reviewed the main results obtained in Romania on the breeding of resistance to scab (FHB) in winter bread wheat. The main topics of researches performed in two centers located in the South (Fundulea) and the North (Turda) of the country, have been:

- development of reliable screening methods under artificial field inoculation;
- identification of new FHB resistant sources;
- recombination of resistance genes from various sources;
- combining FHB resistance with yield capacity, quality and adaptation by stepwise hybridization, recurrent selection or the DH approach.

As results of these breeding approaches, a reliable screening protocol for resistance to FHB in winter bread wheat and improved resistant lines, not related to the sources previously described in spring wheats of Asian and Brazilian origin, were obtained. Some of the most advanced lines, particularly *Fundulea 201 R* and *Turda 195* have shown in several environments high levels of resistance Type I, Type II and reduced DON content as well. Following successive years of selection at Fundulea, promising derivatives of crosses between the scab resistant parent F 201 R and donors for good bread making quality (Dropia and Delabrad), combining reasonable levels for the both traits, were identified. Selection of new sources of resistance to scab in bread winter wheat derived from complex crosses is in progress.

INTRODUCTION

Fusarium head blight (FHB, scab) has emerged in the past decades as a very destructive wheat disease with a worldwide distribution. In addition to the significant reduction of grain yield and quality, accumulation of toxic compounds (DON, NIV etc) in infected grains and in end use products, is detrimental for health of humans and animals upon consumption. Breeding wheat cultivars that combine high levels of resistance to FHB with other agronomic traits, remains the most reliable and friendly to environment strategy for scab control. Current resistance to scab reduces to just a few sources, mostly spring types of Asian and Brazilian origin, containing each only a few genes (1,2, 10,11). In Romania natural occurrence of *Fusarium* scab (FHB) in wheat is not very common. However the damaging potential of this disease is considerable when humidity is high at anthesis. In the past decades breeding researches for resistance to FHB are conducted under artificial field inoculation in two centers located in the South (Fundulea) and the North (Turda) of the country (4). As results of breeding approaches to develop new winter bread wheat cultivars with improved resistance to FHB, advanced lines, not related to the sources previously described in spring

wheats of Asian and Brazilian origin, were identified (7,8). The objective of this report is to review the main results obtained in Romania according to the following topics: screening for resistance; identification of new FHB resistant sources and recombination of resistance genes from various sources; analysis of resistance; combining FHB resistance with other important agronomic traits.

RESULTS

Screening for resistance to FHB. Our breeding strategy for resistance to FHB is based on combining this character with other desirable agronomic traits: complex resistance to pathogens, yield capacity, quality and adaptation. In this respect, we start the field evaluation for resistance to FHB in F5 at Fundulea and Turda. Following single head artificial inoculation, two assessment criteria are frequently utilized to characterize the FHB resistance Type II (resistance to fungal invasion within plant tissues): the area under disease progress curve (AUDPC, % of bleached spikelets at 10 and 20 days postinoculation) and Relative Weight of Inoculated Spikes (RWIS, % of control). RWIS, % of control provides also reliable information on tolerance to FHB being constantly found to be correlated with other yield components. Selected entries with higher levels of resistance to FHB are artificially inoculated, in parallel, in advanced yield trials and local FHB nursery, as single head rows. After trials performed for two years in each center, the best entries from each location are interchanged, in order to be evaluated also in the other center (Fig. 1).

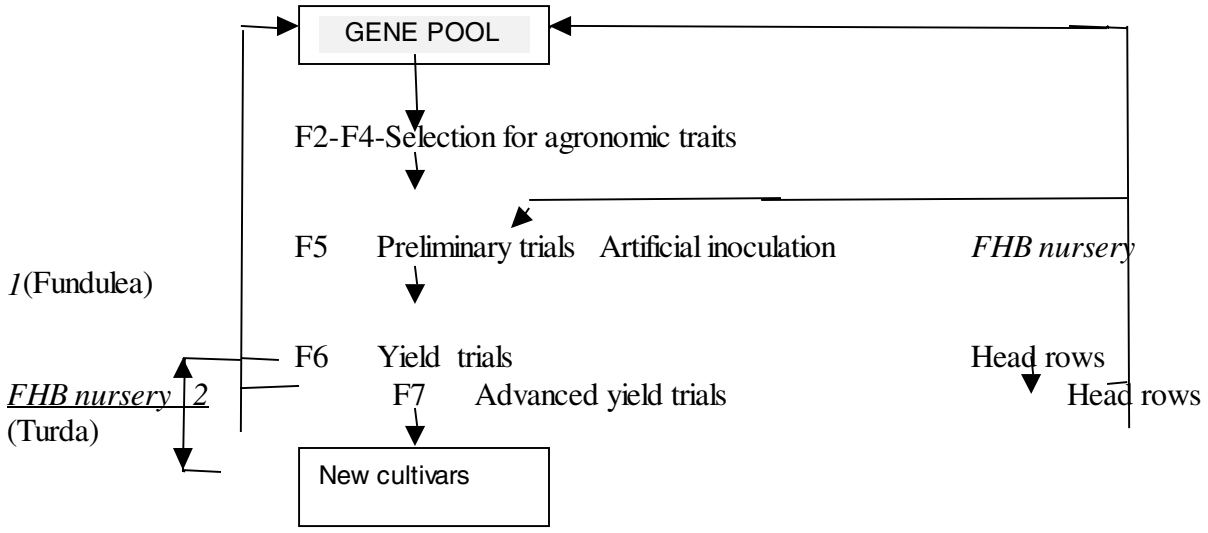

Fig. 1 Screening protocol for resistance to FHB in wheat

Evaluation of aggressiveness in local Fusarium isolates.
The current evaluation of aggressiveness among local *Fusarium* isolates became quite necessary in order to determine the pathogenic potential and to select the most suited isolates for assessment of resistance under artificial inoculation. Evaluation of aggressiveness in a collection of 57 Romanian *Fusarium* isolates revealed a large diversity of this trait in two bread wheat cultivars with different responses to FHB, *Fundulea 29* (moderately resistant) and *Fundulea 4* (very susceptible). Experiments were performed in seedling stage by a rapid screening method (3). ANOVA of data regarding the length of coleoptyle (% of control) showed significant differences between wheat cultivars and Fusarium isolates. The

main sources of variance were the genotypes of host (F=319:68**) and pathogen (F=54:03**), respectively. However, we found some differential host plant/pathogens combinations among the *Fusarium* isolates from our collection. These results suggested that preliminary selection of *Fusarium* isolates, according to their pathogenic potential to wheat be recommended in order to reduce the risks of non-accurate estimation of resistance. In the same experiment evidence for the existence of association between resistant host genotype/aggressive isolate was observed. The isolates originated from the moderately resistant cultivar *Fundulea 29* reduced more drastically the length of the coleoptyle in seedlings of susceptible *Fundulea 4*, as compared to its own isolates. This could be the effect of selection pressure into the pathogen population, as result of cultivation host genotypes with higher resistance (5).

Identification of new FHB resistance sources and recombination of resistance genes from various sources.

Efforts to select new sources of resistance to FHB in winter wheat were initially directed toward the breeding gene pool. We identified new sources of resistance in old Romanian (Montana, Turda 195) and foreign more or less adapted wheat cultivars (Libelulla, NS 732, Amigo). New cultivars with improved resistance to FHB obtained at Turda (Turda 95) have in their pedigree old Romanian wheat breeding lines, not particularly bred for resistance to FHB. As a result of a program to recombine resistance to FHB from various sources, several winter bread wheat lines with better levels of resistance were obtained (9). *Fundulea 201 R*, (F 201R) is a selection of complex cross between several sources of resistance *(F156I5-2112/F2076 W12-11)*. It cumulates resistance genes derived from cultivars NS 732 and Amigo, having no relation to any of previously described sources of resistance. It has shown high levels of resistance, in several environments from Romania and other European countries, Mexico etc, when various methods of inoculation were utilized. HPLC analysis of DON content, performed in Poland, showed low amounts of DON (0.70µg g^{-1}), in kernels of F201 R from heads inoculated with two Romanian *Fusarium graminearum* isolates (6). Repeated multiple location investigations on resistance to FHB including other advanced lines are in progress.

Analysis of resistance to FHB.

Recombinant inbred lines (RIL's) approach. Recombinant inbred lines derived from the cross F 1054 W (*susceptible*)/Sincron (*moderately resistant*) were investigated for three years, in order to study the genetic control of resistance to FHB and to identify possible associations of resistance with several marker loci. Frequency distributions for AUDPC (area under disease progress curve) and RWIS (relative weight of inoculated spikes) indicated different patterns among years and a quantitative inheritance of resistance. When lines were grouped according to alternative alleles at marker loci for height-reduction (*RhtB1 & Rht 8*), gliadin content (*Gli B1* & *Gli D1*) and waxy appearance of leaves (*W2*), significant association between FHB resistance and some gliadin loci (*Gli D1 b & Gli R1)* was documented. These results emphasized the involvement of chromosome *1B* in the control of FHB resistance and provided additional evidence for the possible role of *1D* (7).

Double haploid lines (DH) approach.

Based on DH analysis, the inheritance of resistance to FHB in winter bread wheat line *Fundulea 201 R* was investigated. Three years of investigation of response to FHB in 108

DH lines from crosses between this line and both, medium resistant (*F 249 T*) and susceptible (*F 135 U*) parents, suggested a quantitative inheritance. Transgressive segregation was observed in both crosses with *F 201 R*, mainly when the response to FHB was assessed as relative weight of inoculated spikes (RWIS, % from control). These data demonstrated the reliability of DH approach in breeding wheat for resistance to FHB. On the other hand the possibility to improve concomitantly the economical level of resistance to FHB and to maintain the desirable agronomic type was demonstrated (8).

Combining FHB resistance with bread making quality.

Line *F 201 R* is characterized not only by a good level of resistance to FHB, but also to other pathogens as rusts, powdery mildew and Septoria leaf blotch, but has a low bread making quality. Following successive years of selection at Fundulea, promising derivatives of crosses between the FHB resistant parent F 201 R and donors for good bread making quality (*Dropia and Delabrad*), combining reasonable levels for the both traits were obtained.

The distribution of 15 lines derived from crosses of *F 201R* with two high quality parents Dropia and Delabrad, according to AUDPC and sedimentation index suggests that, although a higher resistance is usually associated with low quality, several lines combining good levels of both resistance and quality were obtained.

Next step of breeding is expected to recombine higher values for both traits, FHB resistance and bread making quality respectively. All evidence reported to date suggests that further progress in FHB resistance is possible through the cumulation of resistance genes from different sources.

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PROBLEMS ENCOUNTERED IN TRANSFERRING SCAB RESISTANCE FROM WILD RELATIVES INTO DURUM WHEAT

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THE PROBLEM

Scab or Fusarium head blight (FHB), caused primarily by the fungus *Fusarium graminearum* Schwabe, is a serious disease of both hexaploid and tetraploid wheats. The fungus infects wheat heads from flowering through grain fill, causing enormous losses to growers in the northern plains area of the United States (McMullen et al., 1997). It is estimated that the losses may approach a billion dollars in the U.S. alone in some years. The combined direct and secondary economic losses suffered by wheat and barley producers in scab-affected regions of the U.S. during the 1998 to 2000 period were estimated at 2.7 billion dollars (Nganje et al., 2001); North Dakota and Minnesota account for about 55 percent of the total dollar losses. Reduction in kernel density and the presence of tombstone kernels (Figure 1) make wheat unmarketable. Moreover, the accumulation of the mycotoxin deoxynivalenol (DON) in the grain renders it unfit for human or even animal consumption. The need for breeding FHB resistance into wheat cultivars cannot, therefore, be overemphasized. Some of the problems encountered in transferring FHB resistance from wild species into durum wheat are outlined in this article.

Figure 1. Healthy and scab-infected seeds of durum wheat. [Photograph by Jim Miller, USDA-ARS]

SOURCES OF SCAB RESISTANCE

Most modern wheat cultivars lack resistance to FHB. Durum cultivars, in particular, have no resistance to this devastating disease. However, some of the wild relatives of wheat are rich sources of genes for resistance to diseases including FHB (Jauhar and Peterson, 1996, 1998, 2001; Friebe et al., 1996). We found that two wild relatives of wheat – the tetraploid wheatgrass (*Thinopyrum junceiforme* (Löve & Löve) Löve, $2n = 4x = 28$; $J_1J_1J_2J_2$ genomes) and diploid wheatgrass (*Lophopyrum elongatum* (Host) Á. Löve, $2n = 2x = 14$; EE) – are excellent sources of resistance to FHB. Thus, *L. elongatum* shows a mean infection of

3.8%, compared to infections of about 60% to 90% in susceptible durum cultivars. Several other wheatgrasses, e.g., *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve ($2n = 2x = 14$; JJ), may also prove excellent donors of FHB resistance to wheat. Chinese scientists have attempted to transfer scab resistance from *Roegneria komoji* Koch. ($2n = 6x = 42$; SSHHYY) into bread wheat (Liu et al., 2000). From the tertiary gene pool of wheat, Canadian workers have found one accession of *Elymus humidus* to be immune to FHB (Fedak, 2000).

It is not known whether the resistance in wild species is due to genetic factors or mechanical reasons. In diploid *Th. bessarabicum*, for example, the resistance may be due to the powdery coating on its leaves and stems. This powdery substance seems to be toxic to the fungal pathogen and even to aphids. But then the production of this metabolic by-product must be under genetic control. Other wild species like *Th. junceiforme* and *L. elongatum* must have genetically controlled resistance to FHB. An appropriate donor of FHB resistance must be selected. The problem with polyploid donors is the high number of undesirable chromosomes they bring into the wheat genome. It is preferable, therefore, to use diploid species as potential donors of FHB resistance. We are and plan on using the diploid wheat grasses *L. elongatum* and *Th. bessarabicum* for resistance breeding into durum wheat.

LACK OF CHROMOSOME PAIRING

Synthesis of hybrids between wheat and alien donors is an essential first step for alien gene transfer. Using embryo rescue techniques, wheat can be crossed with most wild grasses in the tribe Triticeae. Pairing between wheat chromosomes and alien chromosomes is a prerequisite for gene transfer into wheat. However, such chromosome pairing is generally low or even absent primarily because the pairing regulating gene, *Ph1*, located in the long arm of wheat chromosome 5B, suppresses pairing among homoeologous (less related) or unrelated chromosomes (Sears, 1976; Jauhar and Joppa, 1996). This poses a serious problem from the standpoint of alien gene transfer into wheat.

This problem may be overcome in durum wheat by using a Langdon 5D(5B) substitution line that lacks chromosome 5B and hence the *Ph1* gene. Hybrids between the 5D(5B) substitution and most alien species show high chromosome pairing, accelerating the chances of alien gene transfer into wheat. Another method of at least partially solving this problem is to use appropriate genotypes of wild species that suppress the activity of *Ph1*, resulting in promotion of chromosome pairing in wheat - alien species hybrids (see Jauhar, 1992; Jauhar and Almouslem, 1998; Jauhar and Peterson, 2001). In very difficult cases when chromosomes fail to pair in synthetic hybrids, gamma radiation may be employed to induce translocations between wheat and alien chromosomes.

STABLE INTEGRATION OF ALIEN CHROMATIN

Occurrence of pairing between wheat chromosomes and alien chromosomes leads to genomic reconstruction in wheat, resulting from integration of alien chromatin into the wheat genome (see Jauhar and Peterson, 2000, 2001). Such integrations may confer FHB resistance but may not be stable and hence lost in subsequent generations, resulting in loss of FHB resistance. This is a serious problem. It is advisable to synthesize a relatively large

number of hybrids with alien integrations, thereby increasing the chances of finding a stable integration. In some cases, a monosomic addition (full chromosome complement plus one alien chromosome, i.e., $2n = 28 + 1$) has been found to have resistance to scab. However, monosomic additions are not stable because the unpaired alien chromosome is lost. To obviate this problem, monosomic addition lines may be selfed to produce stable disomic additions. The production of a disomic addition would, however, depend upon the transmission of the monosome through the male and female gametes.

ASSOCIATION OF UNDESIRABLE TRAITS WITH FHB RESISTANCE

In some (perhaps most) cases, alien chromatin conferring FHB resistance may, at the same time, bring undesirable traits to the recipient parent. The severity of this problem may depend on the size of the alien chromosome fragment integrated. The smaller the size of alien chromatin, the lower are the chances of bringing undesirable characters into wheat. Smaller integrations would also be generally stable.

PROBLEM OF SCREENING FOR TYPE-2 FHB RESISTANCE IN WILD GRASSES

Screening for Type-2 FHB resistance is done by introducing 10 ¼l of inoculum (100,000 spores per ml) of three different isolates (biotypes) of *F. graminearum* into the florets at the time of anthesis. Inoculated plants are then grown under warm, humid conditions favoring *Fusarium* growth and spread (see Jauhar and Peterson, 2001) and individual spikes are scored for percent infection once after two weeks and then after three weeks (Stack and McMullen, 1994).

The grass florets are generally small and tightly compact, making it difficult to introduce the desired amount of inoculum, thereby posing a problem for optimal screening. Increasing the concentration of spores per ml may help solve this problem. Spraying the florets with the inoculum may offer another alternative.

CONCLUSION

In transferring FHB resistance from wild gasses into wheat we confront several problems, which are not insurmountable. Wide hybridization does offer an excellent tool for breeding scab resistance into wheat cultivars. Transgenic approaches to combating scab are also being pursued in several laboratories including ours (Dahleen et al., 2001). Standardization of transgenic technology for durum wheat in our laboratory (Bommineni et al., 1997) has paved the way for direct introduction of antifungal genes into otherwise desirable durum cultivars. All available approaches should be adopted to combat Fusarium head blight in cereals.

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CHARACTERIZATION OF WHEAT GERMPLASM FOR SSR MARKER ALLELES NEAR THE FUSARIUM HEAD BLIGHT RESISTANCE QTL ON CHROMOSOME 3BS

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ABSTRACT

Previously, we reported SSR markers associated with Sumai 3-derived FHB resistance. The objective of this research was to characterize wheat germplasm using SSR markers in the region of a major QTL on chromosome 3BS. Seventy-four FHB resistant and susceptible lines from throughout the world were genotyped with four SSR markers. Cluster analysis of a genetic similarity matrix was performed using the unweighted pair-group mean algorithm (UPGMA). The SSR markers GWM389, GWM533, GWM493 and BARC102, detected 14, 12, 9 and 12 alleles, respectively. Sumai 3 type alleles are unique. Only 19 out of 54 FHB resistant lines have Sumai 3 type alleles for at least one SSR marker, and none of the FHB susceptible lines have the Sumai 3 type alleles. Six FHB resistant lines have the same genotype as Sumai 3 for all four SSR markers. All of these lines contain Sumai 3 or derivative in their pedigree, and, therefore most likely contain this major QTL. Five FHB resistant lines have the Sumai 3 type alleles at three of the four marker loci. The five lines likely have the same QTL as Sumai 3 in this 3BS region. All the other 42 FHB resistant lines and all 20 susceptible lines tested were distinct from Sumai 3 in this region. These resistant lines may carry novel FHB resistance genes, and further genetic study is worthwhile.

MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE QTL IN THE WHEAT LINE WUHAN3

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ABSTRACT

The best method of controlling Fusarium head blight (FHB) is through resistance and therefore, it is necessary to identify sources of resistance. The objectives of this study were to determine the associations between disease (symptomatic spikelets) and molecular markers and to confirm QTLs already reported in other populations. A population of 110 F7 derived RIL from the cross Wuhan 3 (resistant) /Norm (susceptible) was evaluated for reaction to the fungus *Fusarium graminearum*, in inoculated mist-irrigated field conditions, (2 locations, 2 years) and under greenhouse point-inoculation, (2 experiments, 5 replications). The number of symptomatic spikelets was recorded 21 days post inoculation in both the field and greenhouse experiments. Wuhan 3/ Norm displays a normal distribution, transgressive segregants and significant variation among RILS 7for FHB severity. The FHB severity evaluations are well correlated and the correlations are statistically significant. Preliminary mapping of this population has been done with DNA SSR markers located on chromosomes 3BS, 2A, and 5A. The interval analysis of the 3BS markers shows a putative QTL that is associated ($P<0.01$) with FHB resistance. This confirms the findings of Anderson et al (2001). The marker has an average R2 value of 0.27 and a range of 0.11 to 0.41 over the five experiments.

RESISTANCE TO FUSARIUM HEAD BLIGHT IN ACCESSIONS FROM THE BALKANS: A PROGRESS REPORT

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OBJECTIVES

To evaluate, under greenhouse and field conditions, accessions from the Balkans contained in the National Small Grains Collection at Aberdeen, ID for resistance to *Fusarium graminearum.*

INTRODUCTION

Host resistance has long been considered the most economical and effective means of control for *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab (Schroeder and Christensen, 1963; Martin and Johnston, 1982), but breeding has been hindered by a lack of effective resistance genes and by the complexity of the resistance in identified sources (Mesterházy, 1997). No source of complete resistance is known, and current sources provide only partial resistance, often in genetic backgrounds with inferior agronomic type. The identification of different sources of resistance and their incorporation into adapted wheat varieties is critical to the continued improvement of Fusarium head blight resistance in winter wheat. Research funded by the National Wheat and Barley Scab Initiative has led to the systematic evaluation of resistance to scab in winter wheat accessions from targeted geographical regions of the world where resistance has been identified or where environmental conditions are conducive to scab development including those from Eastern Europe. Approximately 2,000 winter wheat accessions from the Balkans were identified in the USDA National Small Grains collection for evaluation.

MATERIALS AND METHODS

In 1999 and 2000, approximately 2000 accessions from the Balkans representing winter wheat landraces, breeding lines, cultivars and cultivated genotypes from Yugoslavia, Croatia, Macedonia, Bosnia Herzegovina, Serbia, and Montenegro were acquired from the USDA-ARS Small Grains Collection at Aberdeen, Idaho. Lines were screened in both field and greenhouse screening programs at Missouri. A sub-sample of lines were jointly screened in the greenhouse at North Carolina State University in order to expedite verification of resistant accessions.

Disease Resistance Screening - Greenhouse*:* Vernalized seedlings (4 per accession) were planted in the greenhouse. At first anthesis, plants were inoculated with 10µL of a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was placed in a single central floret using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously

determined to be the most aggressive Missouri isolate on our most resistant cultivar, Ernie. Plants were incubated in a mist chamber (100% relative humidity) for 72 h post-inoculation to promote disease development and then returned to the greenhouse bench. Ratings for disease spread in the spike were made at 21 d after inoculation. Plants were identified for further evaluation that had low spread in the head (mean spread $\leq$ 2 spikelets), and good kernel quality relative to an uninoculated head. Resistant check cultivars were Ernie, Sumai 3, Ning 7840, and MO 980525. The susceptible check was MO 94-317.

Disease Resistance Screening – Field: Accessions were planted as head rows in the field at the Agronomy Research Center near Columbia, MO. Plants were sprayed at 75% heading with a macroconidial suspension concentrated to 50,000 macroconidia/mL. Head rows were maintained under overhead mist irrigation through heading and evaluated for scab incidence 7-10 d post inoculation and severity18 - 21 d after inoculation. A field scab index was determined as the product of incidence and severity. Checks were again Ernie, MO 980525 and MO 94-317.

RESULTS AND DISCUSSION

Table 1 provides information on the accession, improvement status, and resistance data for accessions identified as having good field and greenhouse resistance in both the Missouri and North Carolina evaluation programs. Data are presented for accessions that had either reduced spread in the spike following greenhouse inoculations, a low scab index in the field (determined as the product of incidence and severity) or both. Resistance in approximately 90 additional accessions will be verified in 2002. Resistance identified in a further group of accessions screened and verified only at Missouri are given in Table 2. The majority of resistant accessions (>90%) from the Balkans are landraces. Many are tall and late and significant pre-breeding will be required to transfer their otherwise excellent levels of resistance into adapted backgrounds.

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Table 1. Winter wheat accessions, originating from Balkans, screened at the University of Missouri and at North Carolina State University in and 2001.

			Missouri Data				North Carolina Data
		Improvement	2001 Field Data			Greenhouse	Greenhouse
MO ID	Accession	Status	Incidence	Severity[1]	Index	Type II[1]	Type II[1]
1947	Cltr 11214	Breeding	80	0.29	23.28	0.09	0.09
2012	PI 278623	Cultivated	70	0.27	18.72	0.14	0.07
2036	PI 345009	Landrace	100	0.29	28.68	0.32	0.04
2037	PI 345010	Landrace	100	0.27	26.70	0.21	0.22
2048	PI 345023	Landrace	100	0.15	15.18	0.15	0.22
2070	PI 345047	Landrace	100	0.14	14.00	0.07	0.08
2084	PI 345063	Landrace	80	0.13	10.79	0.11	0.08
2105	PI 345086	Landrace	100	0.28	27.85	0.09	0.23
2122	PI 345110	Landrace	90	0.18	16.28	0.24	0.06
2170	PI 345168	Landrace	50	0.32	16.10	0.10	0.07
2229	PI 345235	Landrace	100	0.21	21.10	0.20	0.09
2230	PI 345236	Landrace	100	0.26	26.23	0.15	0.08
2255	PI 345274	Landrace	90	0.26	23.48	0.16	0.23
2294	PI 345334	Landrace	100	0.11	11.34	0.17	0.07
2295	PI 345335	Landrace	80	0.15	12.31	0.27	0.08
2304	PI 345346	Landrace	80	0.24	18.96	0.06	0.14
2314	PI 345356	Landrace	40	0.14	5.69	0.08	0.06
2329	PI 345377	Landrace	90	0.15	13.25	0.18	0.21
2416	PI 345490	Landrace	100	0.16	15.53	0.18	0.14
2429	PI 345504	Landrace	100	0.28	27.51	0.28	0.19
2539	PI 350118	Landrace	90	0.16	14.26	0.13	0.11
Ernie	Resistant check (cultivar)		82.5	0.22	18.2	0.14	
Sumai 3	Resistant check		not grown in the field			0.06	
Ning 7840	Resistant check		not grown in the field			0.16	
MO 94-317	Susceptible check (cultivar)		100.0	0.47	47.0	0.93	

[1] Mean ratio of infected spiketlets to total spikelets on inoculated heads.

Table 2. Winter wheat accessions, originating from Balkans, screened and verified at the University of Missouri in 2000 and 2001.

MO ID	Accession	Improvement status	2001 Field Data[1] Incidence	Severity[3]	Scab Index	2000 Field Data[2] Scab Index	Greenhouse Type II[3]
939-2	CI 11225	Landrace	100.0	0.21	21.1	20.0	0.30
940-1	CI 11228	Landrace	90.0	0.26	23.3	30.0	0.16
1083-1	PI 316425	Breeding	100.0	0.42	42.0	9.5	0.19
1143-1	PI 349928	Landrace	100.0	0.45	45.0	22.5	0.08
1158-2	PI 349964	Landrace	80.0	0.36	28.6	18.0	0.05
1173-1	PI 350002	Landrace	100.0	0.29	29.3	25.5	0.11
1182-1	PI 350018	Landrace	90.0	0.33	29.6	27.0	0.19
1184-1	PI 350020	Landrace	90.0	0.35	31.8	38.0	0.16
1186-1	PI 350022	Landrace	100.0	0.34	33.5	38.0	0.17
1223-3	PI 350134	Landrace	100.0	0.25	24.5	28.5	0.34
1225-1	PI 350146	Landrace	100.0	0.19	19.2	24.0	0.20
1240-2	PI 350258	Landrace	100.0	0.37	37.3	36.0	0.11
1274-2	PI 362459	Landrace	70.0	0.31	21.8	14.0	0.14
1280-2	PI 362469	Landrace	100.0	0.29	28.8	95.0	0.15
1301-1	PI 362503	Landrace	70.0	0.12	8.5	22.5	0.07
1306-1	PI 362512	Landrace	100.0	0.16	16.3	35.0	0.08
1312-3	PI 362519	Landrace	80.0	0.21	17.0	18.0	0.23
1423-4	PI 362676	Landrace	100.0	0.15	15.5	27.0	0.16
1487-2	PI 374515	Landrace	100.0	0.27	26.8	17.0	0.07
1507-1	PI 374545	Landrace	60.0	0.09	5.6	18.0	0.21
1529-1	PI 374577	Landrace	90.0	0.46	42.0	38.0	0.12
1579-2	PI 374676	Landrace	90.0	0.41	37.0	25.5	0.09
1580-1	PI 374677	Landrace	100.0	0.42	42.0	25.5	0.10
1588-2	PI 374689	Landrace	100.0	0.24	24.0	27.0	0.30
1600-1	PI 378265	Landrace	90.0	0.33	29.6	18.0	0.13
1607-1	PI 378278	Landrace	100.0	0.22	21.8	25.5	0.16
1644-2	PI 378330	Landrace	100.0	0.43	43.0	45.0	0.07
1645-1	PI 378331	Landrace	100.0	0.32	32.0	38.0	0.07
1652-3	PI 378342	Landrace	90.0	0.40	36.4	25.5	0.10
1673-1	PI 378398	Landrace	100.0	0.32	31.7	34.0	0.14
1682-1	PI 378415	Landrace	100.0	0.20	20.2	12.0	0.12
1734-2	PI 378528	Landrace	80.0	0.18	14.1	18.0	0.22
1750-1	PI 405862	Landrace	80.0	0.16	13.0	36.0	0.28
1752-1	PI 405864	Landrace	50.0	0.13	6.5	10.0	0.14
1838-1	PI 420587	Landrace	80.0	0.30	23.7	24.0	0.20
1842-2	PI 420591	Landrace	60.0	0.20	11.9	27.0	0.12
1845-2	PI 420594	Landrace	90.0	0.19	17.4	8.0	0.18
1846-2	PI 420595	Landrace	90.0	0.17	15.4	20.0	0.16
1891-3	PI 434658	Breeding	90.0	0.22	19.7	27.0	0.03
Ernie	Resistent check	Cultivar	82.5	0.22	18.2	11.5	14.0
MO 94-31´	Susceptible check	Cultivar	100.0	0.47	47.0	60.0	92.0
980525	Resistent check	Cultivar	63.3	0.08	5.2	-	10.0

[1] Data are for progeny from individual plant selected for good type II greenhouse score.

[2] Data are for the accession prior to selection for an individual plant within the accession exhibiting a good type II greenhouse score.

[3] Mean ratio of infected spiketlets to total spikelets on inoculated heads.

TYPES I AND II RESISTANCE TO FUSARIUM HEAD BLIGHT IN ASIAN AND ITALIAN GERMPLASM

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ABSTRACT

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab, is a devastating disease of wheat and barley in warm and humid regions of the world. Host plant resistance has long been considered the most practical and effective means of control but breeding has been hindered by a lack of effective resistance genes and by the complexity of the resistance in identified sources. No source of complete resistance is known, and current sources provide only partial resistance, often in unadapted types. The identification of different sources of resistance in winter wheat through a systematic evaluation of accessions maintained in the National Small Grains Collection at Aberdeen, ID has been identified as a key objective of the US Wheat and Barley Scab Initiative's germplasm research area. The objective of this research was to confirm type I and II resistance in 45 accessions from Asian and Italy that we had previously identified as having potentially useful levels of type I and/or type II resistance. Vernalized seedlings were arranged in a split-plot design with genotype as the main plot and type of resistance as the sub-plot. For each accession, 10 plants per treatment were planted and the experiment was replicated six times. For evaluation of type II resistance, plants were inoculated at first anthesis with 10µL of a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was placed in a single central floret using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously determined to be aggressive on our most resistant cultivar, Ernie. Plants were incubated in a mist chamber (100% relative humidity) for 72 h post-inoculation to promote disease development and then returned to the greenhouse bench. Ratings for Type II resistance (disease spread in the spike) were made at 21 d after inoculation. For evaluation of type I resistance, heads were inoculated with a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was sprayed directly on the head using a Pulmo-Aide nebulizer as the power source and an atomizer (Model 163, DeVilbiss Sunrise Medical, Somerset, PA 15501-0635, USA). Inoculum was delivered to each head, spraying one side and then the other. Plants were incubated in a mist chamber as described above. At 10 d post-inoculation heads were rated for symptoms of Fusarium head blight. Total spikelets in the head were recorded followed by the number of spikelets in the head showing disease. The type I rating for each head was determined as the number of spikelets with disease divided by the total number of spikelets on the head. Ratings were taken again at 21 d post-inoculation to determine the scab index (incidence x severity) for the head. The type I rating (10 d) was taken as a measure of incidence. The 21-d rating (total number of infected spikelets/total spikelets in the inoculated head) provided an estimate of severity on the inoculated head. Data presented indicated independence of type I and II reactions in this range of plant material. Correlations with field based evaluation of incidence and severity will be presented.

FUSARIUM HEAD BLIGHT RESISTANCE IN FALL-SOWN TRITICALE

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OBJECTIVES

1) To evaluate Type II resistance in advanced generation breeding lines and cultivars of triticale adapted to the Southeastern United States and,
2) to evaluate the impact of inoculation with different spore concentrations on estimates of Type II resistance.

INTRODUCTION

Fall-sown triticale (X *Triticosecale* Wittmack) is grown on a limited acreage in North Carolina and is utilized as a winter cover crop, a forage and a grain component in hog and poultry diets. Much of the wheat crop in eastern North Carolina is utilized as an animal feed and, in the event of repeated FHB epidemics, triticale may offer an alternative for the small grain producer.

N.C. State has a small cultivar development effort in triticale and we released our first cultivar, Arcia, in 2000. The methodologies we utilize to develop breeding populations include cultivated x cultivated triticale crosses and the development of primary triticales [(wheat x rye) x triticale] combinations. An evaluation of advanced generation lines and cultivars is appropriate. In addition to providing information on the inherent resistance of these triticales, novel sources of resistance may be identified in triticale germplasm which can be incorporated into wheat breeding populations.

The head morphology of triticale differs from that of soft red winter wheat. The larger and more lax heads of triticale may provide a different environment for disease establishment in infected florets. An evaluation of inoculation techniques, particularly number of macroconidia per inoculation, was considered timely.

MATERIALS AND METHODS

Three triticale cultivars, two wheat check cultivars and 28 advanced generation triticale breeding lines were evaluated in greenhouse tests during spring 2001 (Table 1). Twenty-four triticale breeding lines ('NC') were selected from conventional triticale x triticale crosses and four breeding lines ('NCPT') were selected from primary triticale populations.

A randomized complete block design with two replications was utilized. An experimental unit was a single plant. Four plants were evaluated per entry. Ten ml of a spore suspension containing 50,000 spores per ml were injected into a floret in the center of the head at the time of the first anther extrusion. The inoculation suspension consisted of three aggressive North Carolina isolates of *Fusarium graminearum* identified by Walker et al. (2001). Following inoculation, the plants were placed in a mist chamber for 3 days. Twenty-one days post-

inoculation, heads were rated for the number of diseases spikelets/total number of spikelets/ head and expressed on a percentage basis. After harvest, inoculated heads were threshed and kernels were divided into five categories: 1 = sound, 2 = slight shriveled, 3 = moderately shriveled, 4 = very shriveled, and 5 = tombstone. A kernel quality score was calculated as the weighed average of the number of kernels in each category.

Table 1. Type II resistance to Fusarium head blight and kernel quality of 31 fall-sown triticale cultivars and breeding lines plus two wheat cultivars evaluated in greenhouse tests in spring 2001.

Cultivar/ designation	Infected spikelets	Total spikelets	Type II resistance	Rank	Kernel quality	Rank
NC 99-424	1.2	25.3	4.6	1	4.0	5
NCPT 97-1017	2.0	21.8	9.2	2	4.1	6
NC 97-1311	2.4	25.8	9.3	3	4.7	21
NC 99-609	2.5	25.0	10.0	4	4.9	28
NC 99-728	2.5	24.8	10.1	5	4.7	24
NCPT 97-1005	2.5	24.3	10.4	6	4.1	7
NC 99-937	2.0	18.0	11.7	7	4.2	8
NC 99-786	3.2	25.8	12.1	8	4.3	12
NC 99-643	2.8	23.0	12.5	9	4.8	26
ARCIA	3.0	23.0	13.0	10	4.2	9
NC 97-1305	3.9	28.3	14.2	11	4.4	14
NC 99-530	3.6	25.0	14.2	12	4.5	16
NC 99-797	3.2	21.5	14.3	13	4.6	19
NC 99-618	3.3	22.5	14.4	14	4.9	29
TRICAL498	4.0	26.3	15.1	15	4.6	20
NC 99-647	3.8	25.0	15.2	16	5.0	31
NCPT 97-1008	3.9	25.0	15.7	17	4.5	17
NC 99-810	2.5	15.8	15.9	18	5.0	32
NC 99 348	4.3	25.3	16.5	19	3.9	4
NC 99-11	3.9	23.0	16.7	20	4.2	10
NC 99-745	4.3	25.0	17.1	21	4.3	13
NC 97-1425	4.5	26.0	17.3	22	4.2	11
FLPFT 215	3.7	20.8	17.5	23	2.4	1
NC 99-628	4.6	23.0	20.0	24	4.8	27
NC 99-647	4.3	21.5	20.0	25	5.0	33
NC 98-1737	4.7	23.0	20.2	26	3.9	3
NC 99-859	4.2	20.3	20.7	27	4.5	18
NC 99-554	5.2	23.3	21.8	28	5.0	30
ERNIE (check)	2.3	10.0	22.2	29	3.4	2
NC 99-815	5.9	22.0	27.0	30	4.8	25
NC 99-857	5.7	19.8	28.0	31	4.4	15
NCPT 98-103	6.7	21.8	31.2	32	4.7	22
C9663 (check)	6.7	13.3	50.4	33	4.7	23
Mean	3.7	22.5	17.2		4.4	
LSD	--	--	17.0		0.5	

A subset of three triticale cultivars and seven advanced generation lines was chosen for the spore concentration study. The experimental protocol was similar to that described above, except a split block design was utilized. Whole plots were three spore concentrations: 50,000 spores/ml, 100,000 spores/ml and 150,000 spores/ml. Subplots were the 10 cultivars and breeding lines.

RESULTS AND DISCUSSION

The two wheat check cultivars performed as expected; Ernie, the resistant check, had significantly better Type II resistance and kernel quality than the susceptible check C9663 (Table 1). The greater overall resistance of the triticale germplasm to these isolates is reflected in the relative rankings of Ernie (29th) and C9663 (33rd) for Type II resistance. All of the triticale entries were significantly superior to C9663.

No triticale had significantly superior kernel quality to Ernie, the resistant wheat check, but six had superior kernel quality to C9663, the susceptible check. A difficulty we observed with rating kernel quality for triticale versus wheat was the inherently greater shriveling of triticale seed that is observed, even in the absence of FHB infection. This resulted in a nonsignificant rank correlation between Type II resistance and kernel quality ratings.

No significant differences were observed for mean Type II resistance or kernel quality at the three inoculation concentrations (Table 2). Neither genotype nor genotype x spore concentration sources of variance were significant for Type II resistance estimates. Genotype variance was significant for kernel quality, but genotype x spore concentration was not significant.

Table 2. Mean Type II and kernel quality scores for 10 triticale cultivars and breeding lines evaluated at three spore concentrations.

Spore concentration	Mean Type II (%)	Mean kernel quality (1-5)
50,000 spores/ml	13.6	4.4
100,000 spores/ml	19.2	4.4
150,000 spores/ml	14.9	4.5
LSD	ns	ns

The results of this preliminary greenhouse evaluation of southeastern triticale cultivars and breeding lines suggested that triticale germplasm is inherently more resistant to FHB isolates in the region than is wheat germplasm. In addition, triticale germplasm may serve as a source of resistance alleles that could be introgressed into wheat breeding populations. Finally, it appears that current wheat protocols regarding spore concentration in greenhouse evaluations of Type II resistance are adequate for similar evaluations of triticale.

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NEW SOURCES OF RESISTANCE TO FUSARIUM HEAD BLIGHT OF WHEAT

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OBJECTIVE

The goal of this research is to identify wheat accessions whose resistance to Fusarium head blight differs from that in Sumai 3 and Ning 7840.

INTRODUCTION

Scab has become a serious disease of wheat and barley in many areas of the US. Resistant cultivars will be an important component of an integrated disease management strategy. Many wheat-breeding programs are utilizing resistance from Sumai 3 or the closely related cultivar Ning 7840. While this resistance appears to be the best available, and reasonably effective, it does not totally prevent disease development. Total reliance on this one source of resistance is also a concern. If any of the *Fusarium* species capable of causing scab were to adapt to this resistance, millions of acres of wheat could become vulnerable to the disease. This project is designed to find other sources of resistance to scab, with two objectives: to provide genetic diversity for resistance and to enhance the degree of resistance conferred by the Sumai 3 source of resistance.

MATERIALS AND METHODS

We selected several lines from accessions in the USDA wheat germ plasm collection for resistance to *F. graminearum*. We also evaluated accessions from other sources. The original accessions were heterogeneous for reaction, but by repeated cycles of inoculation and selection, we developed lines with a high degree and consistent expression of Type II resistance (Table 1).

Table 1. Wheat accessions from which lines with a high degree of Type II resistance to *Fusarium graminearum* were selected.

Accession	Accession
Chokwang	CIMMYT 211
Futai 8944	Funo
Mentana	Paula VZ 434
Oscar V	Y 5418

We crossed these resistant selections to susceptible cultivars, to Sumai 3 or Ning 7840, and to each other. During the spring of 2001, we evaluated progeny from several test cross and

backcross populations for Type II resistance. We also evaluated Typemon (10^4 conidia/ml) was used as inoculum. The inoculated spike was covered with a small, clear polyethylene bag for 48 hours to provide moisture for infection. Blighted spikelets were counted 10 and 20 days after inoculation.

RESULTS

The initial goal of the crossing program with these new sources of resistance was to combine them in order to seek transgressive segregates for greater resistance than shown by either parent. Because of the unreliability of single-plant selection, even following controlled inoculation in the greenhouse, identification of transgressive segregates must await confirmation in progeny tests, which are now underway. Nonetheless, several crosses included some progeny that were highly resistant. These showed no symptoms, or discoloration of only part of the inoculated floret. Based on this preliminary observation, genes for resistance in Mentana and Paula VZ 434 may differ from those in Sumai 3. CIMMYT 211, Futai 8944, and Y5418 probably share at least one resistance gene with Sumai 3.

Genes for resistance do not appear to be completely dominant. For example, when the F1 of Futai 8944 x Norm was backcrossed to Futai 8944, progeny ranged from highly resistant to moderately susceptible. Likewise, when the F1 of Futai 8944 x Paula VZ 434 was backcrossed to Futai 8944, progeny ranged from highly resistant to moderately susceptible. When the F1 of Futai 8944 x Sumai 3 (or Ning 7840) was backcrossed to Futai 3, all progeny were resistant or moderately resistant, further suggesting that Futai 8944 has one or more genes in common with Sumai 3.

Distributions of the backcross of the Chokwang x Clark F1 to Clark and of the test cross to Norm were trimodal (Figs. 1 and 2)

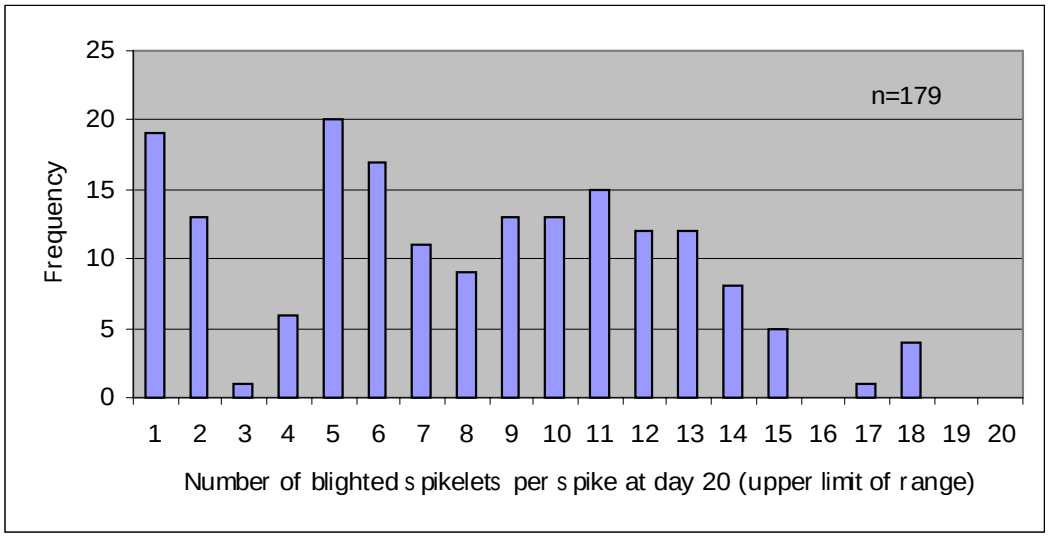

Fig. 1. Distribution of Fusarium head blight severity among F1 progeny of Clark//Chokwang/Clark

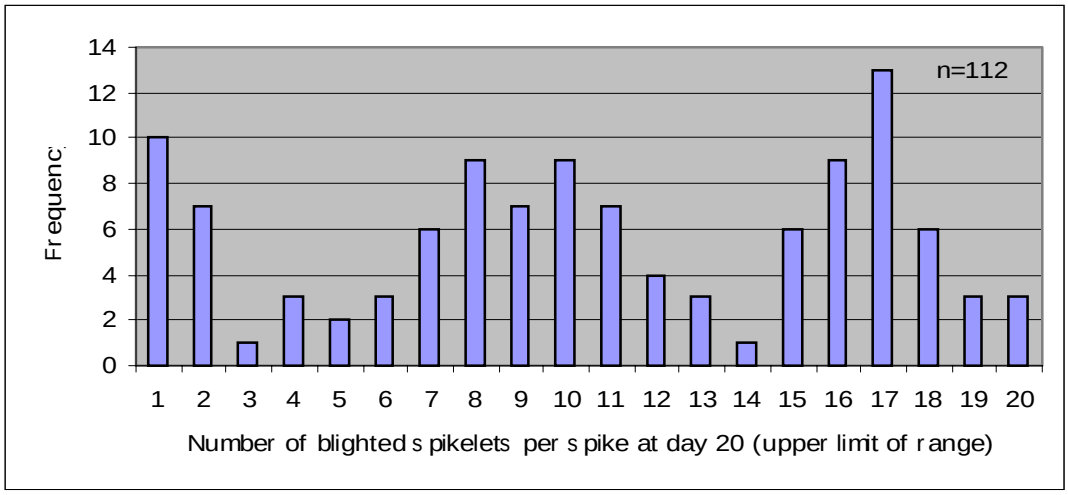

Fig. 2. Distribution of Fusarium head blight severity among F1 progeny of Clark/Chokwang// Norm

None of the progeny of the backcross to Chokwang were fully susceptible, but 62% fell into an intermediate category (Fig. 3).

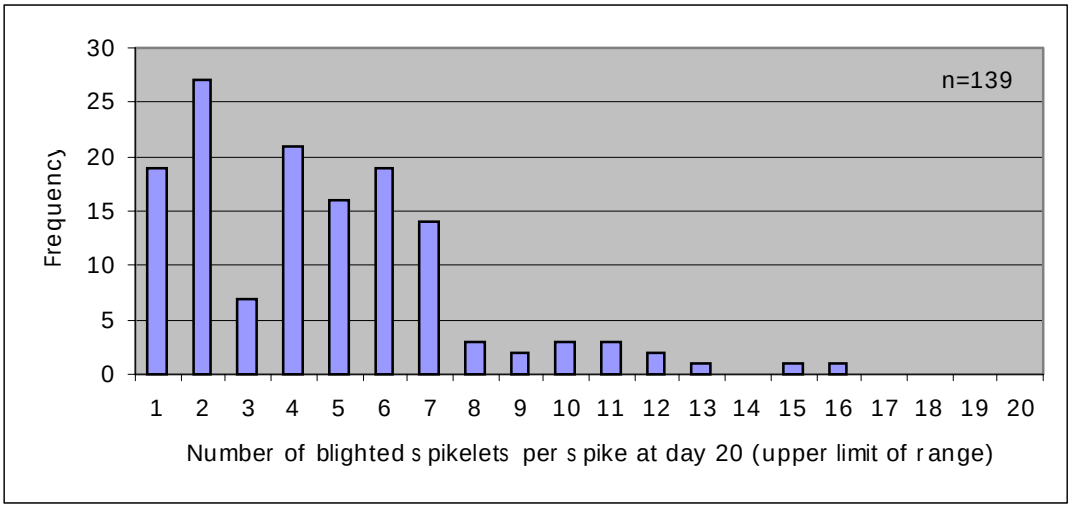

Fig. 3. Distribution of Fusarium head blight severity among F1 progeny of Chokwang// Chokwang/Clark

DISCUSSION

Preliminary results suggest that at least some of the accessions selected for resistance to *Fusarium graminearum* have genes not found in Sumai 3 or Ning 7840. The Type II resistance of these latter cultivars is generally quite good in greenhouse tests, leaving only a limited range for detection of transgressive segregation for enhanced resistance. Progeny tests may allow us to confirm whether backcross F1s with essentially complete resistance will be stable for this trait.

Based on evaluation of a small recombinant inbred population derived from Chokwang/Clark, we speculated that at least 2 genes in Chokwang conferred resistance to *F. graminearum*, but that the interactions among genes were not simply additive (Buechley and Shaner, 1999). In the study reported here, highly resistant progeny were most frequent in the backcross to Chokwang, and least frequent in the test cross to Norm. The fact that all 3 crosses gave rise to some highly resistant progeny further supports the notion that these genes do not act in a simple additive manner.

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COMBINING ABILITY OF FHB RESISTANCE FROM DIFFERENT SPRING WHEAT SOURCES

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ABSTRACT

Fusarium head blight (FHB) has been a serious concern in the spring wheat region of the northern great plains for nearly a decade. In response to this problem, both public and private spring wheat breeding programs have directed considerable resources to adding FHB resistance to their lines. As sources of FHB resistance, many programs have used the Chinese lines such as Sumai 3 or its derivatives. FHB resistance in wheat is a quantitative trait of complex inheritance. None of the known FHB resistance source lines possess sufficently strong resistance to prevent economic damage under conditions of high disease pressure. This study was an attempt to determine if the testing of F1's would be a suitable method to decide which crosses have potential for major improvement in resistance to FHB. In this study we used a half-diallele set of five parents: all pairings were included but not the respective reciprocal crosses. Three parents were derived from Sumai 3 through different lineages and two were of other origin. Using spikelet inoculation in the greenhouse, we tested the ten F-1 hybrids among these 5 lines. Sufficient hybrid seed was made so that there could be replicated row plantings of F1 plants. Values for FHB severity for each parent and F1 represent the mean of 45-50 individual head scores Analysis of Variance for the FHB severity scores were partitioned by Griffing's model to show general and specific combining ability effects. Some pairings of lines showed major additivity of resistance while others, supposedly of different origin, did not. All but one parent showed significant general combining ability for FHB severity. The two crosses that showed the greatest differences between F1 severity score and parent mean score were also those with the largest effect for specific combining ability. The ability of partially resistant lines to show useful combining ability for FHB response is encouraging. It also suggests that that various partially resistant lines are likely to have different genetic makeup for that resistance – a factor which may aid the stability of resistance to FHB. The lack of combining ability between the two least susceptible lines was disappointing, and may suggest that major improvement in FHB resistance may be more difficult to achieve. Proper choice of parents will be needed to obtain major improvement in FHB resistance.

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EVALUATION OF *HORDEUM* ACCESSIONS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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OBJECTIVE

To identify diverse sources of resistance to Fusarium head blight (FHB) in Hordeum.

INTRODUCTION

FHB has devastated the barley (*Hordeum vulgare*) crop in the Upper Midwest region over the past nine years (1993-2001) (Salas et al. 1999; Steffenson 2002). Deployment of resistant cultivars is the most effective and environmentally sound means of managing the disease. Thus, the objective of this study was to identify diverse sources of resistance to FHB in the primary (*H. vulgare* and *H. vulgare* subsp. *spontaneum*) and secondary (*H. bulbosum*) gene pools of cultivated barley.

MATERIALS AND METHODS

The primary and secondary *Hordeum* gene pools are potential sources of useful resistance genes for cultivated barley (Pickering 2000). Over the past three years, we evaluated about 8,100 six-rowed spring and 600 six-rowed winter accessions of *H. vulgare* for resistance to FHB in the United States (North Dakota and Minnesota) and China (Zhejiang Province), respectively. Additionally, 510 accessions of wild barley (*H. vulgare* subsp. *spontaneum*) and 28 *H. vulgare ´ H. bulbosum* (bulbous barley grass) introgression lines were screened in China in 2000-2001. FHB nurseries were inoculated using the "grain spawn" method, except in St. Paul where the "foliar spray" method was used (Steffenson 2002). For the grain spawn inoculation, equal amounts of two to six regional *F. graminearum* isolates were applied uniformly to plots. The first inoculation was made when the flag leaves of the earliest maturing plants were expanding. One to four additional inoculations were made during the course of the season to ensure that sufficient inoculum was available for infection of later maturing accessions. To promote FHB infection, overhead irrigation was applied to plants in the morning and evening. For the foliar spray method, plants were uniformly inoculated twice with a back-pack sprayer at the late milk stage of development. Overhead irrigation was applied to promote infection. Disease severity was assessed on each accession at the mid-dough stage. Accessions exhibiting low levels of FHB infection were also assayed for deoxynivalenol (DON) concentration.

RESULTS AND DISCUSSION

Six-rowed spring barley evaluations. Over 8,100 six-rowed spring barleys were initially screened for FHB resistance at two locations in North Dakota in 1999 and 2000 (~4050 per

year). Only 27 accessions exhibited FHB severities less than 30% in one of the evaluations in 1999 or 2000 (Scholz and Steffenson 2000). These accessions were then evaluated in the greenhouse under more controlled conditions and also in additional field environments. Data on the infection levels of the five most resistant accessions identified in each year are given in Tables 1 and 2, along with the resistant (Chevron and CIho 4196) and susceptible (Foster or Stander) controls. FHB severity and DON concentration varied considerably in the selected accessions across different evaluation tests (Table 2); however, a few accessions (CIho 6613 and CIho 11526) consistently exhibited resistance levels that were similar to Chevron. Several of the most resistant six-rowed accessions identified to date originate from Switzerland. This includes PI 370919, PI 371317, the resistant control Chevron (CIho 1111) and a Chevron selection (CIho 11526)(Table 1).

Table 1. Accvessions of six-rowed spring barley exhibiting resistance to FHB in initial field screeing in Langdon and Osnabrock, North Dakota in 1999 and 2000.

Accession Number	% FHB Severity			Origin	
	Langdon	Osnabrock	Name	Country	Region
Screening 1999					
CIho 4095	40	20	--[2]	Georgia	
CIho 4530	40	20	7603	China	Jilin
CIho 6613	30	10	Seed Stocks 1148-1	USA	WI
CIho 9114	40	20	184	Yugoslavia	Serbia
CIho 11526	10	10	Chevron Selection	USA	MD
CIho 1111[1]	17	14	Chevron	Switzerland	
PI 592758[1]	--[2]	54	Foster	USA	ND
Screening 2000					
CIho 9699	20	30	9241	Ethiopia	Gonder
PI 328642	20	20	HOR 1390	Romania	
PI 370919	30	30	569C	Switzerland	Valais
PI 371317	30	40	194D	Switzerland	Uri
PI 565567	40	30	Chun Gong Mai	China	Shandong
CIho 1111[1]	28	18	Chevron	Switzerland	
PI 592758[1]	60	47	Foster	USA	ND

[1]Chevron (CIho 1111) and Foster (PI 592758) are resistant and susceptible six-rowed controls, respectively.

[2]Not available or not tested.

Table 2. FHB severity (%) and DON concentration (ppm) of selected six-rowed barley accessions evaluated in greenhouse and filed tests, 2000-2001.

	Greenhouse 2000		Field 2000		Field 2001	
	Fargo		Fargo		St. Paul	
Accession	FHB	DON	FHB	DON	FHB	DON
CIho 9114	1.4	0.1	4.2	13.7	4.5	11.6
CIho 11526	2.8	0.0	3.9	17.6	6.0	--[2]
CIho 6613	4.7	0.9	1.7	14.7	2.9	9.2
CIho 4530	6.4	1.1	14.9	60.4	2.8	16.0
CIho 4095	11.8	2.2	13.1	55.1	2.9	12.2
Chevron[1]	4.7	0.6	3.7	16.4	3.4	3.6
Stander[1]	39.7	7.0	14.5	81.6	5.3	35.6
CIho 4196[1]	2.3	1.4	6.4	7.0	4.4	12.8
CIho 9699	10.1	--	--	--	9.8	17.2
PI 328642	7.1	--	--	--	9.9	10.0
PI 370919	8.0	--	--	--	5.4	3.7
PI 371317	16.4	--	--	--	2.3	7.4
PI 565567	14.7	--	--	--	0.7	6.0
Chevron[1]	7.4	--	--	--	3.4	3.6
Stander[1]	73.6	--	--	--	5.3	35.6
CIho 4196[1]	8.1	--	--	--	4.4	12.8

[1]Chevron (CIho 1111) and Stander (PI 564743) are resistant and susceptible six-rowed controls, respectively. CIho 4196 is the resistant two-rowed control.

[2]Not tested.

Six-rowed winter barley evaluations. Six hundred winter barley accessions from diverse regions of the world were evaluated for FHB resistance in China in 2001. Less than 1% (56) exhibited FHB severities less than 30% under heavy disease pressure. Many of these accessions headed very early and may have escaped severe infection. Only three accessions (CIho 39516, CIho 2339, and CIho 14296) in this group of 56 had an intermediate heading time and a DON concentration less than 3 ppm (susceptible control Stander=5.4ppm).

***Hordeum vulgare* subsp. *spontaneum* evaluations.** One hundred and ten wild barley accessions from Israel and Jordan were tested in China in 2000. Twenty-three accessions from this group exhibited less than 10% FHB incidence and severity under light disease pressure. In 2001, four hundred additional *H. vulgare* subsp. *spontaneum* accessions from across the Fertile Crescent were evaluated, and forty exhibited FHB severities less than 30% under heavy disease pressure. Two accessions from Israel (PI 391056 and PI 466519) had DON concentrations that were less than 3 ppm compared to 16 ppm in the susceptible cultivar Stander. *Hordeum vulgare* subsp. *spontaneum* exhibited a high degree of genetic diversity for FHB reaction as disease severities ranged from <10% to over 80%. This wild

species may be a useful source of alternative FHB resistance alleles in barley breeding programs.

***H. vulgare´ H. bulbosum* evaluations.** Data were obtained on 26 of the 28 introgression lines planted in China in 2000. Only two lines (38P18/22/3 and 219W3) exhibited less than 10% FHB incidence and severity under light disease pressure. It is not know whether the lower disease severity in these lines was due to the *H. bulbosum* introgression because the *H. vulgare* parent also exhibited relatively low disease levels.

An emphasis was placed on screening six-rowed barley germplasm for resistance to FHB because this type is preferred by the malting and brewing industries in the Upper Midwest. Our results indicate that resistance in six-rowed *H. vulgare* germplasm (both spring and winter types) is very rare. To achieve the highest level of FHB resistance possible, it is best to pyramid resistance genes from different sources including two-rowed types, six-rowed types, and wild barley.

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CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANT GERMPLASM WITH SSR MARKERS LINKED TO FHB RESISTANCE IN SUMAI 3

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INTRODUCTION

Fusarium head blight (FHB) resistant materials have been identified from the USDA spring wheat collection (Zhang et al. 2000a). Analysis of geographical origin and pedigree indicated that diversity exists in this group of resistant materials (Zhang et al. 2000b). DNA markers linked with FHB resistance in Sumai 3 related materials have been identified (Waldron et al. 1999, Bai et al. 1999, Anderson et al. 2001). The most significant genomic region associated with FHB resistance in Sumai 3 has been located on chromosome 3BS. SSR markers Gwm533, Gwm493 and Gwm389 were reported to be closely linked with FHB resistance from Sumai 3 in this region (Anderson et al. 2000, 2001, Zhou et al. 2000). We used these SSR markers to screen putative new sources of FHB resistance identified from the USDA spring wheat collection in an attempt to characterize the relatedness of these new resistant sources with resistance from Sumai 3.

MATERIALS AND METHODS

One hundred and thirty-nine accessions of spring wheat from the USDA spring wheat collection, selected for resistance to FHB based on field and greenhouse inoculations, were used in this study. Sumai 3 and Wheaton were used as the positive and negative check, respectively. DNA was extracted from 0.3g young leaf tissue. SSR primers of Gwm533, Gwm493 and Gwm389 were synthesized by Life Technologies Inc. according to the sequence information published by Roder et al. (1998). The PCR products were visualized by silver staining.

RESULT AND DISCUSSION

The materials were highly polymorphic for these three markers. The average allele at a given locus is 1.1. The DNA fragments amplified by primers Gwm389, Gwm493, and Gwm533 were 176bp, 135bp, and 145bp, respectively. Nine percent of the materials (13 accessions) were positive for all three markers (Table 1). Three of these lines are landraces from Europe, and the rest are from South America. Five of the lines from Uruguay, PI 225375, PI 225376, PI 225384, PI 225444, and PI 225448 shared same pedigree, Sinvalocho/Petiblanco. One of the parents, Petiblanco was moderately resistant in the field, and very resistant to point-inoculation in the greenhouse (Zhang, *unpublished*). Two of the lines in this group, Excelsior and 69Z108.42, have exhibited resistant levels equivalent to, or better than, Sumai 3 in the field.

Twelve lines (8.6%) were positive for the two tightly linked markers, Gwm493 and Gwm533 (Table 2), including several of the most resistant lines (i.e. Tokai 66, Nobeoka Bozu, and Abura) identified from our germplasm project (Zhang et al. 1999, 2000a). Three of the lines from Uruguay had the same pedigree, Sinvalocho/Petiblanco as those in Table 1. Ban (2000) reported that Nobeoka Bozu has one FHB resistance gene identical to Sumai 3, and our result may corroborate that finding. Seven lines (5%) were positive for Gwm389 and Gwm493 (Table 3). Fifty-eight lines (41%) had one of the three markers, and 50 lines (35%) did not have any. Part of the FHB resistant lines that were negative for any of these markers is listed in Table 4. This group of materials is of particular interest because the FHB resistance is likely not related to the major component of resistance from Sumai 3. Results from this study further support our hypothesis that genetic diversity for FHB resistance exists in the USDA spring wheat collection.

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Table 1. Accession, origin, and pedigree of lines with three SSR markers, Gwm389, Gwm493, and Gwm533.

Accession ID	Origin	Pedigree
Citr 5103	274	Argentina Landrace
PI 163424	Argentina	
PI 104131	EXCELSIOR	Argentina Arminda/Virtue
PI 214394	COLOTANA 1085/50	Brazil Colonista/Frontana
PI 225375	Uruguay	Sinvalocho/Petiblanco
PI 225376	Uruguay	Sinvalocho/Petiblanco
PI 225384	Uruguay	Sinvalocho/Petiblanco
PI 225444	Uruguay	Sinvalocho/Petiblanco
PI 225448	Uruguay	Sinvalocho/Petiblanco
PI 264927	220	Greece Landrace
PI 337151	MAGNIF 100	Argentina
PI 349494	1112 BS	Switzerland Landrace
PI 35076	869Z108.42	Austria Landrace

Table 2. Accession ,origin, pedigree of lines with Gwm493 and Gwm533.

Accession ID	Origin	Pedigree
PI 182561	SIN CHUNAGA	Japan
PI 225396	Uruguay	Sinvalocho/Petiblanco
PI 225519	Uruguay	Sinvalocho/Petiblanco
PI 225525	Uruguay	Sinvalocho/Petiblanco
PI 344465	A. A. L.	Argentina complex
PI 351998	TA 3332	Finland
PI 382140	ABURA	Brazil
PI 382153	NOBEOKA BOZU	Japan
PI 382161	TOKAI 66	Brazil
PI 584926	PANTANEIRO	Brazil Sonora63*2/Lagoa Vermelha
PI 83729	MAGYAROVAR 81	Hungary
PI 92387	BELOZIORNAYA NO. 604	Russian

Table 3. Accession, origin, and pedigree of lines with Gwm389 and Gwm493

Accession ID	Origin	Pedigree
PI 214396	COLOTANA 2107/50	Brazil Colonista/Frontana
PI 344454	BUCK AUSTRAL	Argentina complex
PI 344467	ONCATIVO INTA	Argentina complex
PI 35086969Z108.164		Austria landrace
PI 351743	CLUJ 49-926	Romania
PI 351748	JASI 10T	Romania
PI 81791	PI 81791	Japan

Table 4. Accession, origin, pedigree of lines negative for Gwm389, Gwm493, or Gwm533.

Accession ID	Origin	Pedigree
Citr 11215	Belgrade 4	Yugoslavia
Citr 12002	RenacimientoUruguaycomplex	
Citr 13136	Rio Negro	BrazilSupresa/Centenario
PI 163429	Argentina	
PI 168727	BahienseArgentina	K./E.
PI 184512	H 51	ArgentinaA./Favorito//U.
PI 185383	3084	Argentina
PI 185843	SurpresaBrazi	Polyssu/A.
PI 192634	TrintecincoBrazil	A./A.
PI 192660	Prodigio Italiano	Italy
PI 197128	Shinchunaga	Japan
PI 197664	ArgentinaThatcher	//L./R6
PI 213682	Buck 62/52	Argentina
PI 225457	Uruguay38	M.A./Petirrojo
PI 225504	Uruguay	Sinvalocho/Petiblanco
PI 225516	Uruguay	Sinvalocho/Petiblanco
PI 285933	Chudoskaja	Poland
PI 349534	533b	Switzerland
PI 351476	Vaulion	Switzerland
PI 351649	263.25-2	SwitzerlandHuron/Newthatch
PI 351993	Z.88.54	Switzerland
PI 352000	Z.89.37	Switzerland
PI 382154	Nyu Bai	Japan
PI 434987	Estanzuela Y.	Uruguaycomplex
PI 519798	PF79782	Brazilcomplex

A PROCEDURE OF PRODUCING *FUSARIUM GRAMINEARUM* CONIDIA IN LARGE QUANTITIES

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INTRODUCTION

Fusarium graminearum is the major pathogen causing Fusarium head blight (FHB) on wheat. We apply conidial inoculum of *F. graminearum* in the field screening nurseries to ensure successful disease development. However, field spray inoculation requires large quantities of conidia. We use solid medium to produce conidia because it is easier to make and culture is less likely to be contaminated than liquid medium. On the solid medium, it was observed that the amount of mycelium growth is negatively associated with the ability to develop conidia and streaking the surface of the culture can suppress the mycelium growth and promote conidial production (Zhang, *unpublished*). This report summarizes our research efforts in developing a procedure of producing large quantities of inoculum.

MATERIALS AND METHODS

Four isolates of *F. graminearum*, Fg1, Fg4, Fg62, and Fg63, part of the composite consisting of 10 isolates for field inoculation, were used in this study. They were cultured on three types of medium, 1/2 PDA (19.5g potato dextrose, 10g agar, 1L dH_20), 1/2 PDA with 0.5ml/L (or 0.05% in volume) of lactic acid, and 1/2 PDA with 2.0ml/L (or 0.2%) of lactic acid. Each petri plate contained 20ml of medium. Each isolate was cultured on three plates of each medium. Isolates were transferred and spread evenly onto agar plates with a sterile glass hokey stick. Plates were incubated at 23C with 12hr light. Conidia were harvested every three days until day 15 by adding 5ml of sterile distilled water into a plate, and sweeping the agar surface with a sterile glass hockey stick. The conidial suspension was collected with a micropipette. The number of spores from each plate was determined based on haemacytometer sampling. The number of spores/plate on each harvest day was the average of the three plates. After each harvest, the plates were re-incubated. The experiment was conducted three times at one-week interval. Data were subjected to ANOVA after proper data transformation.

RESULTS AND DISCUSSION

The number of conidia harvested over time was presented in Figures 1 to 4. Log-transformation was conducted for conidia/plate on each isolate before data were subjected to analysis. Analysis of variance did not detect differences among the three trials, thus, the number of conidia/plate on each medium was averaged over the three trials. The effect of isolates was significant ($P<0.0001$). Among the four isolates of *F. graminearum*, Fg1 and Fg4 were most productive. After first three days of incubation on 1/2 PDA, for instance, 1.1×10^8 conidia/plate were produced by Fg1 (Fig. 1) and Fg4 (Fig. 2) while half of that were produced by isolates Fg62 (Fig. 3) and Fg63 (Fig. 4). The number of conidia harvested from different days varied greatly, as expected. The second harvest after another three-day incubation period could still

yield substantial number of conidia. The number of spores produced after the second harvest, however, was minimal.

Although medium with a high concentration of lactic acid (0.2%) suppressed conidial production slightly at the beginning, this medium sustained a higher spore production in the subsequent harvests. For example, the second harvest of isolate Fg4 on the acid medium yielded 70% spores of the first harvest (Fig. 2), while only 28% was obtained from the medium with no addition of lactic acid. Similar trends were also observed on other isolates (Fig. 3 and Fig. 4). Analysis of variance indicated that the number of conidia harvested from different media was significantly different for a given isolate except for the first harvest.

SUMMARY

Results from this research suggested that secondary harvests of *F. graminearum* conidia on solid media could be productive, and addition of lactic acid (0.2%) could sustain conidial production better than regular medium. Based on our experience, this is a time/supply saving protocol for large quantity inoculum production.

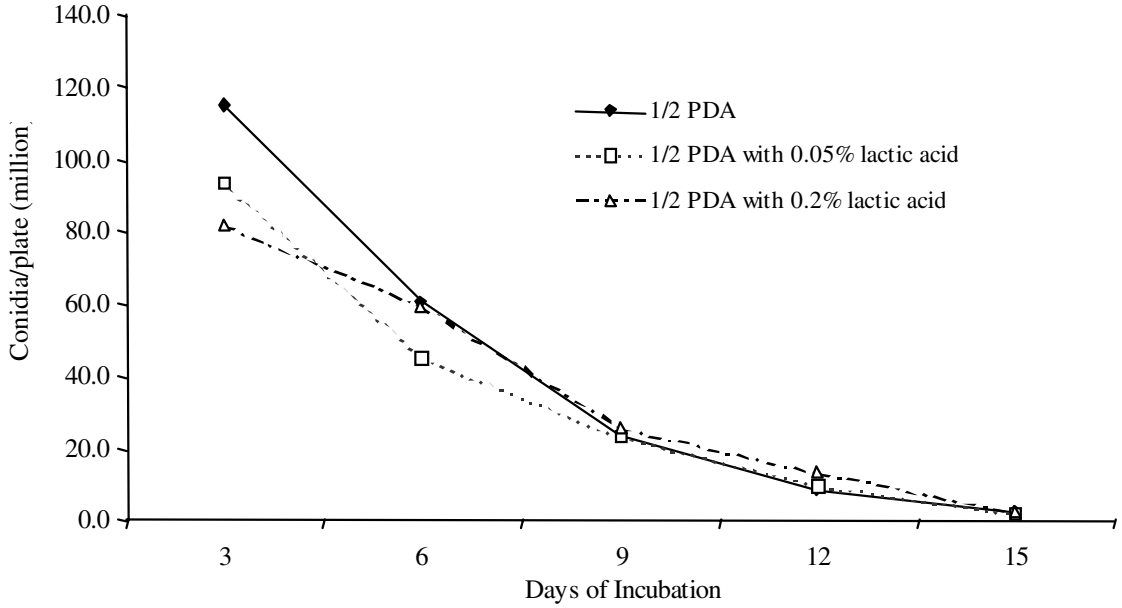

Fig. 1. Conidial production of isolate Fg1.

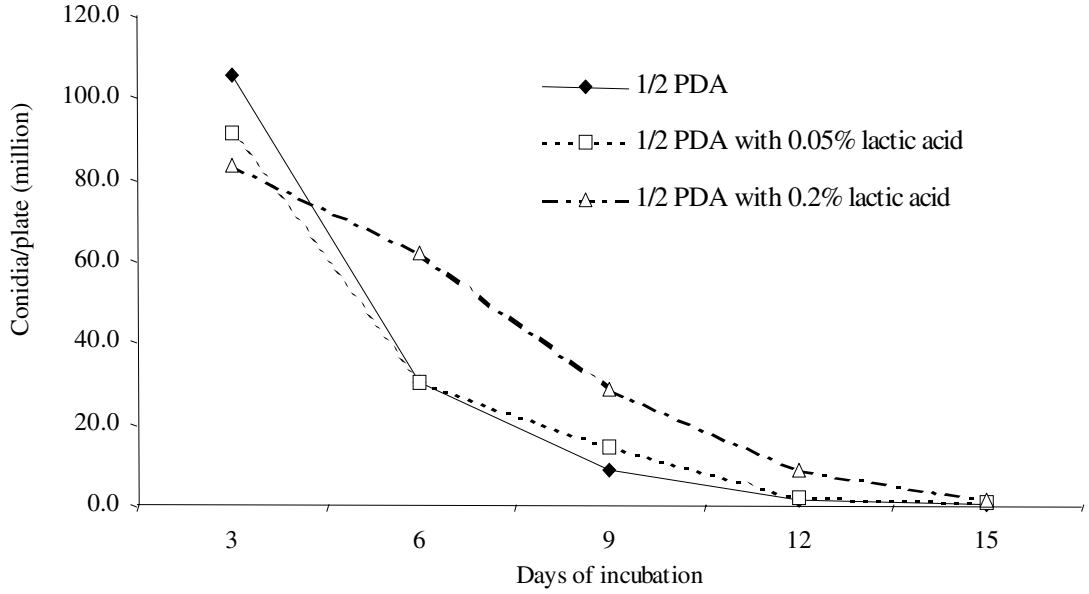

Fig. 2. Conidial production of isolate Fg4.

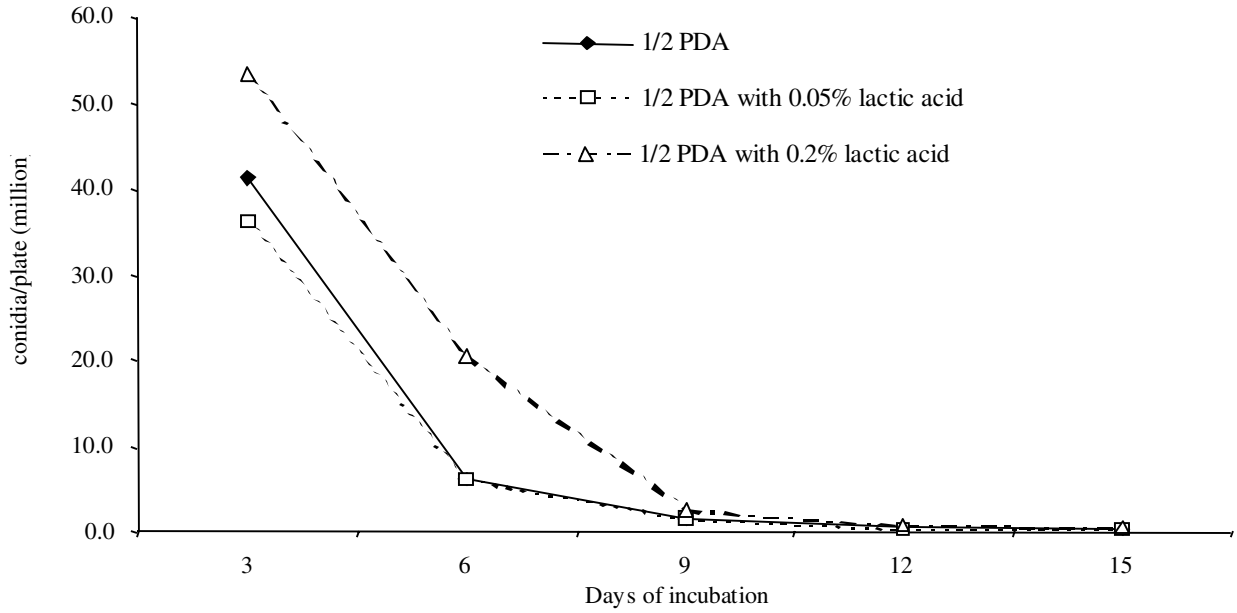

Fig. 3 . Conidial production of isolate Fg62.

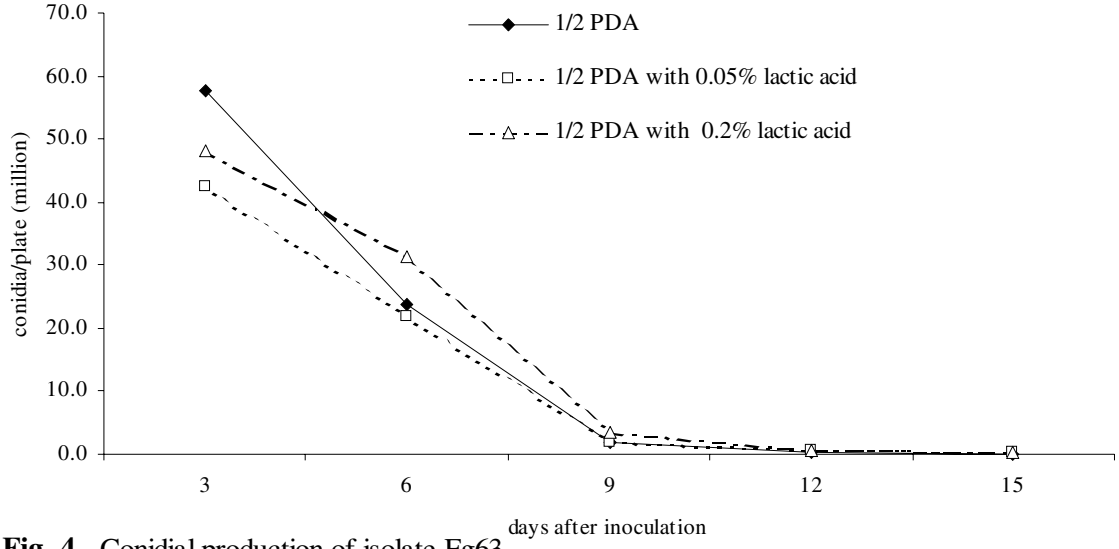

Fig. 4. Conidial production of isolate Fg63.

EVALUATION OF USDA SPRING WHEAT GERMPLASM FOR FUSARIUM HEAD BLIGHT RESISTANCE.

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INTRODUCTION

The use of host resistance will be one of the major components in managing Fusarium head blight (FHB) of wheat. Since 1998, we have been systematically evaluating the USDA spring wheat collection in an attempt to characterize the variations for FHB resistance in this germplasm pool, initially focusing on germplasm originated from regions where FHB has been problematic. A number of accessions were found to be moderately resistant to resistant (Zhang et al. 2000a). Analysis of geographical distribution and pedigree of these selections indicated that considerable diversity for FHB resistance exists in the USDA spring wheat collection. Since 2000, our search for new sources of resistance has been extended to germplasm from most of the European countries while we continue our evaluation of the eastern Asian and South American materials. This report summarizes the progress on the USDA germplasm screening, and the screening methodology we used in 2001.

GERMPLASM SCREENING METHODS

In 2001, our FHB screening project followed a germplasm screening system we have developed (Zhang et al. 2000a) with a few modifications as described bellow.

Preliminary Screening Nursery (PSN): Spring wheat germplasm from the USDA collection (Aberdeen, ID) were first evaluated in the PSN nursery. This is a non-replicated nursery with entries planted into rows (ca. one meter in length). ND 2710 and BacUp were used as resistant checks and Sonalika and Wheaton as susceptible checks with a check-to-entry ration of 1:28. The nursery management, inoculation, and data collection were as described previously (Zhang et al. 1999, 2000a) except second spray inoculation was applied four days after the first spray. Selections were made based on FHB index and seed infection and increased in off-season nursery. After row/plant selection, the entire PSN was harvested in bulk, and mass selection was made based on kernel weight. The composite will be evaluated and selected under FHB pressure for several cycles (years) before individual lines will be derived.

Elite Germplasm Nursery (EGN): Resistant selections from the previous year's PSN is advanced to EGN. The entries of EGN were planted into row plots (one meter in length) with three replicates and arranged into split-plot design, with maturity as the main plot and genotype as the subplot. In 2001, there were four maturity groups based on days between planting and flowering: early (£55), intermediate early (55-60), intermediate late (61-65), and late (³66). Nursery management, inoculation and data collection were the same as in PSN. Data on Fusarium damaged kernels (FDK), DON, and seed weight/row were collected after

harvest. Selections evaluated for two years in EGN were entered into an advanced EGN nursery (with doubled plot size) for a final evaluation.

Greenhouse confirmation and selection: Selections from PSN except the composite are evaluated in the greenhouse with point and spray inoculations (Zhang et al. 1999). A floret at the middle of a spike is injected with a *Fusarium graminearum* conidial suspension (ca. 70,000 conidia/ml) when the plant is at full heading to beginning of anthesis. Spray inoculation (ca. 50,000 conidia/ml) was done at the beginning to half anthesis stage. Inoculated plants are incubated in a mist chamber in the greenhouse for 72h. Approximately 15 to 30 spikes of each entry were tested by each of the two methods.

Uniform Regional Scab Nursery (URSN): Each year, five most FHB resistant lines in second year EGN nursery are selected and contributed to the URSN for spring wheat. These entries are selected based on origin, pedigree, and agronomic traits, which may imply uniqueness of the resistance in addition to selections based on FHB resistance. Testing these resistant sources across the region allows a vigorous evaluation of the elite germplasm through replicated trials over multiple locations. This approach also provides the accessibility to individual programs for utilizing these resistant sources if they desire to do so.

RESULTS AND DISCRUSSION

In 2001, a total of 1262 spring wheat accessions were evaluated in PSN and the origin of the germplasm is listed in Table 1. One hundred forty-one accessions were selected for further testing in the greenhouse and as entries of the 2002 EGN.

One hundred thirty-one entries of the 2001 EGN were from the 2000 PSN selections. A small portion (11 lines) were eliminated. Interestingly, only one of the discarded lines entry was in the early nursery maturity group. Apparently Thus, maturity had a significant effect on FHB field infection. Although a combination of resistance to field floret tissue infection, seed infection, point-inoculation, and toxin was not common, lines possess a few of those components were readily found in this group of materials. A few of these linesLines with high level of resistance in the field (low FHB index and low FDK) and resistant to point inoculation in the greenhouse are listed in Table 2.

Table 3 lists lines evaluated for two or more years in the EGN. Selections were grouped into three categories: 1) lines with low FHB indices (≤40%) AND low FDK (≤40%); 2) lines with low FDK (≤40%) but high FHB indices (>40%); and 3) lines with low FHB indices (≤40%) but high FDK (>40%). We have observed that most lines from group 1 exhibited stable FHB reaction over years, whereas materials in group 2 were variable. Materials in group 3, namely Sin Chunaga, Norin 61 and several other lines originated from Japan, consistently showed a lower disease index, but high seed infection (in the form of bleached seed, not tombstone kernels). The level of geographical diversity was unexpectedly high. Diverse origins of these selections may imply that potential genetic diversity for FHB resistance exists in the spring wheat gene pool (Zhang et al. 2000c). A number of selections were landraces, likely possess different resistant genes. Studies are in progress to elucidate the

genetics of resistance and allelic relations among these new resistant sources (Zhang et al. 2000a).

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Table 1 . Country of origin and number of accessions tested in the 2001 PSN

Country[a] of origin	Number of accessions	Country of origin	Number of accessions
Europe	705	South America	557
Austria	51	Argentina	50
Finland	44	Bolivia	53
France	30	Brazil	44
Greece	54	Chile	100
Portugal	150	Colombia	50
Sweden	50	Ecador	66
Switzerland	57	Uruguay	27
Yugoslavia	38		

[a]Countries with less than 20 entries were not listed.

Table 2. Accessions in the 2001 EGN exhibiting low infection in the field and greenhouse evaluations.

Accession or ID	Origin	FHB index (%)	FDK (%)	DON (ppm)	FHB (%) with point inoculation
PI 225396	Uruguay	19.0	10.0	13.3	17.0
PI 225516	Uruguay	18.8	6.0	5.9	32.6
PI 225467	Uruguay	15.4	8.3	5.8	17.2
PI 225382	Uruguay	16.4	13.0	8.4	18.6
ND 2710--CK	ND	14.2	15.0	11.1	15.0
Wheaton--CK	MN	85.0	95.0	36.7	95.0

Table 3. Fusarium head blight resistant accessions of spring wheat selected from the USDA collection that have been evaluated for the second year in the EGN.

Accession	ID	Country of origin	FHB[c] index (%)	FDK[d] (%)	DON[e] (ppm)	Maturity[f] group
PI 478282	Sonalika --CK	India	85.7	79.9	37.0	Early
PI 469271	Wheaton--CK	MN, USA	87.7	95.7	22.5	Early
	ND 2710--CK	ND. USA	11.5	19.4	5.0	Early
PI 596533	BacUp--CK	MN, USA	21.0	30.4	7.7	Early
PI 382161[a]	Tokai 66	Brazil	8.5	14.0	1.9	Interm.
PI 349478[b]	193C	Switzerland	8.9	14.0	5.0	Early
PI 382154	Nyu Bai	Japan	10.6	15.0	2.2	Interm.
PI 350768[b]	69Z108.42	Austria	12.8	19.8	4.6	Early
PI 382153	Nobeoka Bozu	Japan	14.0	14.9	4.2	Interm.
PI 382140[b]	Abura	Brazil	16.4	35.7	3.7	Early
PI 182568	Norin 34	Japan	18.7	38.3	6.8	Interm.
PI 382167[a]	16-52-9	Brazil	19.7	20.7	4.9	Late
CItr 5103	274	Argentina	20.1	21.2	11.2	Late
PI 434987	Estanzuela Young	Uruguay	21.5	42.0	10.1	Early
CItr 12002	Renacimiento	Uruguay	23.0	39.2	9.3	Interm.
PI 519790	274-1-118	Uruguay	24.1	36.7	8.5	Early
PI 345731	Tezanos Pintos Precoz	Argentina	24.9	20.0	9.2	Early
PI 462151	Shu Chou Wheat No. 3	China	24.9	19.4	5.8	Interm.
PI 192660	Prodigio Italiano	Italy	27.3	20.8	10.8	Late
PI 163429		Argentina	28.4	27.5	10.0	Early
PI 185380[b]	Prodigio Italiano	Italy	28.6	21.8	8.2	Late
PI 351256	Japon 2	Japan	28.7	34.2	8.0	Early
PI 81791a	Sapporo Haru K.	Japan	30.2	19.8	15.3	Late
CItr 12021	Centenario	Uruguay	32.0	35.8	8.4	Early
PI 285933	Chudoskaja	Poland	32.6	24.2	16.8	Interm.
PI 382144	Encruzilhada	Brazil	33.5	39.2	8.0	Late
PI 351221	Newthatch Sel.	Switzerland	34.6	20.0	9.9	Late
PI 104131[b]	Excelsior	Argentina	37.4	17.8	5.8	Interm.
PI 168727	Bahiense	Argentina	37.8	22.5	12.9	Early
PI 192634	Trintecinco	Brazil	38.4	34.2	9.5	Early
CItr 13136	Rio Negro	Brazil	39.0	36.0	12.6	Early
CItr 2492	Manchurian	China	40.0	22.5	9.4	Early
PI 264927	220	Greece	40.0	18.3	4.6	Interm.
CItr 17427	16-52-2	Brazil	41.6	30.0	19.4	Early
PI 362437	III/14-B	Yugoslavia	43.9	27.9	8.1	Late
PI 83729	Magyarovar 81	Hungary	44.1	34.5	9.0	Late
PI 185843	Surpresa	Brazil	44.4	33.3	10.8	Interm.
PI 264998	628	Greece	45.8	28.3	8.2	Interm.
PI 294975	Artemowska	Bulgaria	45.8	20.0	10.4	Late
PI 163428		Argentina	47.3	37.3	7.1	Late
PI 344467	Oncativo Inta	Argentina	49.2	31.9	5.2	Early
PI 256958	Academia 48	Romania	50.7	23.3	11.8	Interm.
PI 264940	111a	Greece	51.5	33.8	9.3	Early
PI 351743	CLUJ 49-926	Romania	52.0	28.0	9.1	Late
PI 519798	PF 79782	Brazil	53.8	25.8	14.3	Interm.
PI 351748	JASI 10T	Romania	56.3	36.7	10.2	Late
PI 184512	H 51	Argentina	57.2	26.2	14.8	Early

Table 3. (Continued)

Accession	ID	Country of origin	FHB[c] index (%)	FDK[d] (%)	DON[e] (ppm)	Maturity[f] group
PI 351993	Z.88.54	Switzerland	57.8	27.5	6.8	Interm.
PI 344465	Laureano Alv. Laah	Argentina	59.3	33.3	7.8	Interm.
PI 362043	Arnaut De Toamina	Romania	60.5	28.3	10.7	Late
PI 349534	533B	Switzerland	60.8	23.3	11.8	Interm.
PI 168716	Klein Condor	Argentina	61.8	33.0	14.0	Interm.
CItr 11215	Belgrade 4	Yugoslavia	61.9	32.0	9.9	Late
PI 584934	Whestphalen	Brazil	63.5	37.1	11.8	Interm.
PI 192219	Hatvani	Hungary	64.0	30.0	8.7	Late
PI 351187	Taillens Velu Sel.	Switzerland	64.5	30.3	15.0	Late
PI 351476	Vaulion	Switzerland	65.6	32.5	13.4	Late
PI 113949	Stepnjachka	Ukraine	66.9	31.2	8.6	Late
PI 352000	Z.89.37	Switzerland	67.2	35.0	8.1	Interm.
PI 192229	Gran Commune Ung.	Romania	67.8	35.8	22.7	Late
PI 349447	1882A	Switzerland	69.1	33.3	17.5	Late
PI 344454	Buck Austral	Argentina	70.8	29.4	14.2	Late
PI 113948	Kooperatorka	Ukraine	76.3	34.8	7.1	Late
PI 182561	Sin Chunaga	Japan	21.4	81.7	14.4	Interm.
PI 360869	Fujimi Komugi	Japan	22.2	51.7	--	Late
PI 182586	Norin 43	Japan	25.3	53.0	11.4	Early
PI 411132	Gogatsu-Komugi	Japan	25.9	72.1	10.6	Early
PI 197128	Shinchunaga	Japan	26.6	78.3	11.4	Interm.
PI 182583	Chuko	Japan	29.9	76.9	7.1	Interm.
PI 351816	Froment Du Japon	Japan	31.1	58.5	8.4	Early
PI 182591	Norin 61	Japan	34.6	53.8	7.1	Interm.

[a] & [b] Lines were entered into the URSN for Spring Wheat in 2000, and 2001, respectively.
[c] & [d] FHB index and FDK data were averages between 2000 and 2001.
[e] DON data were based on 2001 field nursery only.
[f] Maturity groups were based on days between planting and flowering: ≤60 (early), ≥64 (late), and 61-63 (intermediate).