SESSION 5:

VARIETY DEVELOPMENT AND HOST RESISTANCE

Co-Chairpersons: Steve Harrison and Brian Steffenson

VALIDATION OF QTLASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE IN THE SOFT RED WINTER WHEAT, 'ERNIE'. Z. Abate¹, S. Liu² and A.L. McKendry^{1*}

¹Division of Plant Sciences, University of Missouri, Columbia, MO 65211; and ²Crop and Soil Environmental Sciences Department, Virginia Polytechnic Inst. and State Univ., Blacksburg VA 24061-0404 *Corresponding Author: PH: (573) 882-7708; Email: mckendrya@missouri.edu

OBJECTIVES

To validate, QTL on 2B, 3BSc, 4BL and 5AS associated with type II resistance (FHB severity; FHBS) and low deoxynivalenol (DON) in the soft red winter wheat 'Ernie'.

INTRODUCTION

Fusarium head blight (FHB) caused by Fusarium graminearum Schwabe [telomorph: Gibberella zeae Schw. (Petch)] reduces yield and quality of wheat (Triticum aestivum L.) when persistent rainfall occurs during heading. Although a significant amount of genetic variation exists for FHB resistance in winter wheat, breeding has been hindered by the complexity of resistance, the difficulty associated with screening large numbers of wheat genotypes at heading and the need for multiple screening environments to both identify and confirm resistance. Marker-assisted selection (MAS) applied at the seedling stage, should enable breeders to discard large numbers of susceptible lines earlier in the breeding stream thereby accelerating the development of FHB resistant genotypes. Provided markers linked to FHB resistance genes are available and validated, MAS should also facilitate the rapid introgression of multiple FHB resistance genes into individual lines and the quick recovery of recurrent parent genetic backgrounds thus avoiding the linkage drag that commonly plagues the use of exotic resistance sources. In the past decade more than 100 QTL associated with different types of FHB resistance have been reported, distributed on 20 out of the 21 wheat chromosomes with multiple QTL often being located in same genomic regions (Buerstmayr et al., 2008). The only exception is chromosome 7D. To date, however, only a limited number of QTL have been validated and thus most

are not being used for MAS. The U.S. cultivar, Ernie, is a widely-used source of FHB resistance in an adapted soft red winter wheat background. In Ernie, four QTL have been identified on chromosomes 2B, 3BSc, 4BL and 5AS that are associated with FHBS (Liu et al., 2007), low deoxynivalenol (DON), and kernel quality retention (Abate et al., 2008), however, none has been validated in genetically related populations or breeding lines. This study was designed to validate these four QTL using advanced breeding lines derived from crosses involving Ernie and other susceptible parents. Marker-trait associations were used to confirm the effect of each QTL on both FHBS and DON content.

MATERIALS AND METHODS

Thirty-one F_4 -derived F_7 and F_8 lines from three crosses made between Ernie as the resistant parent and AgriPro Hickory, Pioneer ® Variety 2510, and IL 87-1917-1 as susceptible parents were used for this validation study. Lines within crosses were selected based on their FHBS reaction. Across all three crosses 17 resistant lines and 14 susceptible lines were included. Ten plants per line arranged in a randomized complete block design with three replications were evaluated in the greenhouse for type II resistance. Lines within each cross were classified as resistant, moderately resistant, moderately susceptible, and susceptible based on the respective LSD $_{(0.05)}$. Seed from each plant was bulked within line and replication and evaluated for DON content at Michigan State University in East Lansing, MI. Deoxynivalenol content was quantified in ¹/₄g g⁻¹ using the mycotoxin extraction kit Veratoxin for DON 5/5 (Veratox®, Lansing, MI). Thirteen Xgwm (Röder et al., 1998), Xbarc (Song et al., 2005), or *Xwmc* (Somers et al., 2004) SSR markers flanking QTL on chromosomes 2B,

3BSc, 4BL and 5AS were used to genotype the Erniederived lines and validate previously identified QTL. Markers spanned < 5, ~ 17, ~12 and ~50 cM on chromosomes 2B, 3BSc, 4BL and 5AS, respectively. Genotyping followed procedures described by Liu et al. (2007). Alleles were scored as derived from Ernie (E), non-Ernie (N) or as heterozygous (H). Dummy variables were defined for markers that amplified two (E or N) and three (E, N, and H) alleles according to the following formula where, X_is are marker codes and a, b, c represent variables for lines carrying the E, N and H alleles, respectively.

$$\begin{array}{ccc} & 1 \\ X_{ia} = & \\ & 0 \\ \end{array} \left. \begin{array}{ccc} \text{If Ernie} & 1 \\ & X_{ib} = & \\ & 0 \\ \end{array} \right. \begin{array}{ccc} \text{If not Ernie} & 1 \\ & X_{ic} = & \\ & 0 \\ \end{array} \right. \left. \begin{array}{cccc} \text{If not Ernie} & 1 \\ & X_{ic} = & \\ & 0 \\ \end{array} \right. \right\}$$

Further statistical analyses were carried out using these dummy variables. Marker variables were selected using stepwise regression analysis with PROC REG (SAS Institute 2007). Markers included in the model for the two resistance traits were determined based on 5 % significance level, adjusted R^2 , and C_p criterion, where C_{p} measures the predictive ability of a fitted model. Multiple regression analysis was carried out using the selected markers and the variation explained by each marker was obtained from the partial R² in the sequential analysis option of PROC REG (SAS Institute 2007). Regression models were used to estimate the predicted values of lines carrying combinations of markers. The predicted values for the two resistance traits were compared with the respective observed values of lines carrying similar combinations of alleles to determine the usefulness of markers in selection programs. Useful markers linked to each QTL associated with FHBS and DON were considered important if lines carrying favorable QTL alleles had significantly lower FHBS and/or DON accumulation. Final marker-trait association was determined based on genotypic and phenotypic association in resistant and susceptible lines from each cross.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) conducted on combined data across the three crosses showed significant differences for the both FHBS and DON content among lines derived from Ernie crosses. Within crosses, lines also differed significantly for both traits. Single factor ANOVA on combined data across the three crosses indicated a significant marker-trait association for most of the tested markers. Of the six 5AS markers, however, only one marker (*Xbarc165*) was significant across combined data reflecting the lower marker density on that chromosome arm. Sample data for 4 markers associated with QTL peaks for FHBS and DON (Liu et al., 2007; Abate et al., 2008) are given in Table 1. Within crosses, marker-trait associa-

If heterozygous Otherwise tions were less consistent. Most markers on 2B, 3BSc were significant in Ernie/AgriPro Hickory and Ernie/ IL87-11917-1 while those on 4BL were significant in Ernie/AgriPro

Hickory and Ernie/Pioneer ® variety 2510. Markers on 5AS were significant only in Ernie/AgriPro Hickory, probably due again to the lack of marker density on 5AS and the lack of tight linkage of markers to the QTL on that chromosome arm. Reduction in FHBS associated with *Xgwm319* on 2B was 33% in Ernie/ AgriPro Hickory lines and 46% in lines derived from the Ernie/IL87-1917-1 cross (Table 1) whereas the average reduction associated with *Xgwm285* on 3BSc was 34 % and 53 % in lines derived from the two crosses, respectively. The Ernie allele on 4BL was associated with reduced FHBS in Ernie/AgriPro Hickory and Pioneer ® Variety 2510/Ernie lines, but was not significant in lines derived from Ernie/IL 87-1917-1. Similar trends were observed for DON.

Multiple regression analysis was used to identify combinations of markers significantly associated with reduced FHBS and DON accumulation. Across crosses, results suggested that the critical markers were Xgwm319 on 2B, Xgwm285 on 3BSc, and Xgwm495on 4BL which together were predicted to reduce FHBS by 67% and DON by 69%. Where marker combinations were actually observed in the data set, predicted and observed reductions in both FHBS (R²=0.97) and DON (R²=0.93) were highly correlated. **Table 1.** Mean Fusarium head blight severity (FHBS) and deoxynivalenol (DON) content of thirty-one F_7 or F_8 lines derived from Ernie crosses. Lines were greenhouse inoculation with *Fusarium graminearum*, genotyped with SSR markers linked to 4 QTL associated with FHBS and DON content in Ernie, and classified according to whether or not they carried alleles from Ernie (E), non-Ernie (N), or were heterozygous (H).

		Markers linked to QTL for FHBS and DON							
		2B - X	gwm 319	$3BSc - \lambda$	Kgwm 285	$4BL - X_2$	gwm 495	$5AS - \lambda$	Kbarc56
Cross	Allele	FHBS	DON	FHBS	DON	FHBS	DON	FHBS	DON
Combined	Е	24±4	9±3	27±4	10±2	24±4	8±2	28±4	12±2
	Ν	36±4	17±3	76±7	56±5	50±5	28±3	36±5	13±4
	Н	-	-	-	-	-	-	29±5	10±4
Significance		**	**	***	**	***	***	NS	NS
Ernie/AgriPro Hickory	Е	19±3	6±3	23±2	9±3	19±3	7±3	22±3	9±3
	Ν	52±5	30±3	61±10	37±7	53±5	30±4	49±7	27±5
Significance		***	***	**	**	***	***	***	**
Ernie/IL87-1917-1	Е	21±7	8±8	31±6	10±3	35±10	10±9	35±10	41±9
	Ν	67±7	44 ± 8	84±9	74±5	53±10	42±9	53±10	10±9
	Н	-	-	-	-	-	-	-	-
Significance		**	*	**	***	NS	NS	NS	NS
Pioneer 2510/Ernie	Е	-	-	27±4	9±3	24±4	7±3	27±6	9±4
	Ν	27±4	9±3	-	-	44±6	19±4	24±6	6±4
	Н	-	-	-	-	-	10 ± 5	29±5	10±3
Significance		NA	NA	NA	NA	***	***	NS	NS

Understanding the genotypic difference with respect to markers present in resistant and susceptible lines is central to validating QTL and eventually recommending markers for marker-assisted-selection programs. Phenotypic and genotypic information for resistant and susceptible lines are given in Table 2. For all crosses, most resistant lines inherited all three alleles from the resistant parent, Ernie, while the majority of susceptible lines carried two or three non-Ernie (N) alleles.

Across crosses, lines classified as moderately resistant or moderately susceptible were less consistent. These results were not unexpected in that breeders frequently have difficulty classifying lines that have intermediate levels of resistance.

In summary, results suggest that QTL on 2B, 3BSc and 4BL have significant effects on both FHBS and DON accumulation in lines derived from Ernie. As such, they should be useful in combination with phenotypic selection for identifying resistant lines and eliminating susceptible lines from breeding streams. These markers were less useful in differentiating among moderately resistant and moderately susceptible lines. Markers on 5AS may also be useful; however, more tightly linked markers must be identified prior to using this QTL for MAS.

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DISCLAIMER

Any opinions, findings conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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Table 2. Phenotypic and genotypic differences among resistant and susceptible lines derived from crosses with the resistant parent Ernie and four susceptible soft red winter wheat lines. Lines were phenotyped by point-inoculation with *Fusarium graminearum* in the greenhouse at the University of Missouri and genotyped with significant markers at each of four QTL on chromosomes 2B, 3BSc, 4BL and 5AS.

	Line	Resistance	FHBS	DON	2B	3BSc	4BL
Cross	no.	$ eve ^{\dagger}$	$(\%)^{\ddagger}$	$\mu g g^{-1}$	Xgwm319	Xgwm285	Xgwm495
Ernie/AgriPro Hickory	F ₈ -11	R	9.0 a	4.8 a	Е	Е	Е
	F ₈ -21	R	11.6 a	2.7 a	E	E	E
	F ₈ -14	R	12.2 a	2.8 a	E	E	E
	F ₈ -23	R	12.6 a	5.4 a	E	E	E
	F ₈ -15	R	15.0 a	2.6 a	E	E	E
	F ₈ -22	R	15.3 a	2.4 a	Е	E	E
	F ₈ -20	R	16.0 a	4.1 a	E	E	E
	F ₈ -12	MR	19.3 b	4.5 a	Е	E	E
	F ₈ -16	MR	27.1 b	6.7 a	E	E	E
	F ₈ -13	MS	28.7 c	9.3 a	Е	E	E
	F ₈ -19	MS	31.9 c	15.8 a	Е	E	E
	F ₈ -24	MS	33.1 c	16.5 a	E	E	E
	F ₈ -17	MS	37.6 c	20.6 b	Ν	E	Ν
	F ₈ -18	S	60.4 d	33.3 b	Ν	E	Ν
	F ₈ -10	S	60.7 d	37.0 c	Ν	Ν	Ν
LSD (0.05)			8.9	7.4			
Ernie/IL 87-1817-1	F ₇ -35	MR	20.9 a	13.7 a	Ν	Е	Е
	F ₇ -37	MR	21.8 a	7.2 a	E	E	E
	F ₇ -36	MS	49.8 b	8.2 a	E	E	Ν
	F ₇ -38	S	84.6 c	7.2 a	Ν	Ν	Ν
LSD (0.05)			10.4	9.0			
Pioneer 2510/Ernie	F ₈ -101	R	13.9 a	4.2 a	Ν	Е	Е
	F ₈ -115	R	15.2 a	2.1 a	Ν	E	E
	F ₈ -100	R	17.1 a	4.3 a	Ν	E	E
	F ₈ -107	R	17.9 a	4.3 a	Ν	E	E
	F ₈ -111	R	19.3 a	3.8 a	Ν	E	E
	F ₈ -106	R	20.6 a	8.5 b	Ν	E	E
	F ₈ -96	R	20.8 a	4.9 a	Ν	E	E
	F ₈ -94	MS	34.2 b	9.8 b	Ν	E	E
	F ₈ -98	MS	37.5 b	14.2 c	Ν	E	Е
	F ₈ -105	MS	40.2 b	14.5 c	Ν	E	E
	F ₈ -113	S	42.6 c	21.5 d	Ν	E	Ν
	F ₈ -99	S	44.7 c	17.3 c	Ν	E	Ν
LSD (0.05)			7.4	4.1			

† Resistance level based on resistance in Ernie ($R=\leq 20\%$) and the respective LSD for the cross. ‡ FHBS = Fusarium head blight severity determined as the proportion of infected spikelets on the inoculated head following greenhouse point inoculation with *Fusarium graminearum*.

GENOTYPIC AND PHENOTYPIC SELECTION FOR HEAD SCAB RESISTANCE IN WHEAT. Andres Agostinelli¹, Anthony Clark¹, Gina Brown-Guedira², Yanhong Dong³ and David Van Sanford^{1*}

¹Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546; ²USDA-ARS Raleigh, NC, 27695-7620; and ³Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108 *Corresponding Author: PH (859)257-5020 ext. 80770, Email: dvs@email.uky.edu

OBJECTIVE

To compare phenotypic selection with genotypic selection for FHB resistance in early generations.

INTRODUCTION

Fusarium head blight (FHB), caused by Fusarium graminearum, is a highly destructive disease that affects wheat (Triticum aestivum L.) throughout the world (Mc Mullen et al., 1997). Breeding for FHB resistance is arguably the best way to combat this disease. Historically, the selection process for resistance to scab has been based on phenotypic evaluation of disease incidence and severity in the field, and then estimation of percentage of fusarium diseased kernels (FDK) and deoxynivalenol (DON) content after harvest (Bai and Shaner 1994). However, phenotypic evaluation is time consuming, costly and often inaccurate. Moreover, the inheritance of resistance to FHB is complex and the phenotypic expression is greatly affected by weather (Bai and Shaner, 2004). Given these facts, molecular markers are potentially very useful in breeding for FHB resistance (Van Sanford et al., 2001; Bai and Shaner, 2004). Still, optimizing the balance between phenotypic and genotypic selection remains a significant challenge in the way of improving FHB breeding programs.

MATERIALS AND METHODS

Plant Material - An $F_{2:3}$ population derived from a cross between FHB-susceptible KY93C-1238-17-2 and FHB-resistant VA01W-476 was divided into two subpopulations (Figure 1): one was aimed to be

subjected to phenotypic selection (SPp) and the other to genotypic selection (SPg). The first subpopulation (SPp) comprised 48 $F_{2,3}$ lines planted in headrows in October 2006 in a scab nursery in Lexington, KY. This material was subjected to phenotypic screening: field ratings, incidence and severity were measured in the field; FDK and DON were measured in the seed. For the second subpopulation (SPg), 10 seeds from each of a second group of 48 F_{2.3} lines were planted in pots in the greenhouse in December 2006. Each plant was evaluated for the presence of QTL associated with FHB resistance: the major FHB resistance QTL on chromosome 3BS *Fhb1* (markers used: Xbarc147-3B, Xgwm533-3B and Xsts3B-256) and the resistance QTL on chromosome 2DL (marker used: Xcfd233). Plants homozygous resistant and susceptible for each QTL were selected to be planted in the field in the fall of 2007 (Fig.1).

In October 2007, seed from the greenhouse and field was planted in headrows in scab nurseries located at Lexington, KY (LEX) and Princeton, KY (PRN). The experimental design at each location was a RCB with two replications. Field ratings were recorded. After harvest in June 2008, FDK and DON were measured in kernels harvested from headrows.

Scab Nurseries – the Lexington nursery had an overhead mist irrigation system on an automatic self timer while Princenton nursery was not irrigated. Scabby-corn inoculum (30 g m⁻²) was spread at both locations three weeks before anthesis. At Princeton, plants were additionally treated with conidial suspensions (100,000 spores ml⁻¹) at anthesis at a rate of 30 ml per m of row.



Figure 1. Schematic of the derivation of subpopulations (SP) subjected to genotypic (SPg) and phenotypic (SPp) selection in 2007-2008.

Phenotyping - In 2007, incidence was based on 20 spikes and severity was recorded as an average of 10 spikes (21 days after anthesis). For both 2007 and 2008, field ratings were an estimation of FHB incidence and severity using 1 to 3 scale (1<10%, 2=10% - 90%, 3>90%). FDK was measured using an air separation machine developed from a Precision Machine head thresher and a Shop-Vac vacuum to separate scabby kernels from asymptomatic ones. DON was determined by GC-MS.

Genotyping - Dried leaf tissue samples from 10 seedlings of 48 F2:3 families were submitted to the USDA/ ARS Regional Small Grains Genotyping Lab (RSGGL) at Raleigh, NC in 2006. DNA was extracted and markers amplified by the RSGGL. PCR products were sized using an ABI 3130XL DNA Analyzer and analyzed using GeneMarker (SoftGenetics, LLC).

Data Analysis - For the comparison between genotypic and phenotypic selection, we graphed p-value as a function of the percentage of the population phenotypically selected (Fig.2). The p-value indicates the likelihood that both phenotypically and genotypically selected populations are equal. Thus, when we applied a high phenotypic selection intensity (low % of the population selected), the phenotypically selected population had significantly lower FHB than the genotypically selected one (p<0.05). At low selection intensities (high % of the population selected), it was the other way round. At intermediate selection intensities, both phenotypically and genotypically selected populations were not significantly different (p>0.05).

RESULTS AND DISCUSSION

Phenotypically Selected Subpopulation - The 2007 field experiment represents a typical breeding program selection scheme in which unreplicated headrows are phenotypically selected at one location. Out of the 10 top lines for FDK in 2007, only 4 were among the top 10 in 2008. Out of the 10 top lines for DON in 2007, only 6 were among the top 10 in 2008. This reinforces the general idea that FHB selection should be based on more than one observation.

Despite the fact that in 2007 there was a higher FHB incidence (Mundell, personal comunication), in our study DON was higher in 2008. This may have been due to the fact that the 2007 seed came from the scab nursery. Mean FDK was similar across years and locations. Standard deviations and ranges for both FDK and DON were higher in 2008 (Table 1).

Genotypically Selected Subpopulation - The subpopulation having both resistance QTL (SP1) showed significantly lower FDK and DON than subpopulations having any single resistant QTL (Table 2, 3). The presence of either resistance QTL (*Fhb1* or 2DL) significantly reduced FDK and DON (Table 2, 3). When averaged over both locations, FDK reduction was similar for both resistance QTL but the 2DL QTL showed a significantly higher reduction in DON (mean value, Table 3). Additionally, the 2DL QTL showed a significant (p<0.01) interaction with the environment, while *Fhb1* was stable across environments (data not shown). The relative effectiveness of the 2DL QTL in

Year	2007		20	08	2008		
Location	LEX		LF	X	PRN		
Parameter	FDK	DON	FDK DON		FDK	DON	
Mean	20.65	18.47	19.71	25.37	20.10	25.46	
S.D.	7.14	10.94	11.91	17.42	10.59	14.25	
Range	9.4 - 41.2	4 - 41.4	3.3 - 58.6	2.4 - 68.7	4 - 42.6	2.3 -61.8	

Table 1. Means, Standard Deviations (ST DEV) and ranges for a set of $F_{2:3}$ lines at Lexington in 2007 and their $F_{2:4}$ progeny at Lexington and Princenton in 2008.

Table 2. Means and Standard Deviations (SD) of parents and subpopulations. Subpopulations reflect the presence of resistance alleles at zero, one or both QTL. Different letters indicate significant differences at p<0.05.

		FDK					DC)N	
	Ν	LEX		PRN	ĺ	LEX		PRN	1
Parents		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
VA01W-476	4	3.05 a	1.21	4.05 a	1.47	3.30 a	1.39	2.10 a	0.57
KY93C-1238	4	33.97 b	7.69	32.85 b	8.58	30.15 a	16.17	44.82 b	10.11
Subpopulation									
Fhb1 R + 2DL R (SP1)	30	6.56 a	4.16	7.38 a	4.34	7.49 a	5.07	6.37 a	4.92
Fhb1 S + 2DL R (SP2)	48	12.21 b	3.97	10.36 a	6.75	12.19 b	7.32	8.27 a	5.74
Fhb1 R + 2DL S (SP3)	52	10.07 b	8.11	15.45 b	7.28	14.84 b	7.46	17.71 b	9.99
Fhb1 S + 2DL S (SP4)	60	16.15 c	9.55	22.06 c	11.69	19.88 c	10.06	25.97 c	14.93

this study was surprising. However, results from this one year, one population study must be viewed with caution, although the same trends have been seen in other studies (Jiang et al., 2007; Agostinelli, unpublished).

Genotypic vs. Phenotypic Selection – In contrast with the high level of FHB inoculum of SPp' seed (coming from 2007 scab nursery), SPg' seed came from the greenhouse where it was not exposed to FHB. The different level of inoculum in seed between SPg' and SPp' hindered us from drawing conclusions by comparing both populations. Thus, for making the comparison between phenotypic and genotypic selection we simulated a phenotypic selection using SPg'. To simulate phenotypic selection, one location was treated as the selection environment and the other as the validation environment. For example, entry means ranking from LEX were used to select entries at PRN and vice versa (Fig. 2).

When the percentage of the phenotypically selected population was between 10 and 55 %, the phenotypically selected population did not differ significantly from the subpopulation having both resistance QTL (SP1) for either FDK or DON in either environment. When <10% of the population was selected, the phenotypically selected population was more resistant than SP1. When > 55% of the population was selected, SP1 was more resistant than the phenotypically selected population (Fig. 2I, 2II and Table 3). The results from the comparison between phenotypically selected populations and subpopulations having one resistance QTL varied with parameter measured (FDK or DON) and location (Fig. 2III, 2IV, 2V, 2VI and Table 3).

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DISCLAIMER

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Figure 2. P-value associated with Student's T-test and percentage of the phenotypically selected population (PSP). Higher p-values indicate higher likelihood that PSP is equal to subpopulations with both QTL (I and II), 2DL (III and IV) and *Fhb1* (V and VI) for DON (I, III and IV) and FDK (II, IV and VI) in LEX (dark grey) and PRN (light grey). Between arrows A and B, phenotypic and genotypic selection are not significantly different (p<0.05). To the left of the A arrow phenotypic selection is significantly more resistant and to the right of the B arrow, genotypic selection is more resistant.

Table 3. Means of subpopulations at Lexington (LEX) and Princeton (PRN). Different letters indicate significant differences at p<0.05. *QTL effect was calculated by subtracting the mean of the subpopulation containing the resistance alleles from the mean of subpopulation containing the susceptible alleles and dividing it by the mean of the subpopulation with susceptible alleles.

							EQUIVAL	ENT RANGE**
		Ν	LEX	PRN	Mean Value	QTL Effect*	LEX	PRN
	Fhb1 R + 2DL R (SP1)	30	6.56 a	7.38 a	6.97 a	63.5%	8% - 55 %	9% - 45%
FDK	2DL R (SP1+SP2)	78	10.01 b	9.24 a	9.62 b	40.4%	33% - 100%	25% - 60%
	Fhb1 R (SP1+SP3)	82	8.78 ab	12.56 b	10.67 b	31.7%	14% - 85%	23% - 58%
	MEAN (SP1+SP2+SP3+SP4)	190	11.99 c	15.02 c	13.50 c	-	-	-
	<i>Fhb1</i> R + 2DL R (SP1)	30	7.49 a	6.37 a	6.93 a	69.8%	7% - 56%	8% - 56%
DON	2DL R (SP1+SP2)	78	10.35 ab	7.55 a	8.95 a	54.9%	35% - 79%	21% - 59%
	Fhb1 R (SP1+SP3)	82	12.12 b	13.65 b	12.88 b	25.4%	55% - 99%	71% - 100%
	MEAN (SP1+SP2+SP3+SP4)	190	14.61 c	16.20 b	15.40 c	-	-	-

**Equivalent range: range of selection intensities between arrows A and B in Fig 2.

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PERCENTAGE OF FUSARIUM DAMAGED KERNELS MEASURED BY AIR SEPARATION. Andres Agostinelli, Nicki Mundell and David Van Sanford^{*}

Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546 *Corresponding Author: PH: (859) 257-5020 ext. 80770; E-mail: dvs@email.uky.edu

ABSTRACT

One of the greatest problems in breeding for Fusarium head blight (FHB) resistance lies in the difficulty of assessing the disease. Air separation methods have long been used in the seed industry for seed conditioning purposes and in seed labs to measure the proportion of different components of seed samples. An air separation machine was specifically developed from a Precision Machine head thresher and a Shop-Vac vacuum to separate scabby kernels from healthy ones. Once a sample is loaded into the machine, air-driven elevation of the lighter portion of wheat (i.e. scabby seeds) occurs until it reaches the top of the column where is collected in a receptacle. The heavier portion of wheat (i.e. asymptomatic seeds) is suspended midair and does not reach the top of the column. Once the air is turned off, the asymptomatic seeds fall and are collected in the bottom of the column. Finally, both portions of the sample are weighed separately and FDK is calculated. Time per sample is about a minute.

A population of 128 F3:4 and 48 F2:4 lines derived from a cross between FHB-susceptible KY93C-1238-17-2 and FHB-resistant VA01W- 476 were grown in headrows in October 2007 in scab nurseries located at Lexington and Princeton, KY. In 2008, scab ratings were recorded in the field; percentage of Fusarium damaged kernels (FDK) and deoxynivalenol (DON) concentration was measured in kernels harvested from headrows. FDK was measured using the air separation machine and DON was determined by GC-MS. The correlation between FDK and DON using all data points was 0.852, indicating that FDK measured by air separation can be a highly useful way to assess FHB in scab breeding programs.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture under Agreement No. 59-0790-4-127. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

CHARACTERIZING BARLEY NEAR-ISOGENIC LINES FOR A DON QTL ON CHROMOSOME 3H. K.A. Beaubien¹, R. Dill-Macky², Y. Dong², B.J. Steffenson² and K.P. Smith^{1*}

¹Dept. of Agronomy and Plant Genetics, and ²Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108 *Corresponding Author: PH: (612) 624-1211; E-mail: smith376@umn.edu

OBJECTIVES

Investigate ergosterol and DON content in barley spikelets following point inoculation in lines isogenic for a DON accumulation QTL on chromosome 3H.

INTRODUCTION

Previously, we evaluated a FHB mapping population using a point inoculation assay to assess accumulation of DON in barley spikelets 72 hours after inoculation (Smith et al., 2004). The mapping population segregated for DON concentration. Interestingly, the parent with resistance to FHB, Frederickson, accumulated higher levels of DON compared to the susceptible parent Stander. In this mapping study, we identified a QTL for DON accumulation on chromosome 3H. This QTL for DON accumulation was not associated with field resistance to FHB, and thus suggested independent mechanisms for host resistance to infection and DON accumulation. Possible explanations for a host genetic effect on DON accumulation include a host effect on: fungal growth, fungal production of DON, and degradation of DON. In this study, we use a pair of near-isogenic lines for this 3H QTL region to confirm the 3H QTL effect on DON accumulation and assay ergosterol to determine if the host effects growth of the fungal pathogen.

MATERIALS AND METHODS

DON and Ergosterol assay of spikelets. Inbred lines FEG8-14-083-F(29) and FEG8-14-083-S(1) are isogenic for the 3H DON QTL region. In addition, the parents of the mapping population, Frederickson and Stander, were used as checks (Mesfin et al., 2003). Two seeds of each line were planted in each of five pots in two separate greenhouse experiments as previously described (Smith et al., 2004). Plants were inoculated when at least one head on the plant reached full head emergence or anthesis. A single isolate of Fusarium graminearum (Butte86ADA-11) was used to produce macroconidial inoculum (spore suspension of 100,000 conidia/mL). Two central spikelets of a single barley spike of each plant were injected with 10µL of inoculum and immediately transferred to the dew chamber for 72 hours with conditions as described in Smith et al. (2004). If only one plant in the pot had reached heading, the other plant was not used. At 72 hours, pots were removed from the dew chamber and inoculated spikelets were collected, a visual score was taken on each for the percent necrosis and chlorosis of each spikelet and then stored at -20°C until analyzed. One of the two spiklets harvested from each plant was used for DON analysis (Smith et al., 2004) and the other was used for ergosterol analysis (Dong et al., 2006) using methods described previously.

Data Analysis. Analysis of variance was performed using SAS Proc GLM (SAS, Institute, Inc. 2003). Each pot was treated as the experimental unit, so in the case where both plants were inoculated, the data were averaged. The two greenhouse experiments were analyzed both separately and together. Mean separation was performed using a protected LSD (P=0.05) for heading date, percent necrosis, percent chlorosis, seed weight, DON ppm, 15ADON ppm, and ergosterol ppm.

RESULTS AND DISCUSSION

Comparison of near isogenic lines: As expected, Frederickson headed later than Stander and the NILs. However, there was no significant difference in heading date between the NILs (data not shown). - The NILs did not differ in seed weight, percent necrosis, or percent chlorosis (data not shown).

- As previously observed, the NIL carrying the Stander allele at the 3H QTL had significantly lower DON concentration compared to the NIL carrying the Frederickson allele (Figure 1).

- The NILs did not differ in ergosterol (Figure 2) but were significantly higher than both Frederickson and Stander.

- The NILs did not differ for 15ADON concentration (data not shown)

In our previous study, we showed that the QTL on 3H was significantly associated with accumulation of DON in point inoculated spikelets 72 h after inoculation. Among a set of NILs we observed that on average lines carrying the Frederickson allele had 2.5 fold higher levels of DON compared to lines carrying the Stander allele (Smith et al., 2004). In this experiment using one selected pair of NILs, we saw a 4-fold difference in DON accumulation. However, we did not observe any difference in ergosterol concentration between the NILs. This suggests that the host effect on accumulation of DON in infected spikelets is not related to growth of the pathogen. Future experiments will investigate the role of the host on fungal production of DON and host degradation of DON.

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Figure 1. DON concentration for Frederickson, Stander, and the NILs in single spikelets harvested 72 h after inoculation. A: experiment 1; B: experiment 2. Bars labeled with different letters are significantly different (LSD, P=0.05).



Figure 2. Ergosterol concentration for Frederickson, Stander, and the NILs in single spikelets harvested 72 h after inoculation. Combined analysis of experiments 1 and 2. Bars labeled with different letters are significantly different (LSD, P=0.05).

INVESTIGATING HOST VARIATION FOR DON ACCUMULATION IN WILD BARLEY. K.A. Beaubien¹, R. Dill-Macky², Y. Dong², J.K. Roy², B.J. Steffenson² and K.P. Smith^{1*}

¹Dept. of Agronomy and Plant Genetics, and ²Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108 *Corresponding Author: PH: (612) 624-1211; E-mail: smith376@umn.edu

OBJECTIVE

Assess variation for toxin accumulation and ergosterol concentration in twenty *Hordeum vulgare* ssp *spontaneum* accessions.

INTRODUCTION

Previously, we showed that there was host genetic variation for accumulation of DON in point inoculated barley spikelets (Smith et al., 2004). Interestingly, the FHB resistant parent, Frederickson, accumulated more DON in this assay than the FHB susceptible variety Stander. While we were able to identify host genetic variation within cultivated barley in this mapping study, we were curious as to the extent to which variation for this trait was present in wild barley. In this study, we analyzed a set of 20 ecogeologically diverse accessions from the Wild Barley Diversity Collection (WBDC, Steffenson et al., 2007).

MATERIALS AND METHODS

Wild Barley Diversity Collection. Three hundred and eighteen wild barley (*Hordeum vulgare* ssp. *spontaneum*) accessions were genotyped on a DArT platform (Steffenson et al., 2007). These data were used to calculate the pairwise genetic distance among the 318 accessions in PAUP v 4.0 (Swofford 2003) and were used to construct a radial dendrogram in Dendroscope (Huson et al., 2007). Twenty lines were selected to best represent the diversity of the entire collection. Resistant and susceptible barley cultivars, Frederickson and Stander, respectively, were used as checks. Two seeds of each line were planted in each of five pots in two separate greenhouse experiments as previously described (Smith et al., 2004). Wild barley lines were vernalized on moistened filter paper in sealed petri plates for 28 days at 4°C before being transferred to the greenhouse for transplanting.

DON and Ergosterol assay of spikelets. See the report "Characterizing barley near-isogenic lines for a DON QTL on chromosome 3H" by Beaubien et al. in these proceedings.

Spread of FHB within the spike. Due to preliminary evidence of variation for spread of FHB in the head, FHB severity was assessed as a measure of spread for all lines in experiment 2. After pots were removed from the dew chamber and inoculated kernels were collected, pots were returned to the greenhouse bench. Two weeks after being returned to the greenhouse bench, the number of visually symptomatic kernels and the total number of kernels from each inoculated spike were counted and used to calculate FHB severity.

Data Analysis. For analysis of variance, only the wild barley accessions were considered; the checks were not included. The pot was treated as the experimental unit, so in the case where both plants were inoculated, the data were averaged. The two greenhouse experiments were analyzed both separately and together. Proc GLM (SAS, 2003) was used to assess variation for heading date (including 28 days vernalization for wild barley accessions), percent FHB severity (experiment 2 only), percent necrosis and percent chlorosis for the inoculated spikelets. The two spikelets harvested for DON and ergosterol analysis were averaged for percent necrosis and chlorosis. We also analyzed seed weight, DON ppm, 15ADON ppm, and ergosterol ppm.

RESULTS AND DISCUSSION

Phenotypic variation in wild barley:

- We observed significant (P<0.0001) variation for percent necrosis on inoculated spikelets (Figure 1)
- There was significant (P=0.0102) variation for FHB severity among wild barley accessions. In general, severity was greater in the wild barley accessions than in Frederickson and Stander (Figure 2).
- There was significant variation (Exp1, P=0.0028; Exp2, P=0.0113) for ergosterol accumulation among wild barley accessions. However, some lines were inconsistent from experiment 1 to experiment 2 (Figure 3).
- Significant variation among wild barley accessions for both DON and 15ADON was observed in experiment 1 only (Figures 4 and 5 respectively).
- Wild barley accessions also differed significantly for heading date and seed weight (data not shown).

While we observed some phenotypic variation for DON accumulation among the wild barley accessions, the extent of variation was generally within what we observed for Frederickson and Stander. This suggests that additional variation for this trait may not be available in wild barley. We did observe significantly more variation for FHB severity (spread in the head) in wild barley compared to Frederickson and Stander. It is important to note that the spread that we observed was likely due to surface growth of mycelia rather than growth in the rachis as is typically observed in wheat. In cultivated barley, there is very limited spread through the rachis compared to wheat. While this variation may not be the same as type II resistance in wheat, our results suggest that further investigation in wild barley may be warranted.

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Figure 1. Percent necrosis 72 hours after inoculation of single spikelets. Data is combined for two experiments.



Figure 2. Percent FHB severity 72 hours after inoculation of single spikelets. Data was collected only in experiment 2.



Figure 3. Ergosterol ppm 72 hours after inoculation of single spikelets.



Figure 4. DON ppm 72 hours after inoculation of single spikelets for experiment 1. Variation among accessions was not significant in experiment 2.



Figure 5. 15ADON ppm 72 hours after inoculation of single spikelets for experiment 1. Variation among accessions was not significant in experiment 2.

DISCOVERY AND MAPPING OF SINGLE FEATURE POLYMORPHISMS IN WHEAT USING AFFYMETRIX ARRAYS. A.N. Bernardo¹, P.J. Bradbury², H.X. Ma³, S.W. Hu⁴, .L. Bowden⁵, E.S. Buckler² and G.H. Bai^{5*}

¹Dept. of Plant Pathology, Kansas State University, Manhattan, KS; ²USDA-ARS, Maize Genetic Diversity Laboratory, Ithaca, NY; ³Institute of Plant Genetics and Biotechnology, JAAS, Nanjing, China; ⁴Dept. of Agronomy, Kansas State University, Manhattan, KS; and ⁵USDA-ARS, Plant Science and Entomology Unit, Manhattan, KS ^{*}Corresponding Author: PH: (785) 532-1124; E-mail: guihua.bai@ars.usda.gov

ABSTRACT

Affymetrix arrays have been used to discover single feature polymorphisms (SFPs) in several crop species. To demonstrate the utility of the Affymetrix GeneChip® Wheat Genome Arrays in SFP discovery and mapping in wheat (*Triticum aestivum* L.), complimentary RNAs synthesized from mRNA isolated from seedlings of 71 $F_{8.12}$ recombinant inbred lines (RILs) from the cross of Ning 7840/Clark were hybridized to the Affymetrix array. SFP prediction on the array data was done following the method of Kirst et al. (2006). A total of 955 SFPs were selected and combined with simple sequence repeats (SSR) data for mapping. A high-density genetic map consisting of 923 SFPs and 269 SSR markers and covering 1,944 cM genetic distance was constructed with 877 SFPs assigned to 21 chromosomes. The SFPs were randomly distributed within a chromosome and effectively filled gaps between SSRs, but were unevenly distributed among different genomes. B genome had the most SFPs, and D genome had the least. Map positions of a selected set of SFPs were validated by SNaPshot analysis and comparison with previous EST physical mapping data. Results indicate that Affymetrix array is a cost-effective platform for SFP discovery and mapping using RILs. The new map will be an important source of markers for quantitative trait loci (QTL) detection and high resolution mapping.

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SINGLE NUCLEOTIDE POLYMORPHISM MARKERS FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT. A.N. Bernardo¹, H.X. Ma² and G.H. Bai^{3*}

¹Dept. of Plant Pathology, Kansas State University, Manhattan, KS; ²Institute of Plant Genetics and Biotechnology, JAAS, Nanjing, China; and ³ARS-USDA Plant Science and Entomology Unit, Manhattan, KS *Corresponding Author: PH: (785) 532-1124; E-mail: guihua.bai@ars.usda.gov

ABSTRACT

Fusarium head blight (FHB) is a devastating disease in humid and semi-humid wheat growing regions of the world. The quantitative trait locus (QTL) on 3BS (Fhb1) of Sumai 3 and Ning 7840 has been identified to have the largest effect on FHB resistance. Simple sequence repeat (SSR) markers flanking the Fhb1 are identified. These SSR markers have been widely used for marker-assisted screening of Fhb1. However, the SSR markers flank a relatively large chromosome region of the QTL and more closely linked markers to the QTL may improve selection efficiency. The rich sources of wheat expressed sequence tags (ESTs) and abundance of single nucleotide polymorphism (SNP) markers makes SNP ideal markers for fine mapping. We developed SNP markers based on wheat ESTs that mapped to the 3BS QTL region. A total of 15 SNPs were identified between Ning 7840 and Clark (FHB-susceptible) based on sequence analysis of three different ESTs. SNP primers were designed and the single base extension method was used to analyze the SNPs in 125 Ning 7840 /Clark recombinant inbred lines. Three SNP markers mapped between *Xgwm533* and *Xgwm493*. Two of them, *Xsnp-21-1* and *Xsnp-20-1a*, have higher coefficient of determination (R²) than *Xgwm533* and should be good markers for marker-assisted selection of Fhb1 QTL in breeding programs.

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TOWARDS RAPID CANDIDATE GENE DISCOVERY IN THE BARLEY CHROMOSOME 2(2H) BIN 10 FUSARIUM HEAD BLIGHT RESISTANCE QTL. Christine N. Boyd¹, Richard Horsley² and Andris Kleinhofs^{1,3*}

¹Dept. of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420, USA; Dept. of Plant Sciences, North Dakota State University, Fargo, ND 58105-5051, USA; and ³School of Molecular Biosciences, Washington State University, Pullman, WA 99164-4660, USA *Corresponding Author: PH: 509-335-4389; E-mail: andyk@wsu.edu

INTRODUCTION

The U.S. Wheat and Barley SCAB Initiative has now been funding research for over ten years and the genes controlling resistance to Fusarium Head Blight (FHB) in barley are still unknown. Many sources of resistance have been reported, but the highest, most stable source of resistance known is located on chromosome 2(2H) (Dahleen et al., 2003; de la Pena et al., 1999; Hori et al., 2005; Hori et al., 2006; Horsley et al., 2006; Kolb et al., 2001; Ma et al., 2000; Mesfin et al., 2003; Zhu et al., 1999). Our chromosome 2(2H) map has been delineated by QTL mapping (Horsley et al. 2006) and by phenotyping of our recombinant lines to include only bin 10 (MWG699-MWG503). The importance of this region has been challenged by the recent findings of Sato et al. (2008) who failed to find a FHB resistance QTL in the Vrs1 region but did find a major QTL in the cly1/Cly2 region on chromosome 2(2H) at approximately bin 14. Sato studies were done with crosses between 2rowed lines using a single resistance source and a cut spike FHB assay. Mesfin et al. (2003) also reported a major FHB resistance QTL in the cly1/Cly2 region, detectable only in greenhouse studies. These results suggest that environmental conditions and the source of resistance may play a major role in the detection of the cly1/Cly2 QTL. The vrs1 mutant we have isolated in CIho4196 (see below) should help resolve this controversy.

Though the bin 10 map is quite saturated, genetic mapping continues—a process that has gained momentum with the development of PCR-based markers allowing a cleaved amplified polymorphic sequence (CAPS) marker to be mapped in a single day. Many of our random fragment length polymorphism (RFLP) markers have been converted to CAPS markers, making genotyping faster and safer. Our physical map of the region is also reasonably saturated and with adequate funding we are prepared to sequence 24 bacterial artificial chromosomes (BACs) with the purpose of rapid gene discovery.

Mutagenesis of CIho4196 with subsequent analysis and phenotyping has resulted in several mutants of agronomic interest including 6-row, early, and semi-dwarf, which retain CIho4196-like levels of FHB resistance. The *vrs1* mutant giving a 6-rowed phenotype contains a nine base pair deletion in the homeobox domain, a mutation unlike any previously reported 6-rowed varieties or mutants (Komatsuda et al., 2007).

RESULTS AND DISCUSSION

Phenotyping of quantitative disease resistance is difficult, at best, and FHB phenotyping is no exception. The recombinant lines previously reported (Boyd et al., 2007) were tested for FHB levels in a China and a North Dakota nursery in 2008. There are 22 lines derived from recombinants 07-76 and 07-84 indicating that the FHB resistance QTL resides at marker BG365406 (co-segregates with Uni4780 and BF254012). However, this is not confirmed by recombinants 07-85-1 and 07-97, which appear to have this chromosome region and yet are susceptible (Fig. 1). Recombinants derived from 07-91 indicate that the region at marker ctg15632 (co-segregates with MWG503) may be important, which is supported by the QTL mapping of Zhu et al. (1999). However, recombinants derived from line 07-87 have this marker, lack the marker BG365406, and are susceptible. The simplest explanation may be that both regions contribute. Line 07-90-8 appears resistant in the first trial and susceptible in the next, underscoring the difficulties of phenotyping QTL-controlled resistance and the importance of finding closely linked genetic markers.

Due to the above difficulties in further refining the location of the FHB resistance QTL, we chose to saturate the genetic and physical map for the entire bin 10 region from Vrs1 to MWG503 (Fig. 2). There are 10 loci in this region represented by 26 markers and 152 BAC clones. The region just below Vrs1 was finemapped by Pourkheirandish et al. (2007), providing further markers for BAC identification (markers identified in gray, Fig. 2). We recently mapped the rRN5S1 gene to our target region. Its tandem repeats may account for the lack of genes in the area (Kanazin et al., 1993). We continue to identify and map additional markers and select BAC clones for this region, currently focusing on the recently released Brachypodium distachyon sequence as a possible source (brachypodium.org).

Using the contigs identified in the barley physical map database from the Tim Close lab and hybridization of *Hind*III-digested BAC clone filters, we eliminated redundant BAC clones and those from loci that do not map to this region. The minimum tiling path of 24 BAC clones makes up 14 contigs covering more than 2 Mb of the genomic region (Fig. 2). For rapid marker and candidate gene detection, the 24 total BAC clones must be sequenced.

Gamma irradiation of CIho4196 seed has proved quite successful in developing lines with better agronomic qualities that maintain FHB resistance. The 6-row line, designated g07-014, contains a unique mutation in the *vrs1* gene and is available for use in breeding programs, as are the early and semi-dwarf lines (Boyd et al., 2008).

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DISCLAIMER

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ND '08 FHB: 5 (with lodging problems) 26 BF265762A MWG865 BG2299611 Uni4780/BG2552406 ctg15522 Rrn5S1 year in China and one year in North Dakota. The recombinants were Fig. 1 Genotype of recombinants lines and phenotyping data from one isolated from crosses of A171 or A80 with Morex. China '08 FHB: Height (cm): ctg37907 BG369629 BG416977 BG417014 BG369432 BE216598-BF623140 BF263615-ABC306 S 2.7 126 FOSIEFATOREA Bold = (6.3 CIInos 196 1.6 141 R 6.76 R 1.1 CIho4196 neterozygous Foster/Morex 780 APS or MP ma R 19.4 R 1.7 A131 N/A ·*orCootogay 8.8 R arker R 10.3 R 1.9 142 70507.76 1.9 146 R 12.6 15 07 03,84 140 S 27.4 03,85,1 $\omega \infty$ 3.2 135 S 34.6 3°F07,87 S R 2 132 , 0, 0, ° S 23.3 ² or 07,91 1.5 R R 18.7 130507.97 S 2.8 142 S 25.9 *BF254012, *Uni4780, BG365406 *ctg15522, 7804, 8397-*BE194244-BG345126,*MWG865, *BE601445, *BF625659 BIN 10 *vrs1 -*BF265762A -BF628983-*BG299611-*Rrn5S1— *BI958325 his s including the minimum tiling path of BAC clones. 172.3 -168.9 - 166.5 -169.4 - 167.1--163.6 - 167.8, *BF255635 *B1948584 *BF260018-Vrs1 BAC contig sequenced by Komatsuda et al. (PNAS, 2007) 165.0 *BI955972-*BJ549838--CX626461 -strikethrough = pulled no BACs Fig. 2 Genetic map of the Chr. 2H bin 10 region, ${
m ctg}={
m contig}$ in Tim Close barley BAC fingerprinting August 2007 database Gray BAC = BAC already sequencedBlack = BAC for sequencing Gray marker = map position estimated based on Pourkheirandish et al. (TAG, 2007) BAC fingerprinting database. No ctg indicates no Contigs are based on our analyses and the Tim Close information in the BAC database ≭_____1066 ctg8863 771N23 (185.9kb) * marker has been used to pull BAC clones . 286i1 (100kb) _____703A2 (118kb) ctg5179 ____778K20 (271kb) <u>253E15</u> 200B19 666M22 ctg675 № 82i01 (100kb) (157.3kb) 47908 ctg5229 784i20 (171.6kb) —462N21 ctg5403 (201.5kb) -679J3 no ctg 782117 ctg111 (202.8kb) ctg3419 ctg7769 (169kb) BF625659 Uni4780 ctg593 (153.4kb) BE601445 2 90N8 657C16

MARKER-ASSISTED SELECTION FOR FHB AT THE EASTERN REGIONAL SMALL GRAINS GENOTYPING LAB. Gina Brown-Guedira^{1*}, Jared Benson², Kim Howell¹ and Jared Smith¹

¹USDA-ARS, Plant Science Research Unit, Raleigh, NC 27695; and ²Dept. of Crop Science, North Carolina State University, Raleigh, NC 27695 *Corresponding Author: PH: (919) 513-0696; E-mail: gina.brown-guedira@ars.usda.gov

ABSTRACT

Head scab of wheat, caused by Fusarium graminearum, is a disease that affects wheat production in the Eastern soft wheat growing region of the U.S.A.. Genotyping and marker-assisted selection (MAS) are being applied in the Eastern wheat growing region to develop resistant wheat varieties and to characterize germplasm. Since 2005, the Eastern Regional Small Grains Genotyping Lab has worked with breeders in eastern region to conduct MAS for FHB resistance. During 2008, samples for screening with markers linked to FHB resistance QTL were received from 13 soft wheat breeding programs. The Fhb1 resistance gene is by far the most frequent gene being deployed by MAS. However, marker-assisted selection has also been conducted for resistance QTL on chromosomes 5A, 2D, 4B, 6B, 2B, and 3BS near the centromere. In addition to MAS, genotyping with markers linked to FHB resistance is also done on collaborative regional nurseries. The 2007 and 2008 Uniform Southern Fusarium Head Blight Nursery and Northern and Preliminary Northern Uniform Winter Wheat Scab Nurseries, were screened for SSR markers linked FHB resistance QTL on chromosome 3BS (Fhb1), 5AS, and 2DL using SSR markers. The 2008 nurseries were also screened with markers linked to the 3BS centromere QTL mapped in Ernie, as well as markers associated with genes for resistance to leaf, stripe and stem rust, the Hessian fly, and barley yellow dwarf virus. Markers for the Bx7^{oe} allele for gluten strength, reduced height genes Rht-B1 and Rht-D1 and the short arm of rye chromosome 1R were also evaluated. No lines were found in any nursery evaluation to contain all the scab resistance QTL evaluated. In 2007, seven lines evaluated had at least one scab resistance QTL in the NUWWSN, while the USFHBN had six lines with at least one QTL. In 2008, the number of lines postulated to have a mapped resistance QTL increased to 12 lines and 11 lines in the NUWWSN and USFHBN, respectively.

ACKNOWLEDGEMENT AND DISCLAIMER

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COMPARISON OF TWO FUSARIUM HEAD BLIGHT INOCULATION METHODS IN WHEAT. E.A. Brucker, C.J. Thompson and F.L. Kolb^{*}

Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA *Corresponding Author: PH: (217) 333-9485; E-mail: f-kolb@illinois.edu

ABSTRACT

Fusarium head blight (FHB), or head scab, is a widespread and destructive disease of wheat and barley. Identifying breeding lines with host plant resistance to FHB is an important breeding objective. Many inoculation and evaluation methods are used to identify breeding lines with resistance to FHB. Often, the phenotypic data collected using different inoculation and evaluation methods are poorly correlated. Our objectives in this study were to determine if FHB resistance ratings from two different inoculation methods were highly correlated, and if the same breeding lines with the highest resistance were selected using the two inoculation methods. The two inoculation methods used were a spray-and-bag method using a macroconidial suspension and an infected grain spawn with mist irrigation system to enhance natural infection. If data from the two methods agree, the spray and bag method would provide a way to evaluate breeding lines in multiple environments and locations without establishing misted, inoculated FHB evaluation nurseries at all sites. Both methods were slightly modified from a similar experiment in 2005. These methods were tested in 2008 on 87 lines in three separate experiments. Scab incidence and severity data were collected, and FHB index was calculated. Data from the two methods were combined and analyzed using the PROC CORR procedure of SAS with a significance threshold of $\alpha = 0.05$. Disease pressure was high in 2008 as indicated by the resistant check Ernie. Scab incidence (r = 0.41), severity (r = 0.83), and FHB index (r = 0.76) were significantly correlated between the two methods. Based on these preliminary results it is possible to obtain highly correlated FHB resistance ratings between two different inoculation methods. Analyzing the data for a subset of the lines with a FHB index in the top 20% and bottom 20% based on the grain spawn infection method increased the correlations between the two methods slightly for incidence and FHB index, but not for scab severity. More than half of the breeding lines with the most resistance under the grain spawn/mist infection agreed with the most resistant lines under spray-and-bag infection. Some of the lines with the highest resistance using one method were not selected using the second method. Nevertheless, the high linear relationships between methods indicate that either method is useful for selecting breeding lines; however, additional data collected in multiple environments are required to validate these results. It appears that the spray and bag method may have potential to supplement data from misted, inoculated FHB evaluation nurseries, but should not be used in place of data collected in these nurseries.

EVALUATION OF HOST PLANT RESISTANCE AND FUNGICIDE TREATMENT FOR SUPPRESSION OF FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL. E.A. Brucker, N.H. Karplus, C.A. Bradley and F.L. Kolb^{*}

Department of Crop Sciences, University of Illinois, Urbana, IL 61801, USA *Corresponding Author: PH: (217) 333-9485; E-mail: f-kolb@illinois.edu

ABSTRACT

The use of resistant wheat cultivars is an effective means of reducing losses due to Fusarium head blight (FHB), caused by Fusarium graminearum, and deoxynivalenol (DON) accumulation. Breeding efforts have produced FHB-resistant wheat lines, but many of these lines yield less than FHB-susceptible wheat cultivars when FHB pressure is low. Farmers frequently plant FHB-susceptible wheat cultivars with the intent of spraying fungicides when necessary. Recent fungicide technology has greatly improved control of FHB in wheat and barley, but fungicides do not provide complete control of FHB. Most fungicide efficacy studies report a significant yield increase in treated plots compared to the untreated check. Fungicides with triazole chemistry are the most effective in reducing FHB and DON. Our objectives were to evaluate the effectiveness of host plant resistance and fungicide treatment for suppression of FHB and DON accumulation and the effect of FHB on yield and test weight. Using an inoculated and irrigated disease nursery we tested two triazole fungicides, tebuconazole (Folicur®) and tebuconazole + prothioconazole (Prosaro®), and twelve wheat cultivars ranging from FHB susceptible to FHB resistant. The experiment was a split-plot design with fungicide treatment as the main plot and variety as the sub-plot with four replications. Data were collected on scab incidence, severity, FHB index, Fusarium damaged kernels (FDK), incidence/severity/kernel quality index (ISK index), DON, yield, and test weight were all measured. Both fungicide and cultivar had a significant effect on all variables. Significant interactions between fungicide and cultivar were detected for FHB incidence, FDK, ISK index, DON, yield, and test weight. In individual untreated plots, FHB incidence ranged from 10% to 100% thereby confirming high disease pressure and varying cultivar FHB resistance levels. Yield varied greatly (110.0-67.5 bu/A) and test weights were moderate to low (58.3-48.7 lbs/bu). Averaged over all cultivars both Folicur and Prosaro significantly increased yield and test weight, and lowered ISK index and DON. Folicur increased yield by an average of 9.5 bu/A and decreased DON by an average of 44%, while Prosaro increased yield by 13.8 bu/A and decreased DON by 67%. Prosaro treated plots significantly outperformed Folicur treated plots in yield, test weight, incidence, FHB index, FDK, and ISK index. The three cultivars with the greatest host resistance in the untreated plots realized the lowest yield increase from the addition of fungicides, whereas, except for one cultivar, the most susceptible cultivars realized a greater than 20% increase in yield with Prosaro fungicide. Notably, in the untreated plots, the most resistant cultivar, IL02-18228, had the lowest DON level (0.7 ppm) and the highest yield and test weight. IL02-18828 in the untreated plots yielded more than all but one of the susceptible cultivars even when these six cultivars were treated with Prosaro, the most effective fungicide. This is preliminary data from one year, but our data indicate that under severe FHB pressure, wheat producers can produce high yields of sound grain, with DON below the FDA's guideline of 1 ppm, by use of cultivars with good FHB resistance in combination with either Folicur or Prosaro.

CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN ALSEN-FRONTANA-DERIVED RECOMBINANT INBRED LINES. Rishi R. Burlakoti¹, Mohamed Mergoum², Shahryar F. Kianian² and Tika B. Adhikari^{1*}

¹Dept. of Plant Pathology, and ²Dept. of Plant Sciences, North Dakota State University, North Dakota 58108, USA *Corresponding Author: PH: (701) 231-7079; E-mail: tika.adhikari@ndsu.edu

OBJECTIVES

Evaluate the recombinant inbred lines (RILs) of wheat for resistance to FHB and DON accumulation, and characterize the RILs with known SSR markers.

INTRODUCTION

Fusarium head blight of wheat (FHB), caused primarily by *Gibberella zeae* is a destructive disease of wheat, and other small grains worldwide (McMullen et al., 1997). In the United States, the fungus causes millions of dollars in losses and poses serious socioeconomic problems (Nanje et al., 2004). The fungus produces several mycotoxins that cause serious health problems to both humans and livestock (McMullen et al., 1997).

Mesterházy et al. (1995) reported five different types of resistance to FHB in wheat. Among them, type II resistance has been studied extensively and reported to be more genetically stable than other types of resistance (Bai and Shaner, 2004; Mesterházy, 1995). Chinese wheat cultivar 'Sumai 3' (PI481542) and its derivatives, which exhibit the type II resistance, are widely used in wheat breeding programs worldwide (Bai and Shaner, 2004). Brazilian cultivar 'Frontana' (PI500147) is believed to exhibit type I resistance for FHB and has also been used in breeding programs of wheat (Singh et al., 1995; Van Ginkel et al., 1996).

North Dakota State University (NDSU) has released the FHB-resistant cultivar 'Alsen' (PI615543) (Frohberg et al., 2006) developed from Sumai 3, which is widely grown in the Midwest of the United States (USDA, 2007). To combine the type I resistance in Sumai 3 background, spring wheat cultivar Alsen was crossed with Frontana and W9207 (Chinese wheat line); consequently, $135 F_9$ recombinant inbred lines (RILs) were developed. The long-term goal of this study was to develop wheat germplasms for durable resistance to FHB by combining both type I and II resistance.

MATERIALS AND METHODS

Inoculation and Disease Assessment: One hundred thirty-five F_o RILs developed from crosses between Frontana/W9207//2*Alsen, were evaluated for FHB reactions and DON content during fall 2006 and spring 2007. Nine spikes of each RIL were sprayinoculated at mid anthesis (Zadok's scale 65) in the greenhouse. The FHB severity was assessed 7, 14, and 21 days after inoculation (DAI) as described previously (Stack and McMullen, 1998). The area under disease progress curve (AUPDC) was calculated from FHB severity values taken 7, 14, and 21 DAI (Campbell and Madden, 2006). FHB severity values estimated at 7 DAI were used to measure the resistance to initial infection (type I), while FHB severity values estimated 21 DAI and AUDPC were used to measure the resistance to fungal spread (type II resistance). The terms 'initial disease severity' (IDS) and 'final disease severity' (FDS) referred to the FHB severity 7 DAI and 21 DAI respectively. The inoculated spikes were harvested and threshed manually. The Fusarium damaged kernels (FDK) per spike were counted for parents and each RIL. Deoxynivalenol (DON), 3-ADON (3-Acetyldeoxynivalenol), 15-ADON (15-Acetyldeoxynivalenol), and NIV (Nivalenol) were estimated from these grain samples using gas chromatography (Tacke and Casper, 1996)

at the Veterinary Diagnostic Laboratory, NDSU, Fargo, ND. Analysis of variance (ANOVA) was performed for each greenhouse experiment using SAS (version 9.1, Statistical Analysis System; SAS Institute, Cary, NC).

Molecular Marker Analysis: Genomic DNA was extracted as described previously (Burlakoti et al., 2007) with some modifications. Forty two resistant RILs were characterized with seven SSR markers known to be linked to FHB resistance (GWM493, GWM533, BARC133 and BARC147 on the 3BS, WMC397 and WMC398 on the 6BS, BARC197 on the 5AS). The PCR amplification was performed as described in Roder et al. (1998) in a PTC-100 Thermal Cycler (MJ Research, Watertown, MA).

RESULTS AND DISCUSSION

Data analysis showed that the variances of experiment, RIL, and RIL × experiment were highly significant (P < 0.001) for IDS, FDS, AUDPC, and FDK (Table 1). Among the three parents, Alsen had lowest FDS (28.16%), AUDPC (319.49) and DON (7.90 $\mu g/g$), and Frontana had the lowest IDS (13.47%) (Table 2). The RIL population showed larger variation for all these three FHB parameters and FDK; however, their means did not deviate significantly from the parental means (Table 2). The average value of DON content for the RIL population was lower (10.11 $\mu g/g$) than the parental mean value (14.22 $\mu g/g$) (Table 2). Among the RILs, 22 lines had less than 10% IDS and $5 \mu g/g$ DON content, and 20 lines had 10-30% FDS. Approximately 11% of the RILs showed higher levels of resistance to initial infection (type I), FHB spread (type II), and DON accumulation (type v) than the resistant parents.

In molecular marker analysis, 78.57%, 80.95%, 95.23% and 88.09% of the resistant RILs showed the Alsen type allele for GWM493, GWM533, BARC133, and BARC147 (3BS), respectively. Similarly, 78.57% and 73.80% of resistant RILs showed Alsen type allele for WMC397 and WMC398 (6BS), respectively. On the other hand, 59.53% of resistant RILs showed Frontana type allele for BARC197

(5A). Among the 24 resistant RILs exhibiting overall resistance to IDS, FDS and DON, 83% RILs showed Alsen type alleles for markers from 3BS and 6BS, and Frontana type allele for marker BARC197, indicating that these resistant RILS had markers linked to both type I and II resistance. This result suggests that that the combination of the two sources (type I and type II) of resistance may provide high levels of resistance to initial infection, FHB spreads, and DON accumulation, and these RILs may be useful for FHB resistance breeding programs of wheat.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the USDA.

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Table 1. Mean square of 113 recombinant inbred lines (RILs) and three parents for initial disease severity (IDS) (%), final disease severity (FDS) (%), area under disease progress (AUDPC), and Fusarium damaged kernel (FDK) per spike.

Source of Variation	Df	IDS (%)	FDS (%)	AUDPC	FDK
Experiment [†]	1	7449.12*** [‡]	20427.12***	1564753.79***	0.14**
Rep (Exp)	4	272.99**	1063.07**	160399.90***	183.97***
Treatment	115	277.35***	1116.09***	107736.85***	43.32***
Treatment \times Exp	115	141.29***	640.01***	60749.27***	25.05***
Error	460	70.34	257.07	25333.81	13.77
CV (%)		51.68	38.32	38.33	54.07

[†]After homogeneity test, data from both experiments conducted in greenhouse were combined and analyzed using SAS.

[‡] ** and ***, Indicates significant at P < 0.01 and P < 0.001, respectively.

Table 2. Mean values of 113 recombinant inbred lines (RILs) and three parents averaged for two greenhouse experiments for initial disease severity (IDS) (%), final disease severity (FDS) (%), area under disease progress (AUDPC), Fusarium damaged kernel (FDK) per spike, and deoxynivalenol (DON) content.

Parameter	IDS	FDS	AUDPC	FDK	DON (µg/g)
	(%)	(%)			
RILs mean	16.22	41.83	408.28	6.85	10.11
W9207	21.50	64.59	573.49	8.94	16.60
Frontana	13.47	32.93	331.49	7.46	18.15
Alsen	14.97	28.16	319.49	3.65	7.90
Mean parental value	16.65	41.89	408.11	6.68	14.22
LSD ($P < 0.05$)	9.52	18.19	180.60	4.18	_

THE ICARDA PROGRAM FOR BREEDING FHB RESISTANCE IN BARLEY. Flavio Capettini^{*}

ICARDA Barley Improvement Program, PO Box 5466, Aleppo, Syria *Corresponding Author: PH: 963 21 2213477; E-mail: f.capettini@cgiar.org

ABSTRACT

ICARDA, in cooperation with CIMMYT, has been producing barley with enhanced resistance to Fusarium head blight (FHB) since the early 1980s. ICARDA's germplasm bank in Syria offers a diverse reservoir of genes that are being explored as new sources of resistance for this devastating disease. The crop's wild relatives represent even richer reservoirs of genes for stress tolerance and adaptation, as their history in the Central and West Asia and North Africa (CWANA) region is very long and includes periods with very harsh climate in the Pleistocene Era. The ICARDA barley breeding program started research on FHB resistance in response to the needs of the Andes countries. In 1986, a total of 5,000 barley accessions were screened in Mexico; of these, 23 were found with some level of resistance, and were subsequently intensively introgressed into the main program. Resistance sources were shared with programs worldwide, especially after the FHB outbreaks of the 1990s. Collaboration and cooperative efforts with advanced research institutions, such as the Busch Agricultural Resources Inc. (BARI) and the US Wheat and Barley Scab Initiative (USWBSI), allow the project to make germplasm sources with enhanced levels of resistance widely available. Environmental conditions at the CIMMYT's Toluca Experiment Station in Mexico at the early years and El Batán since 2006 are ideal for FHB development and evaluation. In addition, the project obtains data through collaboration with programs in the USA, Canada, China, Ecuador, Brazil and Uruguay. A recent initiative to comprehensively screen ICARDA's gene bank for unique and undiscovered sources of resistance has identified some potentially promising barley sources. A BARI/ICARDA collaboration line (ADV BARI 57) continues to show lowered levels of DON and is now being used in crossing blocks with superior malting parents. Several other ones have been found as highlights in the nursery network carried out in cooperation. Overall, this collaboration has illustrated the need for multi-year data collection and the usefulness of FHB disease nurseries for barley breeding.

MOLECULAR MARKER-ASSISTED EVALUATION AND CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT GENOTYPES GROWN IN THE PACIFIC NORTHWEST. J. Chen^{1*}, D. See², C.R. Hollingsworth³ and J. Windes¹

¹University of Idaho Aberdeen Research & Extension Center, Aberdeen, ID; and ²Western Regional Small Grain Genotyping Laboratory, USDA-ARS, Pullman, WA; amd ³University of Minnesota Northwest Research & Outreach Center, Crookston, MN *Corresponding Author: PH: (208) 397-4162, ext. 229; E-mail: jchen@uidaho.edu

ABSTRACT

This study was to deploy molecular marker-assisted selection and to evaluate and characterize Fusarium head blight (FHB) resistance in wheat genotypes grown in the Pacific Northwest (PNW). A total of 276 wheat genotypes from all classes except soft red winter wheat were evaluated and characterized with 13 markers flanking the six known FHB QTL (2DL, 3AS, 3BS, 5AS, and 6B) previously identified. Seventy-eight genotypes were released and adapted cultivars from California, Utah, Colorado, Washington, Oregon, and Montana. The rest of 198 genotypes were University of Idaho released cultivars and advanced lines. These genotypes have no Sumai3 related backgrounds. By comparing haplotypes of 276 genotypes with four known resistance sources Sumai 3, W14, Renwood3260, and Ernie, we found that the six known QTL existed in the 276 genotypes. Especially, the known UMN10 marker allele on 3BS was present in 66 lines out of the 276 genotypes studied. Among the 66 genotypes, twenty have combined three QTL of 3BS, 2DL, and 6BS. Eight of the twenty have additional 3AS QTL combined; while five of the twenty have additional 5AS QTL combined. The WMC 152 marker allele on 6BS was common and present in 108 lines; while the wmc264 marker allele on the 3AS QTL was rare and only present in 10 genotypes. The Gwm120 marker allele on the 2BS QTL was present in twenty-three genotypes, the gwm261 marker allele on the 2DL QTL was present in thirtyeight genotypes, the known Barc117 marker allele on 5AS was present in 68 lines. These identified cultivars/ lines having good field FHB resistance and/or known FHB resistance QTL can then be grown in PNW region and be used as adapted resistance sources in the PNW and Great Plains breeding programs.

HAPLOTYPE ANALYSIS OF GENES FOR FUSARIUM HEAD BLIGHT RESISTANCE IN TETRAPLOID WHEAT GERMPLASM. Chenggen Chu¹, Shiaoman Chao², Xiwen Cai³, Shaobin Zhong¹ and Steven Xu^{2*}

¹Department of Plant Pathology, North Dakota State University, Fargo, ND 58105; ²USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105; and³Department of Plant Sciences, North Dakota State University, Fargo, ND 58105 *Corresponding Author: PH: (701) 239-1327; E-mail: steven.xu@ars.usda.gov

ABSTRACT

Haplotype analysis at the molecular marker loci associated with the known Fusarium head blight (FHB) resistance QTL in wheat can be used to identify resistance genes in the resistant germplasm, and thus provides practical information of pyramiding different sources of resistance for the development of resistant germplasm or cultivars. In this research, we analyzed haplotypes of 132 tetraploid wheat accessions with various levels of FHB resistance at 19 molecular marker loci associated with the known FHB resistance QTL on the chromosomes 2B (*Triticum carthlicum* 'Blackbird'), 3A (*T. aestivium* 'Frontana' and *T. dicoccoides* 'Israel A'), 3B (*T. aestivium* 'Sumai 3' and 'Wanshuibai'), 5A ('Sumai 3' and 'Frontana'), 6B ('Blackbird' and 'Wangshuibai'), and 7A (*T. dicoccoides* PI 478742). Among the tetraploid wheat accessions included 40 accessions of *T. carthlicum*, 81 accessions of *T. dicoccum*, and 9 accessions of *T. turgidum*. We found 43 accessions, including two accessions of *T. carthlicum*, one accession of *T. turgidum*, and 39 accessions of *T. dicoccum*, showed different haplotypes at all the marker loci investigated, suggesting that these accessions may carry FHB resistance genes different from those in the known resistance sources. The novel FHB resistance genes carried by the tetraploid wheat accessions identified in this research could be utilized to enhance FHB resistance of durum wheat as well as bread wheat.

INTROGRESSION OF EXOTIC QTL INTO SOFT RED WINTER WHEAT USING MARKER-ASSISTED SELECTION AND EVALUATION OF NEAR-ISOGENIC LINES FOR SCAB RESISTANCE. Jose M. Costa^{1*}, Jing Kang¹, Anthony Clark², David Van Sanford², Carl Griffey³ and Gina Brown-Guedira⁴

¹University of Maryland, PSLA Dept. 2102 Plant Sciences Bldg., College Park, MD 20742-4452; ²University of Kentucky, Dept. of Plant Sciences, Lexington, KY 40546; ³Virginia Polytechnic and State University, Blacksburg, VA 24061; and ⁴USDA-ARS, Plant Science Research Unit, Raleigh, NC 27695 *Corresponding Author: PH: (301) 405-1317; E-mail: costaj@umd.edu

ABSTRACT

Scab of wheat, caused by *Fusarium graminearum*, is a disease that periodically strikes the U.S. mid-Atlantic region. Breeding for resistant wheat varieties is an effective method of disease control. The objective of this study was to develop scab resistant soft red winter wheat germplasm adapted to the US mid-Atlantic region using marker-assisted selection. McCormick, a genotype adapted to the Mid-Atlantic region, was used in a backcross program with the Chinese variety Ning7840. An accelerated backcross scheme was developed to incorporate scab resistance QTL found on chromosomes 3BS, 5A and 2DL in Ning7840. Two rounds of backcrossing were completed using McCormick as the female parent. Progenies from the first round of backcrossing were selected for the presence of the Ning7840 scab resistance alleles at 3BS, 5A, and 2DL and for a high background of McCormick alleles. Two backcross progenies had over 60% McCormick background. Using these two selected BC_1F_1s , 400 BC_2F_1s were produced in a second round of backcrossing. Additionally, the two selected BC₁F₁s were crossed with a wheat line with leaf and stripe rust resistance (Southern States 8641). 800 BC₂F₁ seeds were screened with molecular markers to identify those with Ning7840 alleles and a predominance of McCormick background. A single BC₂F₂ population derived from a selected $BC_{2}F_{1}$ plant was screened with markers to select those homozygous for the resistant alleles. Additionally, we derived eight near-isogenic lines (NILs) from this BC₂F₂ population. Seven BC₂F₂ NILs segregated into both awned and awnless types. A field study conducted in Salisbury, MD in 2007/2008, showed that the combination of scab-resistant QTLs in 3BS and 2DL conferred the lowest deoxynivalenol (DON) content: 1.7 ppm and 1.1 ppm for awned and awnless lines, respectively. We plan to further characterize the BC_2F_4s derived in field and greenhouse studies in 2008/2009 at Maryland and Kentucky. In the fall of 2008, F₄ seed of McCormick and SS8641 derivatives with scab QTLs were distributed to seven breeding programs (AR, GA, KY, LA, NC, VA, and Westbred) for crossing and further evaluation.

ACKNOWLEDGEMENT AND DISCLAIMER

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DEOXYNIVALENOL (DON) ACCUMULATION IN EIGHT WHEAT LINES WITH VARIOUS FUSARIUM HEAD BLIGHT RESISTANCE GENES. Mahua Deb¹, Judy Lindell¹, Lingrang Kong¹, Yanhong Dong² and Herb Ohm^{1*}

¹Dept. of Agronomy, Purdue University, 915 W. State St., West Lafayette, IN 47907; and ²Dept. of Plant Pathology, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108 *Corresponding Author: PH: (765) 494-8072; E-mail: hohm@purdue.edu

ABSTRACT

Breeding new cultivars, particularly with fusarium head blight (FHB) resistance genes from different sources combined is considered to be the most effective and durable approach to reduce crop production losses (Mesterhazy, 1995) and to minimize DON accumulation in the grain. The objective of this study was to compare DON concentration at four dates, at approximately weekly intervals, after grain physiological maturity (during June – early July) in grain of eight wheat cultivars that differ significantly in FHB resistance, in 2007 and 2008 at Lafayette, IN. DON accumulation was significantly greater in FHB susceptible wheat lines than in wheat lines with various resistance genes, both in 2007 (with limited rainfall in June) and 2008 (with more and also more frequent rainfall).
LINKAGE DISEQUILIBRIUM ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANCE IN TUNISIAN DURUM WHEAT. Farhad Ghavami, Melissa Huhn, Elias Elias and Shahryar Kianian^{*}

Department of Plant Sciences, North Dakota State University, Fargo, ND 58105 *Corresponding Author: PH: (701) 231-7574; E-mail: s.kianian@ndsu.edu

ABSTRACT

To expand the number of genes for FHB resistance in gene pyramiding programs, it is necessary to find genetically varied sources of resistance. In this study we used 184 BC1F6 and 189 BC1F7 lines derived from crossing of Tun7, Tun18, Tun34, Tun36 (all lines identified as sources of resistance from Tunisia) with durum cultivars 'Ben', 'Maier', 'Lebsock' and 'Mountrail' for association studies. As the pedigree of Tunisian lines show no relation to the popular Chinese sources of resistance, they could potentially carry different genes or alleles for resistance to FHB. We checked the parents and RILs in the greenhouse in two seasons for type II resistance to FHB by single floret injection inoculation method. The data showed that the Tunisian lines have different amount of resistance varying from 23% to 11% infection rate through the spikes as compared with D8750 (susceptible control) and Sumai3 (resistant control) with 41% and 9% of infection rate respectively.

We conducted the Diversity array (DArT) marker analysis to have a good coverage of the whole genome. DArT analysis used 2300 markers which showed 25% polymorphism between the parents. About 8% of the polymorphic markers were present in all the Tunisian lines but not the susceptible cultivars. The cluster analysis of the polymorphic markers revealed three distinct groups. Tun7 was in a separate group far from the other two and all the other Tunisian lines fell in a separate group from susceptible cultivars. As both Tun7 and Tun18 are more resistant to FHB than others and have different genetic backgrounds, they may be considered as potential candidates for new sources of resistance.

Linkage disequilibrium analysis on DArT markers revealed seven QTL associated to FHB resistance in Tun34 pedigree were located on Chromosomes 3B, 6B, 2A, 5B, 1B, 7A and 7B. Tun18 carries three QTL on 7B, 7A and 1B for FHB resistance. The 1B QTL were exactly in the same location as the one from Tun34 population. We have found three QTL on 3AS, 1BL and 2AL associated to FHB resistance in the Tun7 pedigree but the 2AL region associated with increased resistance was from the susceptible source parent. With this study we revealed some QTL in the same chromosome as had been reported in hexaploid wheat, which may change the belief of lacking the genes for FHB resistance in durum wheat as compared to hexaploid wheat. This may lead to future analysis identifying loci that may act as suppressors of resistance in the durum wheat making FHB resistance genes less effective compared to their action in hexaploid genetic background. Although we found several new potential QTL regions for FHB resistance in durum wheat, two regions located on 5B and 7B have not reported in the hexaploid wheat and may be valuable new sources for pyramiding once transferred into cultivated bread wheat background. This study also shows the power of pedigree based association mapping to find the minor QTL although we had problem with the pedigrees with less than 100 entries especially when there are selections in favor of the other agronomic traits beside FHB resistance.

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DEVELOPMENT OF FHB RESISTANT SPRING WHEAT IN THE NORTHERN GREAT PLAINS. K.D. Glover^{1*}, J.A. Anderson² and M. Mergoum³

¹Plant Science Department, South Dakota State University, Brookings, SD 57007, USA; ²Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA; and ³Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, USA
*Corresponding Author: PH: (605) 688-4769; E-mail: Karl.Glover@sdstate.edu

ABSTRACT

Released in 2000, 'Alsen' was the first Hard Red Spring Wheat (HRSW) cultivar made available to growers in the northern Great Plains known to carry the major Fusarium Head Blight (FHB) resistance QTL, Fhb1 (Qfhs.ndsu-3BS). With the progression of time, cultivars possessing Fhb1 released by the North Dakota State University (NDSU), South Dakota State University (SDSU), and University of Minnesota (UMN) HRSW breeding programs have become more prevalent. Resistance sources derived from Triticum dicoccoides, as an example, have also been utilized. The cultivar 'Steele-ND' would be placed within this category. Although Fhb1 and other resistance genes are presently found within most releases from these programs, their resistance is incomplete, and therefore, losses caused by FHB can still be significant. Continual germplasm screening efforts combined with marker-assisted selection are a requirement if further advances in resistance levels are to be realized. In an attempt to achieve this goal, the NDSU, SDSU, and UMN HRSW breeding programs each operate significant FHB resistance screening programs. Within each program, thousands of early-generation, preliminary, and advanced breeding lines are either screened at multiple field locations or in both field and greenhouse environments each year. Though not to the same extent, phenotypic observations are collected for disease incidence, severity, and disease index values as well as the frequency of Fusarium damaged kernels and deoxynivalenol concentrations. Significant procedural differences exist among the programs, although each is successful in identifying lines with progressively elevated FHB resistance. Classical phenotypic selection techniques coupled with an increased usage of molecular markers should allow resistance levels to gradually increase, although it is anticipated that progress will be more tempered after Fhb1 becomes widely utilized.

VALIDATION OF A FAMILY-BASED QUANTITATIVE TRAIT LOCUS MAPPING APPROACH FOR SELECTION OF FUSARIUM HEAD BLIGHT RESISTANT SPRING WHEAT BREEDING LINES. K.D. Glover^{*}, J.L. Gonzalez-Hernandez, U.R. Rosyara, D. Karki, K. Gedye and J.M. Stein

Plant Science Department, South Dakota State University, Brookings, SD 57007, USA *Corresponding Author: PH: (605) 688-4769; E-mail: Karl.Glover@sdstate.edu

ABSTRACT

Traditional Quantitative Trait Loci (QTL) mapping approaches are based on analysis of bi-parental populations. Mapping populations, however, are not widely known for the creation of new cultivars. In addition, markers linked to QTLs of interest are often not immediately available for use in breeding, and may never be useful within some genetic backgrounds. Use of multiple segregating populations for simultaneous QTL mapping, marker validation, marker-assisted selection (MAS) and prospective cultivar development has recently caught the attention of plant breeders because weaknesses of traditional mapping approaches can potentially be circumvented. Using a non-traditional family-pedigree based mapping approach, we previously localized the well-characterized Fusarium Head Blight (FHB) resistance QTL, Fhb1 (Offhs.ndsu-3BS) within 82 segregating populations. The objective of this study was to demonstrate advantages of the family-pedigree based approach in the context of generating breeding lines for potential cultivar release. In the mapping portion of the study, ten Simple Sequence Repeat (SSR) markers on chromosome 3B, with Gwm389, Gwm533, and Gwm493 being of most interest, were used to genotype F₁ plants. A single spike from heterozygous plants in each population was threshed individually and grown as a head row during winter 2007-2008. Each row was harvested in bulk and grown as a yield trial plot in 2008. Thirty F₃ spikes from each of 18 desirable yield trial plots were selected prior to harvest and a sample of F_4 seed from each spike was sown as a hill for FHB screening in the greenhouse during fall 2008. Four plants from each hill were also genotyped using the SSR marker Gwm533. Results from FHB disease screening and marker genotyping will be presented. Although the location of Fhb1 was known prior to initiation of this study, it was chosen to illustrate the speed with which 1.) this approach can localize QTL using widely applicable molecular markers and 2.) several FHB resistant breeding lines can be selected from within many agronomically acceptable populations.

CHARACTERIZATION AND DEVELOPMENT OF FHB RESISTANT SOFT WINTER WHEAT CULTIVARS IN THE EASTERN U.S. Carl A. Griffey^{1*}, Gina Brown-Guedira², Shuyu Liu¹, J. Paul Murphy³ and Clay Sneller⁴

¹Crop & Soil Environmental Sciences Dep., Virginia Tech, Blacksburg, VA 24061; ²ARS-USDA, Eastern Regional Small Grains Genotyping Lab, Raleigh, NC 27695; ³Crop Science Dep., North Carolina State University, Raleigh, NC 27695; and ⁴Horitculture and Crop Science Dep., OARDC-Ohio State University, Wooster, OH 44691 *Corresponding Author: PH: 540-231-9789; E-mail: cgriffey@vt.edu

Fusarium Head Blight (FHB) epidemics have occurred frequently in many of the eastern soft winter wheat production regions of the U.S. where much of the wheat crop is planted directly into maize residue. Prior to 1990, few winter wheat breeding programs considered it necessary or placed a significant amount of emphasis on the identification and development of cultivars having resistance to FHB. Initially uncertainty prevailed as to whether cultivars having significant resistance to FHB could be derived from existing breeding populations in which parental lines had not been directly selected on the basis FHB resistance. As a result the initial goal of many breeding programs was to incorporate FHB resistance derived from Asian, South American, and other "Exotic" sources into adapted soft winter wheat backgrounds. Initial success was hindered by lack of reliable phenotypic and genotypic (DNA markers) selection capabilities and by the persistence of linkage of unfavorable traits, such as low yields, susceptibility to other prevalent diseases, shattering, poor winter hardiness, etc., to FHB resistance. Subsequently, very effective sources of "Native" FHB resistance were identified within the soft winter wheat germplasm pool. While native resistance remains the genetic base of most breeding programs for developing FHB resistant cultivars, the goal of many programs is to pyramid unique QTL or genes derived from both native and exotic sources to further enhance resistance to FHB and DON toxin accumulation.

FHB Resistance Identified in Native Sources: Upon evaluation of existing adapted winter wheat lines and cultivars in FHB nurseries, several of them were documented as having moderate to high levels of FHB resistance, and subsequently referred to as native resistance sources. Recent analysis of FHB resistance in the variety development program at The Ohio State University infers that genotypes having moderate resistance to FHB derived from native sources are fairly common. The data indicates that FHB resistance alleles come from many parents and exists at a relatively high frequency in soft winter wheat. Similar observations have been made in other soft wheat breeding programs. Many soft wheat lines evaluated in the Uniform Scab Screening Nurseries have moderate FHB resistance and lack or have little exotic parentage.

Soft wheat cultivars with native resistance have been released and some of their QTL have been mapped. The soft red winter (SRW) wheat cultivar Freedom, released by The Ohio State University in 1991, was among the first winter wheat cultivars identified with native resistance. The SRW wheat cultivar Ernie, released by the University of Missouri in 1994, was identified as having a moderately high level of native FHB resistance, which subsequently was mapped and reported to be conferred by QTL on chromosomes 2B, 3BSc, 4B, and 5A in greenhouse single-floret inoculation studies. Recent mapping studies using phenotypic data collected in inoculated, mist-irrigated field tests indicate that the QTL located on chromosome 4B in Ernie has a larger effect on FHB severity and Fusarium damaged kernels, while the awn inhibitor gene B1 on chromosome 5A has a larger effect on FHB incidence. An even higher level of native FHB resistance was identified in the cultivar Truman, released by the University of Missouri in 2003, which currently is being mapped. Mapping studies published to date indicate that the Asian QTL alleles for *Fhb*1 on chromosome 3BS appear to have been absent in native soft winter wheat prior to recent introgression efforts. Collectively, there have been nine chromosome regions implicated to confer FHB resistance in soft winter wheat with potentially coincident QTL on chromosome 2B in Ernie and Goldfield, chromosome 3B near the centromere in Ernie and Freedom, and chromosome 5AS in Ernie and Freedom. The QTL appear to have moderate to small effects with just two QTL producing an R² that exceeds 0.20.

During past eight years, more than 30 SRW and 2 SWW wheat cultivars having resistance to FHB have been released by public and private breeding programs. A majority of these cultivars were evaluated in the Uniform Scab Screening Nurseries and have native FHB resistance including: McCormick and Tribute (released in 2002); INW0304 (QTL on chromosome 2B), IL94-1653 (exclusive release), Neuse, and Truman (2003); INW0411 with QTL on 2AS and 2B (2004); Bess, Coker 9511, Jensen (SWW), USG 3342, and WestBred X00-1079 (2005); IL99-12976, IL00-8061, and IL00-8633 (exclusive releases, 2006); OH02-13567 (exclusive release) and Jamestown (2007); Bromfield, Coral (SWW), GA981621-5E34, IL00-8109, IL008530, IL01-11934, IL01-16170, IL02-19463 (exclusive releases), Malabar, and Pembroke (2008). Lines having native FHB resistant slated for release in 2009 include: B030543 and NC04-20814. While native FHB resistance currently comprises and will continue to provide a base level of resistance in winter wheat cultivars, only a few native sources has been genetically characterized and mapped. This remains a critical priority if genes in these potentially novel sources of resistance are to be effectively used, selected for and combined with genes from other unique native and exotic sources in cultivar development programs.

Incorporation of FHB QTL from Asian and Eu-

ropean Sources: In an endeavor to incorporate novel FHB resistance and/or to enhance current resistance derived from native sources, many programs initiated efforts using a vast array of breeding methods to in-

corporate Type II FHB resistance, derived predominantly from a seemingly diverse array of Asian and other sources, into adapted winter wheat backgrounds. Subsequent emphasis has been placed on identifying diverse sources of Type II resistance as well as other unique types of resistance and their incorporation and combination in elite wheat lines. Of the QTL reported for FHB resistance, those located on chromosomes 1B, 2AS, 2B, 2DL, 3A, 3BS, 3BSc, 4BL, 5AS, 6B and 7B have been postulated as conferring resistance among current winter wheat cultivars and advanced elite lines. Winter wheat cultivars having FHB resistance derived directly from Asian (3BS and 5AS) and/ or European (1B and 3A) sources or from diverse combinations of these with native sources include: Pioneer Brand 25R42 with Fhb1 (2001); 25R35 and 25R54 (2003); INW0412 (2004); Pioneer Brand 25R51 (2005) and; INW0801 (2008) having QTL on chromosomes 1B, 2AS, and 3A. While notably lower, the number of cultivars having FHB resistance derived from exotic versus native resistance sources has increased in recent years. This trend is expected to continue as more desirable cultivars and parental lines having FHB resistance derived from exotic sources have and are currently being used in breeding programs. Increased availability of more diagnostic and broadly applicable high-throughput PCR-based markers for validated FHB QTL is critical as this also will determine success in further enhancements of FHB resistance and variety development efforts.

Genotypic Assessment of FHB Resistance of Entries in Uniform Scab Nurseries: Initially (2001 –2003) entries in the Southern Uniform Winter Wheat Scab Nursery (SUWWSN) were genotyped for markers (Xgwm 493, Xgwm 533, and Xbarc133) first reported to be associated with resistance conferred by Fhb1 (3BS). Few entries in these early nurseries had FHB resistance derived from Asian sources and only a few lines, such as VA01W-476 (Roane/W14) were postulated to possess Fhb1. Beginning with the 2005 SUWWSN, entries were genotyped using markers for both Fhb1 and the 5A QTL. Two entries (NC03-11457 and NC03-11458) were postulated to possess both QTL and three entries (NC03-11465, NC0311561, and VA04W-433) likely possess Fhb1. Among entries in the 2006 SUWWSN, three (NC04-27617, NC04-27618, and NC04-27669) were postulated to possess both Fhb1 and the 5A QTL, and one entry (AR97002-2-1) putatively has the 5A QTL. In the 2007 SUWWSN, entries also were genotyped using markers for the 2DL QTL from Wuhan1. One entry (LA01096D-88) was postulated to possess both Fhb1 and the 2DL QTL, and three lines (NC05-25083, GA991109-6E8, and GA991109-6A7) putatively have the 5A QTL. In the 2008 SUWWSN, entries were genotyped for Fhb1 and for the QTL on 2DL, 3BSc, and 5A. One entry (LA01164D-94-2-B) was postulated to possess Fhb1 and the 5A QTL, six lines (LA01141D-138-4-B, LA01150D-79-7-B, LA01162D-131-8-B, NC05-25059, NC05-25062, and NC05-25066) likely have Fhb1, and two lines (M04-4715 and VA06W-608) putatively have the QTL on 3BSc.

Genotyping of entries in the Northern Uniform Winter Wheat Scab Nursery (NUWWSN) for FHB resistance was initiated by the USDA-ARS Genotyping Lab beginning with the 2007 nursery. One entry (MSU Line E6003) was postulated to possess *Fhb1* and the 2DL QTL, MSU Line E6001 likely has *Fhb1*, MSU Line E6002 likely has the 2DL QTL, and two OSU lines (OH02-12678 and OH02-12686) putatively have the 5A QTL. In the 2008 NUWWSN, one entry (VA05W-775) was postulated to possess *Fhb1* and four lines (MO-050921, VA05W-534, OH02-13567, and OH02-7217) likely have the 3BSc QTL.

Genotype assessment of the entries in the Uniform Scab Nurseries has been useful not only for determining if a particular line may carry a resistance QTL, but also in determining the potential usefulness of markers for conducting marker assisted selection in soft winter wheat populations. The results of the genotyping done on the nurseries are most reliable for lines resulting from crosses where the resistance can be traced by pedigree. For lines having Asian sources of resistance in their pedigrees, the markers linked to *Fhb*1 and the 5A QTL can reliably determine if a chromosome region was inherited from the resistant parent. This is due to the fact that closely linked markers are

selected for genotyping; however, the frequency of the Asian alleles and/or haplotypes is very low in soft winter wheat. For the QTL mapped in native sources of resistance, it is more difficult to find these sorts of markers for genotyping. Therefore, using the results of marker analyses alone to predict the presence of a native QTL is less reliable. For instance, a survey of 250 soft wheat lines found that for markers linked to the 5A QTL mapped in Ernie, the frequency of the Ernie alleles ranged from 0.34 to 0.53. Thus, these markers are not suited for genotyping or for markerassisted selection (MAS) and new more diagnostic markers linked to this QTL need to be identified. In contrast, the frequency of Ernie alleles for markers Xgwm285 and Xwmc612 in the 3BS centromere region ranged from 0.10 to 0.18 and the Ernie haplotype for markers across this region is not common. Thus, these 3BSc markers are better suited for MAS and are included in genotyping the Uniform Scab Nurseries. However, there is not yet conclusive evidence of an effect of this haplotype in conferring FHB resistance in lines unrelated to Ernie.

Availability of genotypic data for marker alleles associated with confirmed QTL governing FHB resistance in entries evaluated in Uniform Scab Nurseries is critical to the success and efficacy of variety development efforts in breeding programs. This information in combination with other agronomic data allows breeders to select parental lines having effective and unique FHB resistance and to implement marker assisted selection in the incorporation, pyramiding, and selection of FHB resistance in subsequent progeny and pure lines. Current lack of such information on a majority of FHB resistant lines identified in Uniform Scab Nurseries greatly hinders breeders' ability to select the best parental lines and subsequent progeny due to the lack of knowledge regarding the QTL/genes conferring resistance and markers to deploy in MAS.

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RESISTANCE TO ACCUMULATION OF DEOXYNIVALENOL IN SOFT RED WINTER WHEAT. M.J. Guttieri^{1*}, R. Jackwood¹, P. Paul² and C. Sneller¹

¹Dept. of Horticulture and Crop Science, and ²Dept. Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH *Corresponding Author: PH: (330) 202-3555 ext. 2656; E-mail: Guttieri.1@osu.edu

ABSTRACT

The ultimate goal of host plant resistance to Fusarium graminearum (Fg) is reduced concentration of the toxin, deoxynivalenol (DON), in the grain. Resistance to spread of infection by Fg (Type II = T2), and to a lesser degree resistance to initial infection (Type I = T1) have been documented in wheat. Combinations of T1 and T2 resistance do not provide complete resistance to Fg, and DON can be accumulated in grain of even our most resistant cultivars. In general, visual symptoms of FHB and are positively related DON, and selection for T1 and/or T2 resistance lowers DON. However, this association becomes highly variable in moderately resistant wheat genotypes. Resistance to accumulation of DON (Type V = T5) has been proposed, but not validated. In a FY07 USWBSI grant we attempted to assess T5 resistance in soft winter wheat. We collected grain from moderately resistant soft winter wheat breeding lines in field-grown, inoculated spikes with 0, 1, 2, or 3 infected spikelets per spike (approximately 0, 6, 12, or 18% infection based on visual symptoms). Fungal biomass was determined by quantitative real-time-PCR (qRT-PCR) for each grain sample from each infection level. For each wheat genotype, we regressed DON on estimated Fg biomass. The slopes were statistically different. In some genotypes, DON increased significantly as Fg biomass increased, while in other genotypes, the response of DON to increasing Fg biomass was negligible. The experiment was repeated in 2008 with three field replications of each wheat genotype. In 2008, Fg biomass ranged from <0.05 copies Fg/copy wheat to nearly 30 copies/copy wheat, and DON concentration ranged from < 0.3 ppm to 33 ppm. In the analysis of covariance, the 28 genotypes differed significantly for DON concentration (p < 0.001), and the response of DON concentration to Fg biomass was highly significant (p < 0.001). The interaction of genotype and Fg biomass also was highly significant. The 13 grain samples among the 345 characterized that had DON concentrations >20 ppm had an average Fg biomass of 9.0 ± 5.3 copies Fg/copies wheat; the 11 grain samples that had Fg biomass of >20 copies Fg/copies wheat had an average DON concentration of 8.8 ± 5.3 ppm. Overall, biomass did not effectively predict DON ($r^2 = 0.02$). Genotypes could be categorized based on response of DON concentration to Fg biomass, as well as by the level of Fg biomass developed, as some genotypes developed little Fg biomass despite visual symptoms of infection. Genotypes were identified that appear to suppress accumulation of DON, despite detectable fungal biomass. Resistance patterns observed in the preliminary 2007 study generally were consistent with the results of the replicated 2008 study. This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-4-101. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

IDENTIFICATION OF WHEAT LINES WITH *FHB1* BY INJECTING DON INTO FLORETS AT FLOWERING. P. Horevaj and E.A. Milus^{*}

Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR, 72701 *Corresponding Author: PH: (479) 575-2676; E-mail: gmilus@uark.edu

OBJECTIVES

To determine if the direct application of DON into florets is a useful method for identifying lines with *FHB1* in different genetic backgrounds and to determine if other FHB resistance genes confer similar resistance to DON.

INTRODUCTION

In North America, the deoxynivalenol (DON) chemotype of Fusarium graminearum is the primary cause of Fusarium head blight (FHB) in wheat. Because DON is toxic to humans and animals, wheat cultivars with low or no DON are desirable for wheat growers, processors and consumers (Bai and Shaner, 2004). DON also acts as virulence factor by enhancing the ability of F. graminearum to spread within a spike (Desjardins et al. 1996). Therefore, wheat lines more resistant to DON should be more resistant to FHB. Lemmens et al. (2005) applied DON directly into wheat spikes and counted the number of bleached florets. Resistance to DON was closely associated with FHB1 that confers resistance to spread within a spike and with detoxification of DON to DON-3-Oglucoside.

MATERIALS AND METHODS

A susceptible check and 15 diverse FHB-resistant winter wheat lines were grown in the greenhouse. For each pot, two pairs of similar-sized spikes in the early flowering stage were selected and marked. One spike of each pair served as a control, and the other spike was treated with DON (250 μ g in each of four primary florets) using a modification of the procedure described by Lemmens *et al.* (2005). The total number of florets per spike was counted at 7 days after

treatment, and the number of DON-bleached florets below and above the treated florets was counted at 7, 14 and 21 days after treatment. Spikes were harvested at maturity and threshed by hand to retain all grain. The relative yield for the treated spikes was calculated as (yield of treated spike / yield of control spike) x 100. The experimental design was randomized design consisting of 16 wheat lines and five replications (pots) in run 1 and four replications in runs 2 and 3. Lines were treated as fixed effects and runs and replications as random effects. Wheat line means were separated using Tukey's HSD test at P=0.05.

RESULTS AND DISCUSSION

The six wheat lines with molecular markers linked to FHB1 had fewer DON-bleached florets 21 days after treatment than the ten lines without FHB1 (Table 1), indicating that presence or absence of this gene can be identified in diverse backgrounds by injecting DON into florets. All lines with FHB1 had type II resistance as measured by the number of FHB-blighted florets (determined in previous experiments), but lines VA04W-433 and Fg 368 had the fewest FHB-blighted florets (Table 1), indicating that these lines may have additional genes for type II resistance. Nine wheat lines without FHB1 had numbers of DON-bleached florets similar to the susceptible check but had fewer FHB-blighted florets than the susceptible check, indicating that these lines had type II resistance that was conferred by genes other than FHB1.

The resistance to DON conferred by *FHB1* did not protect plants from the phytotoxic effects of DON on kernel formation as measured by the relative yield of treated spikes. However, lines Fg 365 and Fg 368 had higher relative yield than other lines, indicating that they may posses one or more genes conferring tolerance to DON and ability to fill kernels in the presence of DON. Line Fg 365 was found to have the highest tolerance against FHB (Mesterházy et al. 1999), and therefore measuring the relative yield loss to DON injection may be a method for identifying lines with tolerance to FHB (type IV resistance). There was only a weak positive correlation ($R^2 = 0.16$) between relative yield and number of florets per spike, indicating that results were not overly influenced by the size of the spikes.

In conclusion, injecting DON into florets can readily detect lines with *FHB1*, which appears to be the only gene that confers resistance to DON. Furthermore, measuring relative yield after DON injection may be a useful method for identifying lines with tolerance to FHB (type IV resistance).

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Line ¹	<i>FHB 1</i> present ²	# 01 DOM- bleached ³ primary florets	# of FHB-blighted ⁴ primary florets	Relative yield ⁵ (%)	1 otat # of primary florets per head
VA04W-433	Yes	$3.2 a^{6}$	1.9 d	29.8 cd	29.5
Fg 368	Yes*	3.7 a	2.4 d	54.0 a	42.0
NC03-11465	Yes	3.9 a	8.1 bcd	32.7 bc	36.7
SZ 13	Yes*	4.1 a	5.9 bcd	25.0 cde	31.3
SZ 14	Yes*	6.2 ab	4.6 bcd	25.5 cde	29.2
ARGE97-1047-4-2	2 Yes: 3 No	12.1 abc	8.0 bcd	16.5 cde	34.9
ARGE97-1064-13-5	No	16.7 bcd	9.9 ab	24.7 cde	38.2
AR97002-2-1	No	20.2 cde	3.7 bcd	18.3 cde	31.1
VA04W-628	No	20.4 cde	3.9 bcd	11.5 de	34.7
ARGE97-1042-4-5	No	21.3 cde	8.1 bcd	23.7 cde	42.0
ARGE97-1048-3-6	No	21.4 cde	8.9 bc	25.1 cde	40.2
Coker 9835 (Susceptible)	No	21.7 cde	15.8 a	8.1 e	31.9
Bess	No	22.0 cde	2.8 cd	24.1 cde	38.9
ARGE97-1033-10-2	No	22.8 cde	2.7 cd	30.9 c	37.8
Roane	No	25.2 de	3.9 bcd	18.4 cde	39.0
Fg 365	No	29.7 e	6.7 bcd	49.5 ab	48.3

Tahla1 d f FHR1 7 9+ 1:-5 f DON-HI 2 <u>1</u> <u>.</u> Þ ١. Ì. F DON ++ es,

⁵ The relative yield for the DON-treated spikes was calculated as yield for treated spikes divided by yield for control spikes and multiplied by 100. ⁶ Means within a column followed by the same letter are not significantly different according to Tukey's HSD test at P=0.05.

RESISTANCE IN WINTER WHEAT LINES TO INITIAL INFECTION AND SUBSEQUENT SPREAD OF DON AND NIV CHEMOTYPES OF *FUSARIUM GRAMINEARUM*. P. Horevaj and E.A. Milus^{*}

Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR, 72701 *Corresponding Author: PH: (479) 575-2676; E-mail: gmilus@uark.edu

OBJECTIVE

To quantify the resistance of selected winter wheat lines to initial infection by deoxynivalenol (DON) and nivalenol (NIV) chemotypes of *F. graminearum* and to subsequent spread of these infections that may be a useful measure of combined type I and type II resistances.

INTRODUCTION

Resistant cultivars are believed to be the key component in any integrated management program for Fusarium head blight (FHB) caused by Fusarium graminearum. Five types of resistance to FHB in wheat have been described (Mesterházy, 1995), however, only two types (type I - resistance to initial infection, and type II - resistance to fungal spread within a spike) have been widely accepted and clearly defined. In wheat, type II resistance is considered to be a major component of the FHB resistance complex (Bai et al. 2000), and breeders more commonly utilize this type of resistance than type I resistance. However, as Bai and Shaner (2004) stated, adding additional type II resistance genes into lines with type II resistance may not increase their overall resistance performance. However, adding type I resistance into lines with type II resistance should increase their field resistance against FHB.

MATERIALS AND METHODS

A susceptible check and 15 winter wheat lines resistant to FHB were inoculated using a spray inoculation technique (1×10^5 macroconidia/ml and 3 ml per spike) with two *F. graminearum* isolates representative of DON and NIV chemotypes in the United States. At

flowering, spikes were inoculated, covered with plastic bags and incubated in a greenhouse at 17 to 22°C. Bags were removed after 48 hr, and plants were incubated at 20 to 24°C for the rest of the experiment. The total number of primary florets per spike was counted 7 days after inoculation (dai), and the number of blighted primary florets was counted 7, 10, 14 and 21 dai. The area under the disease progress curve (AUDPC) from 0 to 21 days was calculated to estimate the combined effects of type I and type II resistances. The experimental design was a randomized design consisting of 16 wheat lines, one DON and one NIV chemotype, and 7 replications (cones) in runs 1 and 2. Lines and chemotype were treated as fixed effects and runs and replications as random effects. Means were separated using Tukey's HSD test at P=0.05.

RESULTS AND DISCUSSION

The line × chemotype interaction was not significant for percentage of blighted primary florets 7 dai (P=0.931) or AUDPC (P=0.685), indicating that lines ranked similarly for both DON and NIV chemotypes. Chemotypes were not significantly different for percentage of blighted primary florets 7 dai (P=0.061) or AUDPC (P=0.124). However, the DON chemotype averaged 15.2% blighted primary florets at 7 dai and 446 AUDPC, whereas the NIV chemotype averaged 9.9% blighted primary florets at 7 dai and 250 AUDPC. The DON chemotype was expected to have a higher AUDPC than the NIV chemotype because DON is a virulence factor that increases spread of infection within spikes. These results indicate that NIV might also be a virulence factor to a lesser extent than DON. Wheat lines were significantly different ($P \le 0.0001$) for both percentage of blighted primary florets 7 dai and for AUDPC. There was much statistical overlap among lines, but Roane, AR97002-2-1, SZ 13, and VA04W-628 were among the most resistant for both variables (Table 1). All except four lines had significantly lower percentages of blighted florets at 7 dai than the susceptible check, indicating that most lines had type I resistance. All lines had significantly lower AUDPC values than the susceptible check, indicating that all lines have type I and/or type II resistances. There was a positive correlation ($R^2 = 0.71$) between AUDPC and percentage of FHB-blighted primary florets 21 days after single floret inoculation in previous experiments to determine levels of type II resistance, suggesting that AUDPC was associated with type II resistance.

Possibilities for improving the ability to quantify resistance to initial infection include 1) counting the number of blighted primary florets at 4, 5, 6 and 7 dai, 2) incubating inoculated plants in a growth chamber to achieve a uniform post-inoculation environment, and 3) utilizing inoculum of similar aggressiveness on each date of inoculation.

In conclusion, most of the winter wheat lines have type I resistance that is effective against both DON and NIV chemotypes. AUDPC following spray inoculation appears to be a useful variable for quantifying the combination of type I and type II resistances. The most resistant lines for both variables were Roane, AR97002-2-1 and SZ 13.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

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winter wheat lines inoculated using a spray inoculation technique with one DON and one NIV chemotype of Fusarium graminearum Table 1. Percentage of blighted primary florets 7 days after inoculation and area under the disease progress curve (AUDPC) for 16 and averaged across two runs of the experiment.

		Dugueu puna y	AUPLO
Line ¹	Pedigree	florets after	across
		7 days (%)	21 days ²
Roane	VA71-54-147 (CI17449)/Coker68-15//IN65309C1-18-2	5.2 a ³	145 ab
AR97002-2-1	AR396-4-2/NING 8026	5.9 a	133 a
SZ 13	Ringo Star / Nobeoka Bozu	6.8 a	233 ab
VA04W-628	Ernie//NING7840/Ernie	7.9 ab	210 ab
SZ 14	Ringo Star / Nobeoka Bozu	8.4 ab	277 abc
ARGE97-1042-4-5	Mason / Catbird	9.5 abc	281 abc
ARGE97-1033-10-2	Freedom/Catbird	10.2 abc	245 ab
ARGE97-1064-13-5	Mason//Freedom/Super Zlatno	10.3 abc	295 abc
ARGE97-1047-4-2	P2684 / 3 NING 7840 // Parula / Veery # 6	11.8 abc	363 abc
ARGE97-1048-3-6	Mason // SHA 3 / Catbird	12.3 abc	377 bc
Bess	MO 11769/Madison	12.6 abc	300 abc
Fg 368	Zugoly / Reka / Nobeoka Bozu	14.9 abcd	341 abc
NC03-11465	NING 7840/P2643//NC95-22426	16.7 abcd	480 c
Fg 365	Ságvári / Nobeoka Bozu // Mini Mano / Sum3	20.2 bcd	484 c
VA04W-433	NING 7840/PION2684//96-54-244 (CK9803/Freedom)	21.1 cd	479 c
Coker 9835	Susceptible check	27.3 d	930 d

¹ Lines were provided by E. Milus, C. Griffey, R. Bacon, A. McKendry, P. Murphy and A. Mesterházy.

² AUDPC was calculated according to the formula AUDPC = $\sum [(y_i + y_{i+1})/2 * (t_{i+1} - t_i)]$ where y= percentage of primary florets blighted at time t and t=0, 7, 10, 14 and 21 days.

³ Means within a column followed by the same letter are not significantly different according to Tukey's HSD test at P=0.05.

DEVELOPMENT OF SCAB RESISTANCE IN SOFT RED WINTER WHEAT. Jerry Johnson^{1*}, Zhenbang Chen¹, James Buck² and Lilian Miranda¹

¹Department of Crop and Soil Sciences, and ²Department of Plant Pathology, Griffin Campus, University of Georgia, Griffin, GA *Corresponding Author: PH: (770) 228-7321; E-mail: jjohnson@griffin.uga.edu

ABSTRACT

Fusarium head blight (FHB) is a potential devastating disease in the southeast region of the United States. Several native sources of Type II resistance (Truman, Roane, Ernie, OH02-12686, and IL00-8530) and from derivatives of Sumai 3 (INW0411, VA04W433, VA 01-461) have being incorporated into GA elite lines. Breeding for Type I resistance is also in progress with populations derived from Truman and Frontana. Several elite breeding lines, GA 991109-6E8, GA 031307DH, GA 031454DH, GA 981621-5E34 have been identified as moderately scab resistant. Marker assisted backcrossing of QTL from Sumai 3 (3BS, 5AS), Goldfield (2BS) and Ernie (5AS, 3BS, and 4BL) have been used to transfer resistance into AGS 2000 background. Several FHB resistant sources were evaluated for Type I resistance in the greenhouse. Bess and Tribute were the two checks that had a low infection spread (1.3 and 1.7, respectively). Lines that were equal to the checks for low infection spread were M04*5109, M03-3616B, NC05-25062, GA 03131354-DH30, GA981621-5E34, and LA01141D-138. Lines that had the lowest DON levels were Bess, Jamestown, M04*5109, M03-3616B, GA 03131354-DH30, and VA 05W-510.

HISTORY OF FHB RESISTANCE EVALUATION IN MICHIGAN STATE PERFORMANCE TRIAL. J. Lewis^{*}, L. Siler, G.L. Jiang and R.W. Ward

Dept. of Crop and Soil Science, Michigan State University, E. Lansing MI, 48824 *Corresponding Author: PH: (517) 355-0271 ext. 1185; E-mail: lewisja6@msu.edu

ABSTRACT

In 1996, the Michigan wheat industry suffered a crippling blow due to a widespread Fusarium Head Blight epidemic. Since this time, FHB has been a priority concern for MI wheat producers, millers and industry. Michigan State University has been conducting FHB screening on the Michigan State Performance Trial for the past 11 years (http://www.css.msu.edu/varietytrials/wheat/Variety_Results.html). Many varieties entered into the Michigan State Performance Trial are evaluated for more than one year, and several varieties were present in over half of these 11 years of trials, and in a few cases, for all of the 11 years. For each year, with the exception of 2003, field trials were successfully conducted and scores of incidence and severity were recorded. In 2003, severity scores were assessed in the greenhouse. Only more recently has toxin evaluation been reported for the State Performance Trial. Over the years that the FHB screening has been conducted, methods of inoculation as well as method of FHB symptom measurement have varied and/or been changed. Our associated poster will consider the trends of genetic resistance in the MI State Performance Trial over time, as well as the consistency of FHB data for genotypes present in the trial over multiple years.

PRELIMINARY SELECTION OF F3 AND F4 BREEDING LINES FOR FHB RESISTANCE AT MICHIGAN STATE UNIVERSITY. J. Lewis^{*}, L. Siler and S. Hammar

Dept. of Crop and Soil Science, Michigan State University, E. Lansing MI, 48824 *Corresponding Author: PH: (507) 355-0271 ext. 1185; E-mail: lewisja6@msu.edu

ABSTRACT

In 2008 we evaluated early generation (F3 soft reds and F4 soft reds and F4 soft whites) breeding lines for FHB resistance at Michigan State University. Our purpose in evaluating these lines was to examine the efficacy of selection in earlier generations, and to do this using a rough evaluation method. Each F3 and F4 breeding line that was evaluated was derived from a single F2 or F3 (respectively) plant. In addition, many of the F3 soft red lines originated from the same crosses as the F4 soft whites, having been generated from segregating F2 populations. During our FHB screening, the breeding lines were observed for FHB index multiple times over the course of approximately 2.5 - 3.5 weeks. Breeding lines that showed clear susceptibility were selected against as early as possible (i.e., not waiting until a specified time after flowering), while lines that showed greater levels of resistance were only identified after a reasonable level of resistance had been maintained until a few weeks after anthesis. Using this rough evaluation system, a line that may have initially appeared resistant could be selected against at a later date if the resistance had appeared to break down over time. For those that were selected, they were objectively categorized on a 1-5 scale, where 1 = very good resistance and 5 =borderline between moderate resistance and moderate susceptibility. 'Truman' was used as a resistant check and was planted regularly in the FHB nursery for comparison across all FHB trials. F3 lines selected this year will be re-evaluated for FHB resistance in 2009 to access the predictability of resistance based on 2008 evaluation. In the associated poster we will highlight trends that were observed in the populations for FHB resistance selection in these F3 and F4 early generations.

IDENTIFICATION OF MOLECULAR MARKERS FOR SCAB RESISTANCE IN WINTER BARLEY USING ASSOCIATION MAPPING. Shuyu Liu¹, Wynse S. Brooks¹, Shiaoman Chao², Carl A. Griffey^{1*} and Marla D. Hall¹

¹Dept. of Crop and Soil Environmental Science, Virginia Tech, Blacksburg, VA 24060; and ²USDA-ARS Biosciences Research Lab, 1605 Albrecht Blvd, Fargo, ND 58105 *Corresponding Author: PH: (540) 231-9789; E-mail: CGriffey@vt.edu

ABSTRACT

Two major approaches to identify molecular markers linked to important traits are traditional mapping in biparental populations and association mapping with a panel of germplasm lines. To date two Barley OPAs, consisting of 3,072 SNPs, have been genotyped in 1,920 barley breeding lines contributed from ten US barley breeding programs collaborating in the USDA-CSREES funded Barley Coordinated Agricultural Project (CAP). Ninety-six advanced lines from the Virginia Tech barley breeding program were screened for about 1536 SNPs in 2006. Phenotypic data for 24 traits have been collected on these 96 lines in Virginia over several years. This presentation focuses on molecular markers linked to scab resistance that were identified via association mapping. A subset of 46 lines having phenotypic data for scab traits collected in inoculated, mistirrigated tests in two years was used in the association mapping study. Tightly linked markers were detected for resistance to scab incidence and DON in both the 2006 and 2007 studies and for scab severity in 2007. Six SNPs on chromosomes 2H and 7H were associated with scab incidence in 2006 (P<0.001) and explained 16.0% to 21.5% of the phenotypic variation. In 2007, eight SNPs on chromosomes 1H, 2H, and 3H were associated with incidence (P<0.01) and explained 10.2% to 13.2% of the phenotypic variation. Seven SNPs on chromosomes 1H, 3H, 4H and 7H were significantly associated with DON levels in 2006 (P<0.0001) and explained 12.7% to 17.8% of phenotypic variation. In 2007, four SNPs on chromosomes 1H, 4H and 5H were linked to DON level (P<0.01) and explained 12.2% to 14.8% of the phenotypic variation. Three markers on chromosomes 3H and 4H were associated with severity in 2007 (P<0.01) and explained 12.7% to 16.3% of phenotypic variation. Among these significant associations, a region containing six SNPs on chromosome 1H was associated with both incidence and DON level in 2007. A region on chromosome 2H with three SNPs was associated with incidence in both years. A region comprised of two SNPs on chromosome 4H was associated with both severity and DON levels in 2007, while another unique region with seven SNPs was associated with DON levels in 2006 and severity in 2007. Three regions on the short arm of chromosome 7H were associated with scab resistance. Region one, comprised of three SNPs was associated with incidence in 2006 and DON in 2007; region two with three SNPs was associated with incidence in 2006 and severity in 2007 and; region three with two SNPs was associated with incidence and DON in 2006. These SNPs associated with scab resistance in the current study will be compared with other known QTL for scab resistance in barley based on chromosome locations. Validation of linked markers for known QTL and identification of novel QTL will further facilitate efforts in breeding for scab resistance in barley.

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MAPPING QTL FOR SCAB RESISTANCE IN THE VIRGINIA WHEAT CULTIVAR MASSEY. Shuyu Liu¹, Marla D. Hall¹, Carl A. Griffey^{1*}, Anne L. McKendry², Jianli Chen³ and David Van Sanford⁴

¹Dept. of Crop and Soil Environmental Science, Virginia Tech, Blacksburg, VA 24060; ²Dept of Plant Science, University of Missouri-Columbia, Columbia, MO 65201; ³Dept of Agronomy, University of Idaho Aberdeen Research & Extension Center Aberdeen, ID 83210; and ⁴Dept. of Plant & Soil Sciences, University of Kentucky, Lexington, KY 40546 *Corresponding Author: PH: (540) 231-9789; E-mail: CGriffey@vt.edu

ABSTRACT

Fusarium Head Blight (FHB) or scab is a serious disease which reduces yield and quality of wheat in warm and humid production areas worldwide. Planting resistant varieties is an economically effective and environmentally sound way to manage this disease. Identifying new sources of resistance and characterizing native sources of resistance are both major components in developing scab resistant wheat varieties. Massey, a cultivar released by Virginia Tech in 1985, has adult plant resistance to powdery mildew as well as being moderately resistant to scab. A set of 589 DArT markers and SSR markers were mapped onto all 21 chromosomes in a Becker/ Massey mapping population comprised of 152 RILs. Phenotypic data for FHB severity were obtained from a greenhouse test conducted in Virginia and FHB incidence, severity, and Index data were collected in field tests conducted in Virginia (2007, 2008), Missouri (2008), and Kentucky (2008). Average FHB severity data from greenhouse evaluations was not correlated with FHB field data. Correlations among data collected from multiple locations and years were not significant. Within each test, FHB incidence was significantly correlated to FHB severity (P < 0.001), and correlations between FHB severity and FHB index were the highest. Mapping data indicate that Massey has QTL on chromosomes 1D and 3B conferring resistance to FHB severity on the basis of single floret inoculation tests conducted in the greenhouse study. Eight QTL conferring resistance to FHB in Massey were located on chromosomes 1A, 2B, 2D, 3B, 4B, 4D, 5B, and 6A on the basis of field data collected from one location in 2007 and three locations in 2008. Among these ten QTL, the major QTL was mapped on chromosome 3B and was associated with greenhouse severity and scab index in field studies conducted in Virginia in both 2007 and 2008. The 3B QTL explained 7.1% and 23.4% of the phenotypic variation for greenhouse FHB severity in 2007 and 2008, respectively. It also explained 9.1% and 8.2% of the field FHB index in 2007 and 2008, respectively. Another QTL associated with greenhouse FHB severity in 2008 is located on chromosome 1D and explained 9.2% of variation. Three other QTL associated with FHB incidence and index in Virginia field studies are on chromosomes 4B, 4D and 6A with R² values of 9.9%, 14.3%, and 19.5%, respectively. The 4B QTL also confers resistance to Fusarium damaged kernels and explained 14.8% of the variation. A major QTL located on chromosome 2B and associated with field incidence (R²=8.1%), severity, (R²=12.4%), and FHB index (R²=11.0%), was identified via analyses of Missouri FHB field data, while the two major QTL identified from analysis of Kentucky FHB field incidence were mapped to chromosomes 2D (R²=8.0%) and 5B (R²=7.1%). The 5B QTL region also was associated with field severity and FHB index in analysis of the 2007 Virginia data and explained 13% of the phenotypic variation. The number of QTL and variation explained among different experiments reflect the complex nature of both phenotypic characterization and quantitative inheritance of FHB resistance. The similarity between QTL mapped in Massey with other known QTL will be compared and potentially novel QTL and/or unique combinations of QTL will be discussed in the presentation.

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SATURATION MAPPING OF SCAB RESISTANCE QTL IN ERNIE AND APPLICATION TO MARKER-ASSISTED BREEDING. Shuyu Liu¹, Carl Griffey^{1*}, Anne McKendry², Marla Hall¹ and Gina Brown-Guedira³

¹Dept. of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, VA 24060; ²Dept of Plant Science, University of Missouri-Columbia, Columbia, MO 65201; and ³Eastern Regional Small Grains Genotyping Lab, USDA-ARS, Raleigh, NC 27695 *Corresponding Author: PH: (540) 231-9789; E-mail: CGriffey@vt.edu

ABSTRACT

Fusarium head blight (FHB), is caused mainly by *Fusarium graminearum* in wheat and results in significant yield and quality losses in humid and warm areas of the world. QTL for scab resistance have been mapped in exotic and native sources. However, only a few QTL have been widely deployed in breeding programs using marker-assisted selection (MAS) due to the lack of diagnostic and tightly linked markers for most QTL. Four major QTL for type II resistance were previously mapped on chromosomes 5A, 4B, 3BSc and 2B of Ernie. A set of 243 Ernie/MO94-317 RILs were evaluated in inoculated, mist-irrigated scab nurseries at Columbia, MO and Blacksburg, VA. The 4B QTL region was associated with field FHB severity ($R^2=4.2\%$), index ($R^2=4.4\%$), kernel quality assessed as 100 grain weight ($R^2=8.0\%$), and fusarium damaged kernels (FDK, $R^2=6.2\%$). The awn inhibitor gene, B_1 , is associated with field FHB incidence ($R^2=4.5\%$) and index ($R^2=5.3\%$) in the Virginia test and with FHB severity ($R^2=4.2\%$) in the Missouri test. Another QTL associated with 100 grain weight is on chromosome 2DS ($R^2=12.4\%$). There is one minor QTL for FDK ($R^2=4.3\%$) on chromosome 5A that is separate from the major QTL for type II resistance and the B_1 gene. Tightly linked markers are being applied for marker-assisted selection in breeding populations for the four QTL in Ernie and the two major QTL on chromosomes 3BS and 6B of Sumai 3. This will facilitate the pyramiding of various QTL for FHB resistance using MAS in variety development.

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INHERITANCE OF FHB RESISTANCE IN SPRING VERSUS WINTER WHEAT GROWTH HABIT BACKGROUNDS. S. Malla¹, A.M.H. Ibrahim², W. Berzonsky^{1*} and Y. Yen¹

¹Plant Science Department, South Dakota State University, Brookings, SD 57007; and ²Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843 *Corresponding Author: PH: (605) 688-4951; E-mail: William.Berzonsky@sdstate.edu

ABSTRACT

Fusarium head blight (FHB) is one of the important diseases of wheat in South Dakota. This study was conducted to determine the inheritance of FHB resistance QTLs in spring and winter growth habit backgrounds. Four genotypes consisting of susceptible winter wheat 'Nekota' and '2137' and moderately resistant spring wheat 'ND2710' and 'BacUp' were crossed and populations derived from the crosses were segregated into spring and winter types following cold treatment of seedlings at -7°C for an hr. A total of six SSR marker (3BS QTL marker: Xgwm389, Xgwm493 and STS256; 5A QTL marker: Xgwm293, Xgwm304 and Barc186) were used to genotype the population. Chi-square analysis showed that there were significant differences in the percentage of the genotypes containing homozygous marker alleles for 3BS and 5A QTLs between spring and winter types in the population ND2710X2137, ND2710XNekota and BacUpX2137. The percentage of the genotypes with homozygous marker alleles for 3BS QTL was less in spring compared to winter growth habit backgrounds in the population ND2710X2137 and ND2710XBacUp. In contrast, spring type in the population ND2710XNekota showed higher percentage of genotypes containing homozygous marker alleles for 5A QTL than winter type. The results indicated that the 3BS QTL was less inherited in spring growth habit backgrounds whereas the 5A QTL was less inherited in winter growth habit backgrounds.

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MAPPING QTLS FOR FUSARIUM HEAD BLIGHT FROM NOVEL SOURCE - TOKAI-66. S. Malla¹, A.M.H. Ibrahim², W. Berzonsky^{1*} and Y. Yen¹

¹Plant Science Department, South Dakota State University, Brookings, SD 57007; and ²Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843 *Corresponding Author: PH: (605) 688-4951; E-mail: William.Berzonsky@sdstate.edu

ABSTRACT

Breeding for resistance is the most effective approach for managing Fusarium head blight (FHB), an important disease on wheat in South Dakota. This study was conducted to identify QTLs linked to FHB resistance in a resistance genotype - Tokai-66. A cross was made between Tokai-66 and Jagalene and single seed descent was used to advance the population. The $F_{2:4}$ and $F_{2:5}$ populations were evaluated by artificially inoculating disease in a mist irrigated nursery in 2006 and 2007. Disease incidence, severity, fusarium damaged kernel (FDK) and deoxynivalenol (DON) content were recorded in the population. Diversity Array Technology (DArT) was used to genotype the population. Preliminary analysis using single marker analysis in MapManager found that each wPt-5672, wPt-7757, wPt-4125 and wPt-5556 markers at 2B explained 11% of the variation in disease index in 2007. For FDK, wPt-2757 and wPt-1081 markers at 3B explained10% of the variation in 2006 and wPt-7984 marker at 3B explained 12% of the variation 2007. Marker wPt-0398 at 3A explained 16% of the variation in DON content in 2006, whereas marker wPt-7984 at 3B explained 13% of the variation in DON content in 2007. We are planning to saturate more SSR markers in the chromosome 2B, 3B and 3A to identify more QTLs linked to FHB.

ACKNOWLEDGEMENT AND DISCLAIMER

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MAPPING QTLS FOR FUSARIUM HEAD BLIGHT FROM SOUTH DAKOTA'S INDIGENOUS GENOTYPE - SD97060. S. Malla¹, A.M.H. Ibrahim², W. Berzonsky^{1*} and Y. Yen¹

¹Plant Science Department, South Dakota State University, Brookings, SD 57007; and ²Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843 *Corresponding Author: PH: (605) 688-4951; E-mail: William.Berzonsky@sdstate.edu

ABSTRACT

Breeding for resistance is the most effective approach to manage Fusarium head blight (FHB), an important disease on wheat in South Dakota. This study was conducted to identify QTLs linked to FHB resistance in an indigenous genotype – SD97060. A cross was made between SD97060 and Jagalene and single seed descent was used to advance the population. The $F_{2:4}$ and $F_{2:5}$ populations were evaluated by artificially inoculating disease in a mist irrigated nursery in 2006 and 2007. Disease incidence, severity, fusarium damaged kernel (FDK) and deoxynivalenol (DON) content were recorded in the population. Diversity Array Technology (DArT) was used to genotype the population. Preliminary analysis using single marker analysis in MapManager indicated that wPt-3132 in 2B explained 22% of the variation for disease index in 2006. Marker wPt-9032 at 3B explained 29% for DON content in 2006. We are planning to saturate more SSR markers in the chromosome 2B and 3B to identify further QTLs linked to FHB.

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USING ASSOCIATION MAPPING TO IDENTIFY FUSARIUM HEAD BLIGHT RESISTANCE QTL WITHIN CONTEMPORARY BARLEY BREEDING GERMPLASM. Jon Massman¹, Rich Horsley², Blake Cooper³, Stephen Neate⁴, Ruth Dill- Macky⁵, Shiaoman Chao⁶ and Kevin Smith^{1*}

 ¹Dept. of Agronomy and Plant Genetics, Borlaug Hall, 1991 Upper Buford Circle, University of Minnesota, St. Paul, MN 55018; ²Dept. of Plant Sciences, 166 Loftsgard Hall, North Dakota State University, Fargo, ND 58105-5051; ³BAR-LLC, 3515 E Richards Lake Rd., Ft. Collins, CO 80524; ⁴Dept. of Plant Pathology, 353 Walster Hall, North Dakota State University, Fargo, ND 58105-5012; and ⁵Dept. of Plant Pathology, 495 Borlaug Hall, 1991 Upper Buford Circle, University of Minnesota, St. Paul, MN 55108; and ⁶USDA-ARS, 1605 Albrecht Blvd, Fargo, ND 58105-5674 *Corresponding Author: PH: (612) 624-1211; E-mail: smith376@umn.edu

ABSTRACT

Utilization of quantitative trait loci (QTL) for Fusarium head blight (FHB) resistance identified through biparental mapping has had limited success in barley. Previously described resistance QTL (identified in wide biparental mapping studies) often have been associated with negative agronomic traits such as late heading and taller plants, thus reducing their overall utility. Mapping within existing breeding populations, however, would identify resistance QTL segregating in elite populations, and represent genetic factors which are immediately available to breeders. A total of 768 breeding lines from 4 programs (UM, BARI, NDSU-2, NDSU-6) were evaluated in four environments over 2006 and 2007. At each location the lines were planted in a randomized complete block design with two replications, inoculated, and overhead mist irrigated to encourage disease development. Each line was genotyped using 1,536 SNP markers, and QTL were mapped using a mixed model approach. Phenotypic variation among lines for disease severity was significant (p<0.0001), but skewed toward resistant. Linkage disequilibrium extended beyond 4 cM on average, and indicated that whole genome mapping was feasible with the available marker density. Multiple QTL with generally small effects ($R^2 < 5\%$) were identified. Overall eight QTL for DON and four QTL for FHB were reproducibly identified. These loci should be useful targets for immediate implementation of marker assisted selection to improve disease resistance.

USING OPTICAL SORTING TECHNIQUES TO SELECT FOR LOWER SCAB DISEASE IN SEGREGATING POPULATIONS. Neway Mengistu¹, P. Stephen Baenziger^{1*}, Stephen Wegulo², Janelle Counsell² and Floyd Dowell³

¹Dept. of Agronomy and Horticulture; ²Dept. of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0915; and ³USDA ARS Engineering Research Unit, Grain Marketing and Production Research Center, Manhattan, KS 66502 *Corresponding Author: PH: (402) 472-1538; E-mail: pbaenziger1@unl.edu

ABSTRACT

Natural epidemics of the Fusarium head blight (FHB), caused by Fusarium graminearum, may result in severe yield losses, reduction in end-use quality, and contamination of the harvested grain by mycotoxins. FHB is an episodic disease in the hard winter wheat region of the Great Plains that is known for its diverse and highly variable climate. In order to diversify our FHB germplasm we created two populations from a soft winter wheat (MO980829) breeding line with a known native FHB tolerance and two hard winter wheat genotypes (Jagalene and NE00564). The objective of this experiment was to estimate FHB damage in the populations and to enrich the segregating populations for FHB tolerance using a kernel optical sorting technique. Our hypothesis was that in a segregating population grown under disease conditions, kernels from the susceptible genotypes would have a higher level of disease than kernels from tolerant genotypes. Of course, kernels with low disease levels might be due to tolerance or escapes and similarly kernels with high disease levels might be due to susceptibility or an overwhelmed tolerance. Because a soft wheat parent was used in these two populations, we first sorted the population for hardness (two classes: hard and soft) and protein content (four classes; low, low to medium, medium to high, and high). We retained for our experiments, the hard segregants. In 2007, the hard kernels of the two populations, each with four levels of protein, were planted in an inoculated scab field with a misting system at Mead, NE. The harvested kernels were then sorted into FHB free (no scab) and FHB infected (scabby) grain. In 2008, the two sorted kernel samples (no scab vs scabby) plus an unsorted kernel sample (control) from each of the four protein classes were grown in a replicated misted scab nursery at Lincoln, NE. The visual scores of incidence (p=0.0017), severity (p=0.0001) and FHB index (p=<0.0001) were significantly different among the 26 lines that consisted of 24 lines from the two populations and two checks (Overland and Wesley). Among the scab-free sorted kernels, scabby kernels, and unsorted kernels there was a slight significant difference for incidence (p=0.045), highly significant difference for severity (p=0.0034), and highly significant difference for FHB index (p=0.0004). The mean of incidence for the no scab and scabby kernels were 41% and 51%, respectively (standard error was 3.1%). There was no incidence difference between the scabby and unsorted kernels. The mean of severity for the no scab, scabby and unsorted kernels were 16%, 32%, and 23%, respectively (standard error was 2.8%). The FHB indexes for the no scab, scabby and unsorted kernels were 7, 15, and 12, respectively (standard error was 1.3). There was no significant difference among the sorted and unsorted kernels for DON test which may reflect the very high CV associated with this trait. Also there were no differences for incidence, severity, FHB index, and DON between the two populations and among the four protein levels within the populations. Based on this study, kernel sorting may be an effective method for in reducing the visual symptoms of FHB, but not for significantly reducing the DON content.

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DEVELOPMENT AND EVALUATION OF THE FIRST *FUSARIUM* INTERNATIONAL ELITE SPRING WHEAT NURSERY (FIESWN) AND THE FIRST *FUSARIUM* INTERNATIONAL PRELIMINARY SPRING WHEAT NURSERY (FIPSWN): PRELIMINARY RESULTS FROM MEXICO AND EUROPE. M. Mezzalama¹, H. Buerstmayr², S. Dreisigacker¹ and E. Duveiller^{1*}

¹CIMMYT, km 45 Carretera Mexico-Veracruz, El Batan, Texcoco, CP56130, Mexico; and ²University of Natural Resources and Applied Life Sciences Vienna, Department IFA-Tulln, Konrad Lorenz Str. 20, A-3430 Tulln, Austria *Corresponding Author: PH: 52-55-5804-2004; E-mail: E.duveiller@cgiar.org

ABSTRACT

Two international spring wheat nurseries, 1st *Fusarium International Elite Spring Wheat Nursery* (FIESWN) and *1st Fusarium International Preliminary Spring Wheat Nursery* (FIPSWN) were prepared and distributed to 13 collaborators in 2008. Of the 188 entries initially received, 28 were included in the FIPSWN and 50 in the FIESWN based on the field performance and DON content evaluation in Mexico during 2006-2007.

In Mexico, both nurseries were inoculated with a mixture of 5 *F. graminearum* isolates and evaluated from May to Sept. 2008. Sumai 3 (resistant) and Wheaton and Flycatcher (susceptible checks) were used as controls. In the FIPSWN, FHB index ranged from 1.4% (Sumai 3) to 49.3% (line 2.49, resistant for crown root rot in the West Asia). Ten lines over 28 scored below 10% and 2 lines from CIMMYT's Fusarium program, EMB16/CBRD//CBRD and SABUF/3/BCN//CETA/AE.SQUARROSA (895)*2/4/BCN, scored around 2.5% FHB index. In Austria, 7 lines over 28 scored below 10% FHB index. Sumai 3 resistance was confirmed and comparable in both locations, confirming the suitability of screening for FHB resistance at CIMMYT El Batan station. EMB16/CBRD//CBRD ranked among the 10 best entries in both locations. Several entries, such as INMIR/NING 8331//INIA BOYERO (Uruguay) and SABUF/3/BCN//CETA/AE.SQUARROSA (895)*2/4/BCN (Mexico) ranked differently in the 2 locations. In the FIESWN 19 out 50 limes scored below 10% FHB index. Line MN00274-2-6 (USA) top ranked at FHB index below 1%; 5 CIMMYT lines (2 of them selected in the Caspian Sea region), 9 USA (MSU and MN) and 5 Canada submitted lines were in this group. In Austria, there was a clear cut separation between resistant (<15% FHB index) and susceptible lines (from 15 to 75% FHB index); 89% of the best 19 lines in Mexico were in the <15% FHB index group in Austria.

These first results show that resistant lines selected by collaborators in North America, South America and the Caspian Sea region were confirmed in Mexico and Austria. As scores of some lines did not correlate in the different regions further research on the pathogen, environmental conditions at screening sites and the level of DON production in the field are needed. Further information will be added from haplotyping research in USDA-ARS Fargo to confirm whether the source of the resistant lines found in these trials differ from Sumai 3.

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THE 2007-08 SOUTHERN UNIFORM WINTER WHEAT SCAB NURSERY. J.P. Murphy^{*} and R.A. Navarro

Department of Crop Science, Box 7629, North Carolina State University, Raleigh, NC 27695 *Corresponding Author: PH: (919) 513-0000; E-mail: paul_murphy@ncsu.edu

ABSTRACT

Most components of Fusarium Head Blight (FHB) resistance are greatly influenced by genotype by environment interaction which limits the heritability of resistance estimated by a single program in any given year. The Southern Uniform Winter Wheat Scab Nursery provides breeders in the public and private sectors with an opportunity for multi-environment, independent evaluations of FHB resistance in advanced generation breeding lines compared with the current most resistant check varieties Ernie and Bess. In addition, the nursery facilitates the sharing of the best resistant materials throughout the breeding community.

The 2007-08 nursery comprised 49 advanced generation breeding lines and three check cultivars, 'Ernie' and 'Bess' (partially resistant) and 'Coker 9835' (susceptible). Six U.S. public programs (Univ. of Arkansas, Univ. of Georgia, Louisiana State University, Univ. of Maryland, N.C. State Univ. and VA Tech.), and one private company, Agripro-Coker, submitted entries. The nursery was distributed to 11 U.S., one Hungarian, and one Romanian cooperator for field and / or greenhouse evaluations. In addition three USDA-ARS laboratories conducted evaluations for Hessian Fly resistance, milling and baking quality and haplotypes based on established SSR markers.

Copies of the full report will be available at the 2008 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

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SEVEN YEARS OF PROGRESS IN THE NORTH AMERICAN BARLEY SCAB EVALUATION NURSERY (NABSEN). S.M. Neate^{1*}, P.L. Gross¹, R.D. Horsley², K.P. Smith³, D.B. Cooper⁴, L.G. Skoglund⁴ and B. Zhang⁵

¹Dept. of Plant Pathology, North Dakota State University, Fargo ND; ²Dept. of Plant Sciences, North Dakota State University, Fargo ND; ³Dept. of Agronomy and Plant Genetics, University of Minnesota, St Paul MN; ⁴Busch Ag Resources Inc., Fort Collins CO; and ⁵Dept. of Plant Protection, Zhejiang University, Hangzhou, China ^{*}Corresponding Author: PH: (701) 231-7078; E-mail: stephen.neate@ndsu.edu

ABSTRACT

The North American Barley Scab Evaluation Nursery (NABSEN) is a regional uniform nursery established to screen in North America and China, elite two-rowed and six-rowed barley germplasm for resistance to Fusarium head blight (FHB). Participants include breeding programs from North Dakota State University (NDSU), University of Minnesota (UM), Busch Ag Resources (BARI), Agriculture Canada and CIMMYT/ICARDA. Each year approximately 50 entries plus resistant, moderately resistant and susceptible checks are grown in either inoculated-misted or dryland sites. Each year one site has been sown in each of Mexico, and Canada, as well as six sites in the upper Midwest of the United States. In the last three years a site has also been established in China. NABSEN participants use different criteria to select elite material for testing, the CIMMYT/ICARDA program is centered on pre-breeding of two-rowed barley and the Agriculture Canada entries includes mostly two-rowed material, either feed or malting barley from several Canadian breeding programs. This abstract reports on the resistance to FHB of entries submitted from the NDSU, UM and BARI six-rowed malting barley programs that were tested in irrigated inoculated experiments between 2002 and 2008. Environment and disease pressure affected the magnitude of DON over the years with 2005 having the lowest DON of 11.2 ppm on the susceptible check Stander, and 2008 having the highest DON of 33.2 ppm also on the susceptible check Stander. Similarly, environment and disease pressure affected disease severity, with the highest severity on Stander in 2008 (27% infected kernels). In contrast to the DON data, the lowest severities on Stander were recorded in 2003 and 2006 (both 11% infected kernels). These data support the hypothesis that DON and FHB severity are not always well correlated in barley, which indicates that active selection needs to occur concurrently for both characters. To reduce the effect of different FHB severities between years, FHB severity was expressed as a percentage of the moderately resistant cultivars Robust/MNBrite The average of all entries from the NDSU program over all years was 89% of Robust/MNBrite, from the UM program was 83% of Robust/MNBrite, and from the BARI program was 110% of Robust/MNBrite. There was variation within the entries from each program, and when only the best entry from each program was included, then the percentages were 63%, 62% and 82% of Robust/MNBrite for the NDSU, UM, and BARI programs, respectively. DON was also expressed as a percentage of the moderately resistant cultivars Robust/MNBrite. The average of all entries from the NDSU program over all years was 85% of Robust/MNBrite, from the UM program was 91% of Robust/ MNBrite and from the BARI program was 115% of Robust/MNBrite. As with severity, there was variation within the entries from each program, and when only the best entry from each program was considered, then the percentages were 58%, 68% and 94% of Robust/MNBrite for the NDSU, UM, and BARI programs, respectively. Overall the average reductions in disease severity and DON have been slight since 2002. Importantly though, good levels of resistance have been maintained while the programs have selected for the yield, maturity and quality characteristics required in a commercial cultivar. Within the average however, are individual lines that are significantly improved in both resistance to infection by FHB and DON accumulation compared to early material, and entries with good resistance are now in the American Malting Barley Association Pilot Scale Evaluation Program in preparation for commercial release.

NIR OPTICAL CHARACTERISTICS OF DEOXYNIVALENOL. K.H.S. Peiris¹ and F.E. Dowell^{2*}

¹Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, Kansas 66506; ²Engineering Research Unit, USDA-ARS-Grain Marketing and Production Research Center, 1515 College Avenue, Manhattan, Kansas 66502 *Corresponding Author: PH: (785) 776-2753; E-mail: Floyd.Dowell@ARS.USDA.GOV

ABSTRACT

We have developed rapid near infra red (NIR) techniques for nondestructive automatic sorting of Fusarium damaged wheat kernels and for estimation of deoxynivalenol (DON) levels in single wheat kernels. We studied NIR optical characteristics of DON to identify NIR absorption bands and to assess the applicability of NIR technique for direct measurement of DON in order to improve the calibrations. NIR transmission spectra of DON (0.5 - 2000 ppm) dissolved in acetonitrile and that of water (0 - 640 ppm) in acetonitrile were studied to identify NIR absorption bands of DON and water and to see how strong NIR absorption bands of water interact with DON NIR absorption bands.

Deoxynevalenol crystals were dissolved in acetonitrile to prepare a 2000 ppm stock solution. It was thereafter serially diluted to prepare a series of DON solutions up to 0.5 ppm. The solutions in IR quartz (10 mm path length) cuvettes were scanned using an ASD spectrometer. Solutions were scanned three times to collect three different spectra per each DON concentration. Likewise, water was added to acetonitrile and spectra were recorded. The collected DON spectra were used to develop a calibration to predict DON levels in acetonitrile solution. Two spectra from each concentration were used for developing the calibration by PLS regression method and the other spectra used to validate the calibration. The optical density spectra of DON and water in various concentrations were used to study DON and water absorption peaks. Difference spectra and second derivative spectra of DON and water were used to identify and resolve absorption peaks.

In the 950 - 2200 nm range two DON absorption bands were identified at 1390 -1440 nm and 1880-1950 nm having peaks at 1410 and 1905 nm respectively. The absorbance at 1905 nm is approximately one magnitude stronger than the absorbance at 1410 nm. Water absorption bands were found around 970 and 1420 nm in increasing intensity. The water absorption bands above 1850nm were much stronger being unable to measure even at 40 ppm using 10 mm path length.

The calibration developed for DON in acetonitrile ($R^2=0.995$ SECV=38.8 with 6 PLS factors) predicted DON levels in acetonitrile with a $R^2=0.998$. This shows that NIR absorbance can be used to accurately estimate DON levels in acitonitrile. However, when it comes to predicting DON in cereal grains such an accuracy is difficult to achieve due to interference with stronger water absorption bands that overlap DON absorption bands. Our present SKNIR technique for scab sorting and DON estimation use 950-1650 nm waveband. Based on the observations of this study it may be possible to further improve calibrations by extending NIR scanning range above 1950 nm to include the stronger DON absorption band at 1905 nm.

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PROGRESS ON DEVELOPMENT AND APPLICATION OF SINGLE KERNEL NIR SORTING TECHNOLOGY FOR ASSESSMENT OF FHB RESISTANCE IN WHEAT GERMPLASM. K.H.S. Peiris¹, M.O. Pumphrey², Y. Dong³, S. Wegulo⁴, W. Berzonsky⁵, P.S. Baenziger⁶ and F.E. Dowell^{7*}

¹Department of Biological and Agricultural Engineering, Kansas State University; ²USDA-ARS, GMPRC Plant Science and Entomology Research Unit; ³Department of Plant Pathology, University of Minnesota; ⁴Dept. of Plant Pathology, University of Nebraska- Lincoln;
⁵Winter Wheat Breeding, South Dakota State University; ⁶Dept. of Agronomy and Horticulture, University of Nebraska- Lincoln; and ⁷USDA-ARS, GMPRC Engineering Research Unit
*Corresponding Author: PH: (785) 776-2753; E-mail: Floyd.Dowell@ARS.USDA.GOV

ABSTRACT

Plant breeders working on developing *Fusarium* resistant wheat varieties need to evaluate kernels coming from a multitude of crosses for Fusarium Damaged Kernels (FDKs). We are developing Near Infrared (NIR) spectroscopic methods to sort FDKs from sound kernels and to determine DON levels of FDKs nondestructively to facilitate rapid varietal screening for *Fusarium* resistance by assessing proportions of sound and FDKs and estimating their DON levels. We report the progress and research highlights of the development and use of our single kernel NIR (SKNIR) scab sorting and deoxynivalenol (DON) estimation techniques since January, 2008.

We have improved the SKNIR scab sorting technique and its feasibility as an objective, rapid and nondestructive method for assessment of FDKs of wheat germplasm demonstrated. Depending on the kernel DON level, FDKs can be sorted into 2-3 fractions. This makes it possible to get an understanding of what fraction and how much each fraction contributes to the final DON level of a composite sample. Moreover, our studies with sorting of North Dakota State University (NDSU) germplasm showed that proportions of SKNIR sorted FDKs in wheat lines affected by FHB correlated fairly well with field FHB assessment indices. Therefore, this technique can be used by wheat breeders as a nondestructive, rapid and an objective method for comprehensive analysis of FDKs when wheat germplasm are screened for *Fusarium* resistance. Since April 2008 we have sorted 108 samples for NDSU and 405 samples for University of Nebraska, Lincoln (UNL) wheat breeders. Another set of samples from the above two institutions will be sorted in November-December, 2008.

A calibration was developed for estimation of DON concentration in single wheat kernels by SKNIR system. This can estimate DON levels in single kernels having more than 60 ppm DON. Experiments will be carried out in collaboration of UNL researchers to further test and refine this calibration to estimate DON levels of FHB affected wheat samples. NIR spectra of pure DON were also studied and DON absorption peaks identified. Results of these experiments are presented in a separate poster. A SKNIR wheat moisture calibration was also developed. It will be integrated to determine moisture content of kernels concurrently when DON levels are estimated so that it is possible to compare DON levels of kernels having different moisture contents or to express DON content of kernels with specific moisture content.

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THE EFFECT OF KEY CHROMOSOME SEGMENTS ON FHB RESISTANCE IN A CROSS OF SOFT-WINTER BY HARD-SPRING PARENTS. A. Phillips¹, C. Sneller^{1*}, J. Lewis³, P. Paul² and M. Guttieri¹

¹Dept. of Horticulture and Crop Science, ²Dept. Plant Pathology, The Ohio State University, Wooster, Ohio 44691; and ³Dept. Crop and Soil Science, Michigan State University, E. Lansing MI, 48824-1325 ^{*}Corresponding Author: PH: (330) 263-3944; E-mail: sneller.5@osu.edu

ABSTRACT

Resistance to FHB is multifaceted and quantitative. New sources of resistance need to be evaluated for their ability to compliment and enhance our current sources of resistance. One potential source of soft winter wheat is a line from CIMMYT termed CASS94 in this study that is derived from a cross of Mayoor by a synthetic hexapoid. CASS94 has better FHB resistance than Mayoor suggesting it may have some FHB resistance alleles from T. Tauschii. Previous greenhouse evaluations for Type II resistance suggested that a sib of CASS94 had a major QTL on chromosome 2DL. Our objective was to assess the effect of key chromosome regions from CASS94 on FHB resistance in the field. We crossed CASS94 to OH685, an adapted soft red winter wheat that has been susceptible to FHB and developed 167 F4-derived RILs. Data on FHB Index (IND) were collected in Ohio in 2007 and in Michigan and Ohio in 2008. Incidence (INC) and severity (SEV) data were collected in both locations in 2008. Heritability was moderate to low (0.49 for IND, 0.44 for severity, and 0.36 for INC). The allele frequencies for many markers were quite skewed in favor of the OH685 allele. We could not phenotype CASS94 in the field due to its spring habit. About 26% of the RILs had lower IND than OH685 which displayed moderate resistance in this test. Nearly 27% had a lower IND than Freedom and 2.4% had a lower IND than Truman. Markers from 3BS and 5AS were not significant indicating that known QTL from these regions were not segregating in this population. We used 13 markers from 2D and formed two linkage groups. A QTL was detected on a group corresponding to 2DL. This QTL accounted for about 10-30% of the genetic variation for IND. Genotyping work is continuing and final results for these regions will be presented at the Forum.

SHORTENING OF THE *LEYMUS RACEMOSUS* SEGMENT IN THE *FHB3* TRANSFER USING *PH1B*-INDUCED HOMOEOLOGOUS RECOMBINATION. L.L. Qi^{1,4}, B. Friebe^{1*}, M.O. Pumphrey², C. Qian³, P.D. Chen³ and B.S. Gill¹

¹Wheat Genetic and Genomic Resources Center, Department of Plant Pathology, Kansas State University, Manhattan KS; ²ARS-USDA, Plant Science and Entomology Research Unit, Manhattan KS; ³The National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing Jiangsu; and ⁴Current Address: ARS-USDA, Sunflower Unit, Fargo ND *Corresponding Author: PH: (785) 532-2364; E-mail: friebe@ksu.edu

ABSTRACT

Fhb3, a new gene conferring resistance to Fusarium head blight disease of wheat, was transferred from Leymus recemosus to wheat in the form of a compensating wheat-Leymus translocation T7AL·7Lr #1S. However, whole arm translocations are often associated with deleterious linkage drag, which usually results in yield reduction and inferior quality. Our previous research indicated that Fhb3 is located in the distal region of the short arm of the Leynus chromosome 7Lr#1. This is encouraging because genetic recombination is known to be high in the distal regions of chromosomes. Therefore, further chromosome engineering aimed at reducing the size of the L. racemosus segment while still retaining the Fhb3 resistance gene appears to be feasible. The translocation stock T7AL·7Lr #1S was crossed twice with the CS ph1b mutant. 154 BC, plants were screened from the cross T7AL·7Lr#1S / ph1b using molecular markers to assay for ph1b and T7AL·7Lr#1S. Sixtyone plants were homozygous ph1b/ph1b and heterozygous for the translocation chromosome T7AL·7Lr#1S/ 7A. These plants were either backcrossed with Overley or selfed. We have developed a large recombinant population of 1,400 BC₂ seeds and more than 8,000 BC₁F₂ seeds. In homozygous *ph1b* genotypes, the alien 7Lr#1S arm is expected to pair and recombine with the homoeologous 7AS arm of wheat. Meiotic pairing analysis in plants homozygous for ph1b and heterozygous for T7AL·7Lr#1S and 7A failed to detect any metaphase I association of T7AL·7Lr#1S with 7A in more than 500 PMCs analyzed, suggesting that the recovery of recombinants is very difficult. A total of 1118 BC, plants were screened by molecular markers and three putative recombinants were identified. These recombinants were further confirmed by genomic in situ hybridization using total genomic L. racemosus DNA as a probe. Rec124 is a proximal recombinant with about the proximal 80% derived from L. racemosus and the distal 20% of the arm derived from 7AS of wheat (T7AL·7Lr#1S-7AS), whereas rec679 and rec989 are distal recombinants with about the proximal 80% derived from 7AS and the distal 20% of the arm derived from 7Lr#1S T7AL 1S (T7AL·7AS-7Lr#1S). Once homozygous recombinant stocks have been obtained they will be evaluated for their resistance to scab and the resistant stock will be crossed with adapted winter wheat cultivars.
MAPPING OF FHB RESISTANCE IN THE JAPANESE WHEAT LANDRACE, PI 81791. E.A. Quirin¹ and J.A. Anderson^{1*}

¹Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN *Corresponding Author: PH: (612) 625-9763; E-mail: ander319@umn.edu

ABSTRACT

The Japanese wheat landrace, PI 81791 (Sapporo Haru Komungi Jugo), has been shown to have consistent resistance to FHB in field and greenhouse studies, and marker analysis has shown that this accession does not contain Fhb1. Because of this, a population of 150 recombinant inbred lines was developed from a cross between this genotype and the susceptible spring wheat variety, Wheaton. Phenotypic data for resistance to initial infection (type 1) and resistance to the spread of infection (type 2) were collected in field experiments for three environments. Type 2 data was also collected from greenhouse experiments. More than 200 SSR markers have been mapped in this population to date. From these data, four markers were found to be significantly (P<0.01) associated with type 2 resistance in field experiments with one marker being significant in all three environments and three being significant in two of the three environments. The most consistent marker, BARC98, has previously been mapped to chromosome 4D, although it's exact position in the map of this cross has yet to be determined. The other markers have been previously located on chromosomes 1D, 3BL/3DL, and 5D. Six markers were found to be significant (P < 0.01) for type 2 resistance in greenhouse experiments, with all six markers being significant for both greenhouse experiments. These markers have been previously identified on chromosomes 1B, 1D, 2A, 2B, and 3A, although their exact locations have not been determined for this cross. Only one marker was found to be significant in multiple locations for type 1 field resistance. Eight RI lines showed high levels of type 1 and type 2 resistance in both field and greenhouse experiments and can serve as breeding parents.

COMBINING RESISTANCE TO YELLOW DWARF DISEASE (*BDV3*) FROM INTERMEDIATE WHEATGRASS, AND RESISTANCE TO FUSARIUM HEAD BLIGHT (*QFHS.PUR-7E*) FROM TALL WHEATGRASS, IN COMMON WHEAT. Kristen Rinehart^{1*}, Xiaorong Shen¹, Lingrang Kong¹, Joseph M. Anderson^{1,2} and Herb Ohm¹

¹Department of Agronomy, Purdue University, West Lafayette, IN; and ²USDA-ARS Crop Production and Pest Control Research Unit, Purdue University, West Lafayette, IN ^{*}Corresponding Author: PH: 567-230-1098; E-mail: kdrineha@purdue.edu

ABSTRACT

The objective of this research is to combine Bdv3 that conditions resistance to yellow dwarf disease (YD) and Qfhs.pur-7EL that conditions resistance to Fusarium head blight (FHB). Bdv3 was introgressed into wheat (*Triticum aestivum* L.) chromosome 7D from intermediate wheatgrass (*Thinopyrum intermedium*) and is located subterminal on the 7E segment that was translocated into 7DS.7DL-7EL. Qfhs.pur-7EL was introgressed from tall wheatgrass (*Thinopyrum ponticum*) and was mapped to the distal region of chromosome 7DS.7DL-7EL in wheat. Bdv3 is located more proximal to the centromere of chromosome 7DS.7DL-7EL than Qfhs.pur-7EL. The wheat line, 216-67, was developed in which the introgressed 7E segment replaced the distal approximately 1/3 of 7DL. The wheat line, 275-4, was developed in which the introgressed 7E segment ontaining Qfhs.pur-7EL replaced the distal approximately 1/3 of 7DL. The wheat line, 275-4, was developed in which the introgressed 7E segment of F₂ plants was genotyped with SSR markers gwm37, associated with Bdv3, and with Qfhs.pur-7EL flanking markers BF145935 and cfa2240 to identify plants that potentially have Bdv3 and Qfhs.pur-7EL. Progenies of selected F₂ plants will be genotyped for presence of Bdv3 and Qfhs.pur-7EL, and their resistance to YD and FHB will be verified by testing putative recombinant plants to YD and FHB.

POWER OF FAMILY-BASED QTL MAPPING: OPTIMIZING FAMILY TYPE, SIZE AND MARKER DENSITY FOR QTLS OF DIFFERENT MAGNITUDES. U. Rosyara, J.L. Gonzalez-Hernandez^{*}, K.D. Glover, K. Gedye and J. Stein

Dept. of Plant Sciences, SNP247, South Dakota State University, Brookings, SD 57007. USA *Corresponding Author: PH: (605) 688-6907; E-mail: jose.gonzalez@sdstate.edu

ABSTRACT

Family-based QTL mapping has been shown to be an expeditious method to map and validate molecular markers using core plant breeding populations. Utilizing the linkage based variance component analysis method, in a previous study; the QTL Fhb1 was accurately mapped to the same chromosomal location identified by conventional QTL mapping methods. However, questions need to be addressed regarding the potential limitations of the Family-based QTL mapping approach. This research details a simulation study, utilizing previous data, which investigates population size (number and size of family) and marker density in relation to the variance explained by QTLs (major or minor) when mapped with the Family-based method. The simulated population consisted of a "typical breeding program" with families derived from three-way and four-way crosses, the computer software package MERLIN was used to perform variance component based linkage analysis on the simulated population. A total of 1,000 simulations were performed using POWQ, a software module based on the variance component engine of MERLIN. The average power to detect a QTL ranged from less than 1 to 100% depending upon family size, family type (three-way or four-way crosses), marker density, variance explained by the QTL and the level of significance. Overall a larger population size led to a higher power of detection. Increasing family size (number of individuals within a family) had higher returns to power gain than increasing the number of families. Thus using a small number of large families, rather than a large number of small families was beneficial in terms of power gain. There was greater power to detect major QTLs than minor QTLs. Also the power was higher for families derived from four-way rather than three-way crosses. As a general rule, a sample size of 500 (20 families with 25 individuals in each family) will provide higher power to detect minor QTLs. Power can also be increased by increasing marker density. As the parameters discussed here effect detection power, the researcher can calculate QTL detection power for a given set of conditions, based on the plant breeding program and the number of molecular markers available. The POWQ module can be used to calculate power in such situation, allowing the researcher to determine if their resources should go into increasing population size or marker density. The results of this study suggest that the family-based QTL mapping method is useful for mapping both major and minor QTLs.

SELECTIVE GENOTYPING IN FAMILY-BASED MAPPING OF FHB RESISTANCE QTLS IN HEXAPLOID WHEAT. U. Rosyara, J.L. Gonzalez-Hernandez^{*}, J. Stein, K. Gedye and K.D. Glover

Dept. of Plant Sciences, SNP247. South Dakota State University, Brookings, SD 57007, USA *Corresponding Author: PH: (605) 688-6907. Email: jose.gonzalez@sdstate.edu

ABSTRACT

Using main-stream plant breeding populations for mapping of QTLs has many advantages including quick mapping, reducing resource requirement and simultaneous validation of QTLs in multiple genetic backgrounds. Family based QTL mapping methods were validated to map *Fhb1* in a previous study. A reduced population size for genotyping will reduce resource requirement. Selective genotyping of individuals displaying "extreme" phenotypes has been considered important for saving costs and time without reducing the power of detection. This study investigates selective genotyping in family based QTL mapping methods, specifically; linkage analysis (variance component and pedigree-wide regression) and association analysis (quantitative transmission disequilibrium test, QTDT). The target QTL of this study was the well characterized *Fhb1* for Fusarium Head Blight (FHB) resistance in wheat (*Triticum aestivum* L.). Individuals with disease value scores falling in the top and bottom 20 percent were selected for genotyping and QTL mapping from a base population of 82 families and 793 individuals. The QTL was mapped to the expected chromosomal location with LOD values comparable to using all the individuals of the population. These results indicate that the selective genotyping approach can be applied to family-based QTL mapping to reduce cost and time requirements for genotyping.

ASSESSING PROGRESS TOWARD BREEDING BARLEY VARIETIES WITH ENHANCED RESISTANCE TO FUSARIUM HEAD BLIGHT. K.P. Smith^{*} and Edward Schiefelbein

Dept. of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108 *Corresponding Author: PH: (612) 624-1211; E-mail: smith376@umn.edu

OBJECTIVES

Use several performance measures outlined in the USWBSI Action Plan to assess progress toward breeding barley varieties with enhanced FHB resistance.

INTRODUCTION

The USWBSI Action Plan states that the primary goal of variety development is to "Increase acreage planted with varieties exhibiting improved FHB resistance". In the case of barley, the first potential releases of new varieties with enhanced FHB resistance could occur in January of 2010 (Smith et al., 2007). Since release and establishment of new varieties is a continuing and long term goal, it is essential that breeding programs assess progress at intermediate points along the way to that ultimate goal. This will provide critical feedback to breeders to assess whether their current practices are likely to meet goals. In goal #2 of the USWBSI Action Plan for varietal development "Increase efficiency of individual breeding programs to develop FHB resistant varieties.", several performance measures were established to assess progress. In this report, we present data from the University of Minnesota using three of those performance measures.

Performance Measures (from Action Plan VDHR Goal #2)

- Total number as well as percentage of crosses made involving FHB resistant parent (native or exotic resistance).

- Average performance of breeding lines (advanced, preliminary yield trial entries etc...) compared to appropriate check varieties for FHB and agronomics.
- Number of variety candidates entered into Uniform or Regional Yield Nurseries or industry quality evaluations with enhanced FHB resistance.

MATERIALS AND METHODS

Assessment of breeding lines entered into first year yield testing was done by assembling data from individual trials conducted from 2001 to 2008. In our program, these lines (PYT entries) are generally evaluated in 2-3 locations for yield and 2-3 misted and inoculated nurseries for FHB severity. Yield trials and FHB trials are arranged in a randomized complete block design with two and three replicates, respectively. Lines included in the analysis were from FHB crosses (ie. involving parents with an exotic source of resistance in the pedigree). The value of each line was normalized for each trial by dividing it by the performance of the mean of three common checks for FHB severity and four common checks for yield. The checks were usually entered twice in a trial so the mean of the checks for any one trial was based on at least 12 plots and as many as 27 plots. The normalized trait values were averaged for each year for FHB severity and yield, plotted over time, and a trend line fit using MS Excel.

RESULTS AND DISCUSSION

Population Development. We make most of our crosses in the fall greenhouse in each year. Crosses involving an exotic source of FHB resistance, or that

include at least one parent with an exotic source of resistance in its pedigree, are referred to as FHB crosses. Since all exotic sources are unadapted they require multiple cycles of breeding to recover enhanced resistance in an elite background. In 1998 about 1/3 of the FHB crosses were 1st cycle, 1/3 were 2nd cycle, and 1/3 were 3rd cycle. In 2007, most crosses were 4th cycle or higher. In the UM program, we have both increased the total number of crosses made each year and also increased the proportion that are FHB crosses (Table 1).

Evaluation of Preliminary Yield Trial (PYT)

Entries. Two years after a cross is made, F4 generation populations are evaluated in inoculated and mist-irrigated FHB nurseries. Each line is grown in two locations with two replications per location. Selection is based on visual assessment of FHB severity and lines with better resistance are harvested and the grain assayed for DON. The most resistant lines are advanced to preliminary yield trials and 2nd year FHB testing in multiple locations. To assess the effectiveness of our first year selection for FHB, we assessed the performance of PYT entries for FHB severity and yield in replicated trials in multiple locations. The data is normalized to a set of common checks and shows significant decrease in FHB severity over the eight year period (Figure 1). During that same period the average yield performance was stable.

Entry of Variety Candidates into Industry Quality Evaluations. For a barley variety to occupy significant acres in the Midwest, it must be approved by the American Malting Barley Association (AMBA). Our program is allowed up to four entries in the AMBA pilot-scale malting evaluation each year. Our first two variety candidates with enhanced FHB resistance to enter the AMBA pilot program were M122 and M123 in 2005 (Table 2). We have entered at least two new entries in each of the following years. Three of the six entries that have completed pilot testing were rated satisfactory. M122 is now in plant scale testing and if

AMBA plant-scale brewing evaluation is satisfactory it will be released as a new variety in January of 2010.

Performance measures presented here indicate that the University of Minnesota breeding program is making progress toward the release of new varieties with enhanced FHB resistance and lower DON. Similar progress is being made by the other Midwest barley breeding programs. Each of these programs uses different sources of resistance and slightly different selection and breeding strategies. Crossing of elite materials with enhanced FHB resistance among the Midwest programs is in progress and should further improve the level of resistance of new varieties.

ACKNOWLEDGEMENTS

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

REFERENCES

Smith, K. P., E. Schiefelbein and G. Velasquez. 2007. Development of Barley Variety Candidate M122 with Enhanced Resistance to FHB. Proceedings of the 2007 National Fusarium Head Blight Forum, The Westin Crown Center, Kansas City, Missouri, Dec. 2-4, 2007, p. 232.

	N	umber of Cros	ses
Year	Total	FHB ²	Percent FHB
1998	49	29	59%
1999	71	37	52%
2000	85	61	72%
2001	76	36	47%
2002	136	71	52%
2003	60	43	72%
2004	61	46	75%
2005	120	93	78%
2006	98	83	85%
2007	134	120	90%

Table 1. Description of crosses made for FHB resistance breeding from 1998 to 2007.

¹number of crosses made in fall and advanced as F1's in the winter greenhouse.

 2 number of crosses involving a parent with an exotic source of FHB in the pedigree.



Figure 1. Mean performance of first year yield trial entries for yield and FHB severity from 2001 to 2008. Trend lines are linear fits using MS Excel 2007. Yield checks are Robust, MNBrite, Stander, and Lacey. FHB checks are Robust, MNBrite, and Stander. Each value is the mean of at least 46 and as many as 90 breeding lines. Lines are from FHB crosses.

Year	Line	FHB Severity	DON Conc.	Evaluation
Entered		(% of Robust)	(% of Robust)	Outcome
2005	M122	47	54	Plant-Scale
	M123	76	81	Unsatisfactory
2006	M128	70	78	Eligible for Plant Scale
	M129	56	83	Eligible for Plant Scale
2007	M130	63	76	Unsatisfactory
	M132	62	78	Unsatisfactory
2008	M134	56	85	In progress
	M135	50	75	In progress
	M136	53	93	In progress
	M137	58	78	In progress

Table 2. Description of UM barley variety candidates entered into AMBA Pilot Testing program.

REPORT ON THE 2007-08 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERIES (NUWWSN AND PNUWWSN). C. Sneller^{1*}, P. Paul², L. Herald¹, B. Sugerman¹ and A. Johnston²

Dept. of Horticulture and Crop Scienc, and ²Dept. Plant Pathology, The Ohio State University, Wooster, Ohio 44691 *Corresponding Author: PH: (330)263-3944; E-mail: sneller.5@osu.edu

OBJECTIVES

This is a summary of the report on the 2007-2008 Northern Uniform Winter Wheat Scab Nursery (NUWWSN) and the Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN). A full report will be available on the USWBSI web site prior to the 2008 forum. The objective of these tests is to screen winter wheat genotypes adapted to the northern portion of the eastern US for scab resistance.

MATERIAL AND METHODS

The traits assessed and locations that reported data are listed in Table 1. Entries for the NUWWSN came from 13 programs while the PNUWWSN entries came from 10 programs (Table 2).

RESULTS

This report presents seven traits using FDK in place of KR and PSS. NUWWSN entries with means that were not significantly different than the lowest mean for five or more FHB traits are shown in Table 3 (eg. entries with at least 5 "I"s). PNUWWSN entries with means that were not significantly different than the lowest mean for four or more FHB traits are shown in Table 4 (eg. entries with at least 4 "I"s). Only three entries had DON < 2 ppm (IL01-34159, KY02C-3005-25, and VA06W-553 from PNUWWSN) and seven had IND < 15% (IL01-34159, KY02C-3005-25, and P.0175A1-37-4 from PNUWWSN; Truman, DH22/24. E6003, and IL02-18828 from NUWWSN). The results for all traits and all entries for the two tests are in Tables 5 and 6.

Code	Trait	Description	PNUWWSN Locations*	NUWWSN Locations*
SEV	Disease severity from field tests	% of infected spikelets in an infected head.	IL,IN,KY,MI,MO,ON,VA	IL,IN,KY,MD,MI,MO,NE,NY,OH, ON,VA
INC	Disease incidence	% of heads with at least one infected spikelets	IL,KY,MI,MO,ON,VA	ILKY,MD,MI,MO,NE,NY,OH, ON,VA
IND	Disease index	IND = (SEVxINC)/100	IL,KY,MI,MO,OH,ON, VA, RO	IL,KS,KY,MD,MI,MO,NE,NY,OH, ON,RO,VA
KR	Kernel rating	A visual assessment of the percent infected kernels	IL	IL,KS
PSS	Percent scabby seed	Percent of scabby seed by weight	KY	KY,MD,MO
FDK	Fusarium damaged kernels	Considers KR and PSS as equivalent estimates of kernel infection	IL, KY	IL,KS,KY,MD,MO
ISK	Composite of head and kernel traits	ISK Index = .3 (Severity) + .3 (Incidence)+.4 (% FDK or PSS)	IL,KY	IL,KY,MD,MO
DON	DON (vomitoxin)	PPM of vomitoxin in grain	IL,IN, VA	IL,IN,KS,KY,MD,NE,VA
GH	Greenhouse severity	Same as SEV except from greenhouse	IL	IL,MO

 Table 1. Traits assessed in the 2007-08 PNUWWSN and NUWWSN tests.

* ON and RO indicate Ontario Canada, and Romania, respectively

PNUWWSN ENTRY	PNUWWSN PEDIGREE	NUWWSN ENTRY	NUWWSN PEDIGREE
ERNIE	Moderate Resistant Check	ERNIE	Moderate Resistant Check
TRUMAN	Mod Resistant/Resistant Check	TRUMAN	Mod Resistant/Resistant Check
FREEDOM	Moderate Resistant Check	FREEDOM	Moderate Resistant Check
PIONEER 2545	Susceptible Check	PIONEER 2545	Susceptible Check
MSU Line E2043	Pioneer 2552/Pioneer 2737W	MSU Line E6002	VA96W-403-WS / CJ9403
MSU Line E6059	D9070 / Pioneer 2552	MSU Line E6001	Pioneer 25W60 / CJ 9306
MSU Line E6042	VA96W-403-WS / CJ9403	MSU Line E6003	VA96W-403-WS / W14
MSU Line E6038	VA96W-403-WS / CJ9403	MSU Line E5011	Caledonia / NY88024-117
MSU Line E5024	D6234 / Pioneer 25W33	P.99600A2-4-93	9560//9811/3/Fdm/201R
P.992192A1-5-4-5-81	92145//201R/Patton	P.0179A1-17	Fdm/Gfd//92829/Patton
P.0172A1-12-1	97395/981129	P.011010A1-15	97395/981129//INW0316
P.0175A1-37-4	981419/97397	P.03112A1-7-3	97395//INW 0315/99794
P.04281A1-4-5	INW 0304/9811//92823/Ernie	KS980512-2-2	(Too long, see final report)
P.04287A1-16	INW0316*2/INW0304//9346/CS5A	KS05HW14-3	KS98HW452/CO960293//KS920709B-5-2
P.03630A1-18	99751/INW0315//981358/97462	MO050143	MO 11769/Madison
MO050600	MO 960903/Bess 'S'	MO050699	950016/3/950016//90X54-1-1/MO 91-1009
MO050261	MO 94-182/VA 91-54-219	MO050921	Ernie/Truman "S"
MO051150	960815/IL 91-14163	MO050101	MO 11769/Madison
MO050617	960815/IL 91-14163	VA05W-425	Roane/3/Ning7840/Coker9904//Pioneer2552
MO041020	960429/960112	VA05W-775	(Too long, see final report)
MO050917	Truman 'S'/960815	VA05W-777	Roane*2//W14/Roane /3/Roane BC3E6
VA06W-553	(Too long see final report)	VA05W-534	Goldfield/Tribute//Gibson
VA06W-558	VA96W-348/P92823A1-1-4-4-5	MD01W233-06-1	McCormick/Choptank
VA06W-561	OH618//Roane/Sisson"S" (VA96W234)	MD01W233-06-16	McCormick/Choptank
VA06W-615	(Too long see final report)	MD99W483-06-11	VA97W358/RENWOOD3260
VA06W-622	(Too long, see final report)	NYCalresel-I	Reselection from Caledonia
	$\sqrt{492-51-39/41}$ 870365 (CK747*2/Amigo)	NY94052-9340	Pio2737w/Harus
	(Too long see final report)	NVW103-1-9100	
BDLS. HONEY-6 SE08 1083-14	PION25R57/OH546	NVW/103-70-0232	
SEKY93 C-1699-14	MO800071-56/PION2545//KY88C	NY93246SP-9070	(Too long see final report)
SE0/ C-0/80-2-2	84C-048-2-1/PION2510//FER555	SE011/02-/	
SE08 1106-6	OH546/SE1694-12	SE80-1873-2	NASW/84-345/Coker0835//0H410/OH380
SE94-1012-25	T814/I 880119	SE98-1089-34	P25R57/SE1694-12
6E94-1012-20 KV02C-3005-25	25P18/MCCOPMICK	SE03-1004-8	OH480/OH400
KY02C-3005-44		NE05/18	(Too long see final report)
KY02C-3008-05	25R18/02C-0010-17	NE05410	(Too long, see final report)
KY02C-3004-04	25R18/Tribute	NE05537	(100 long, see lina report) NI07/35 /NE0/632 /2/ KS80180B-2-1
KY01C-1542-07	Tribute/BL 940582//Tribute/91C-170-3	NE03488	KARIEGA/PRONGHORN//Millennium sib
KY99C-1205-06-1	25R26/ USG 3209//2540	NE01643	Millennium sib//Seward/Archer
M04-4566	BRADI EY/ROANE	KY00C-2059-16	91C-170-3/2552
M04-4715	MASON/ERNIE	KY00C-2143-08	90C-048-59/90C-160-14
M05-1172	M94-1048-1/IO2552	KY00C-2755-03	2552/Allegiance
M05*1589	GA871339/PIO2540	KY97C-0321-05-2	Kristy///A94-52-25//2540
M05-1531	L 487167-D8-/P92118B4-2	M04*5109	VA94-54-479/PIO2628
		M04-4802	FER518//ELKHART/M\/18
OH04-213-39		M03-3616-B11	
OH04-264-58		M03-3616-C10	
OH04-268-39	HOPEWELL/VA96-54-372	01100 40507	(Too long, soo final report)
OH04-176-29	P.92227C5-1-1/BL930390	OH02-13567	
OH03-41-45	IL91-14167/0H599	OH03-235-2	OH552/HOPEWELL
BCUOCE110202D/4	SD07060 x Bingo Stor	OH02-12678	FOSTER/HOPEWELL//OH581/OH569
	SD07060 v Eroodom	0H02-7217	(Too long, soo final report)
			(Too long, see final report)
DCATTE202/2			
RCATI 31	(Too long see final report)		AU KUN/WEKUUUUUU XAU KUN
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104-7074	903201/1290-12212 11 94-1653/11 97-3578	104-10110	1235-2310/1230-12212 11 95-4162/11 97-7010
IL 04-17204	IL 07-3578/ Emio	10721	IL 95-/162/ IL 97-7010
			160 - 102/ 1601-1010

Table 2. Entries in the 2007-08 PNUWWSN and NUWWSN.

NAME	INC		SEV		IND		FDK		ISK		DON		GH		#I	#h
IL02-18228	41.9	I	15.3	Ι	13.0	I	10.7	I	28.1	I	5.5	I	24.1	I	7	0
DH 22/24	42.4	Ι	19.5	Ι	12.5	I	17.0	1	31.6	I	7.8	Ι	9.0	Ι	7	0
TRUMAN	47.4	I.	21.6	I.	14.5	1	10.9	I.	31.8	1	6.7	Ι	4.5	I.	7	0
MSU Line E6003	48.5	T	17.6	I.	10.5	I	9.1	1	28.8	I	6.7	I	7.0	Ι	7	0
IL04-10741	48.7	I.	24.1	I.	18.9	I	21.6	Ι	38.5	I	7.0	I	29.8	Ι	7	0
MO050921	49.6	T	24.1	I.	16.4	I	13.4	I.	29.8	1	6.1	Ι	4.9	I.	7	0
M03-3616-C10	51.9	T	25.4	I.	17.3	I	24.4	hl	39.1	1	10.7	Ι	17.9	I.	7	1
VA05W-534	52.6	I	24.9	I	17.0	I	14.0	I	38.1		5.3	I	17.9	I	7	0
MO050143	52.6	Ι	28.7		19.4	Ι	11.5	Ι	33.7	1	7.1	Ι	6.7	Ι	6	0
M03-3616-B11	53.4	T	26.6		19.3	I	19.0	I.	41.3	1	9.1	I	13.8	I.	6	0
OH02-13567	54.1	I	28.7		18.3	I	22.8	hl	42.2	I	8.2	I	6.2	I	6	1
IL04-10118	55.9		24.7	T	18.9	I	10.5	Ι	35.7	T	8.2	Ι	14.5	Ι	6	0
MO050101	49.9	Ι	27.3		19.5		14.1	1	33.8	I	7.9	Ι	8.7	Ι	5	0
NE05418	52.0	I	26.3		19.7		24.5	hl	41.5	I	8.3	I	23.7	Ι	5	1
MD01W233-06-1	53.5	I.	26.1		18.8	I	19.4	1	44.0		4.7	Ι	4.8	Ι	5	0
OH02-12678	54.4	Ι	27.0		20.3		16.2	1	41.9	I	8.1	Ι	23.3	Ι	5	0
VA05W-775	55.4		28.8		17.7	I	19.9	1	40.9	I	5.1	Ι	4.1	Ι	5	0
VA05W-777	57.2		28.1		18.5	I	16.9	1	38.7	I	5.7	Ι	4.9	Ι	5	0
DH F/SF, 23	64.7		50.1	h	39.1		37.6	h	55.7	h	33.8	h	43.1	h	0	5
PIONEER 2545	74.9	h	52.2	h	42.8		30.7	h	58.7	h	19		48.3	h	0	5
SE98-1089-34	78.5	h	60.7	h	52.1	h	38.9	h	63.5	h	20.7		40.7	h	0	6
AVERAGE	59.4		34.0		26.2		22.3		44.2		11.6		23.9			
MINUMUM	41.9		15.3		10.5		9.1		28.1		4.7		4.1			
MAXIMUM	78.5		60.7		52.1		38.9		63.5		33.8		70.0			
LSD(0.05)	12.8		10.6		9.0		16.3		15.5		7.3		31.0			

Table 3. Best entries (top) and worst (bottom) from the 2007-08 NUWWSN. Summary statistics are for all entries.

l,h indicate a mean that is not significantly different than the lowest (l) or highest (h) mean in that column

Table 4. Best entries (top) and worst (bottom) from the 2007-08 PNUWWSN. Summary statistics are for all entries.

NAME	SEV		INC		IND		FDK		ISK		DON		GH	#I	#h
P.0172A1-12-1	49.7	Ι	17.4	Ι	9.4	Ι	13.1	Ι	29.6	Ι	3.8	Ι	13.3	6	0
IL01-34159	45.9	T	16.2	T	10.6	T	16.4	T	31.0	I	1.7	I	3.7	6	0
KY02C-3005-25	52.0	I.	18.2	I.	11.8	I.	26.3	I.	39.5	I	2.3	I	4.0	6	0
VA06W-558	55.3	I.	25.4	I.	15.3	I.	19.7	I.	38.8	I	3.9	I	43.5	6	0
TRUMAN	58.4	I	18.0	I	17.2	I	18.4	I	40.3	I	9.5	I	6.4	6	0
IL79-002T-B-B	60.2	T	26.3	Т	17.6	T	26.4	Т	42.1	Т	3.9	I	4.5	6	0
ERNIE	57.9	Ι	24.2	Ι	18.5	I	20.9	Ι	41.9	Ι	10.0	Ι	10.5	6	0
P.03630A1-18	61.5		27.3		18.9		32.5		42.7		4.0		4.0	6	0
P.0175A1-37-4	63.3		23.6	I	15.7	I	29.3	I	43.9	I	5.9	I	4.5	5	0
KY02C-3004-04	63.6		24.6	1	16.7		24.6	1	42.1		3.3		7.7	5	0
VA06W-561	59.7	I.	20.7	Ι	16.8	I.	39.3		47.6		5.7	I	28.8	4	0
VA06W-553	65.1		29.7		16.9	I.	21.5	I.	41.4	I	2.9	I	5.3	4	0
KY02C-3005-44	64.6		25.7	I	18.3	I	35.8	I	47.9		9.5	I	4.0	4	0
SE98 1083-14	64.8		27.6	I	20.5		32.9	Ι	44.4	I	8.3	I	7.8	4	0
MSU Line E6042	50.8	I	30.2		20.7		15.2	Ι	33.3	I	8.8	I	49.7	4	0
IL04-17204	60.4	Ι	33.2		21.2		20.2	Ι	42.1	Ι	8.2	Ι	30.5	4	0
IL04-8445	59.8	Ι	30.5		21.7		28.7	Ι	43.8	Ι	6.0	Ι	28.2	4	0
RCUOGF110202D/4	59.5	I	31.1		23.2		27.9	I	44.3	I	10.1	I	7.0	4	0
SE94 C-0480-2-2	73.4	h	40.6	h	32.6		48.2	h	59.3	h	10.5	I	65.3	1	4
MSU Line E2043	84.5	h	39.1		33.2		64.0	h	66.2	h	24.8	h	10.5	0	4
M04-4566	74.9	h	45.0	h	34.5	h	50.6	h	64.3	h	13.0		65.5	0	5
M04-4715	70.1	h	41.5	h	34.7	h	42.1	h	52.7	h	14.6		76.3	0	5
OH04-213-39	73.2	h	40.6	h	34.8	h	56.5	h	65.6	h	9.7	I	36.5	1	5
SE94-1012-25	72.8	h	45.7	h	37.4	h	53.8	h	60.3	h	12.3		72.0	0	5
BDLS. HONEY-6	75.5	h	46.5	h	39.4	h	43.4	h	62.1	h	10.7	I	60.7	1	5
MSU Line E6059	85.5	h	40.6	h	33.8	h	50.5	h	64.2	h	27.7	h	24.8	0	6
PIONEER 2545	85.8	h	46.6	h	42.5	h	55.3	h	65.1	h	19.6	h	98.8	0	6
KY01C-1542-07	82.4	h	51.8	h	43.6	h	44.7	h	60.9	h	20.9	h	44.3	0	6
AVERAGE	68.3		32.7		25.6		36.8		50.5		10.1		29.2		
MINUMUM	45.9		16.2		9.4		13.1		29.6		1.7		3.7		
MAXIMUM	85.8		51.8		43.6		64.0		66.2		27.7		98.8		
LSD(0.05)	15.6		12.3		9.9		24.5		15.1		9.9				
I.h indicate a mean that is not s	ignificant	lv dif	ferent the	in the	lowest (1) or h	ighest (h)	mea	n in that c	olum	n				

		nts		5 20		S PI		VV C			Davi		<u></u>	,	
NAME	SEV		INC				FDK		ISK		DON		GH	#1	#h
ERNIE	57.9		24.2	1	18.5		20.9		41.9		10.0	1	10.5	6	0
IRUMAN	58.4	I	18.0	I	17.2	I	18.4	I	40.3	I	9.5	1	6.4	6	0
FREEDOM	67.5		34.0		24.9		47.9	h	57.5	h	8.7	I	12.5	1	2
PIONEER 2545	85.8	h	46.6	h	42.5	h	55.3	h	65.1	h	19.6	h	98.8	0	6
MSU Line E2043	84.5	h	39.1		33.2		64.0	h	66.2	h	24.8	h	10.5	0	4
MSU Line E6059	85.5	h	40.6	h	33.8	h	50.5	h	64.2	h	27.7	h	24.8	0	6
MSU Line E6042	50.8	T	30.2		20.7		15.2	Т	33.3	T	8.8	Ι	49.7	4	0
MSU Line E6038	63.3		36.9		25.8		28.9	I.	45.2		12.1		48.5	1	0
MSU Line E5024	76.0	h	37.1		29.1		29.3	I	54.7	h	12.9		26.7	1	2
P.992192A1-5-4-5-81	79.6	h	38.0		32.7		52.7	h	57.5	h	11.3	Т	22.3	1	3
P.0172A1-12-1	49.7	I.	17.4	Т	9.4	Т	13.1	Т	29.6	T	3.8	Т	13.3	6	0
P.0175A1-37-4	63.3		23.6	Т	15.7	T	29.3	Т	43.9	T	5.9	Т	4.5	5	0
P.04281A1-4-5	74.9	h	34.3		27.7		42.5	h	54.7	h	10.8	Т	6.6	1	3
P.04287A1-16	66.5		34.0		26.3		48.9	h	56.2	h	7.7	Т	30.5	1	2
P.03630A1-18	61.5	Т	27.3	Т	18.9	I.	32.5	Т	42.7	Т	4.0	Т	4.0	6	0
MO050600	60.6	i	33.8		24.3	· ·	30.0		/3.5	i	4.0	i	8.0	3	0
MO050261	65.6	'	31.0		24.5		39.0 41.0	h	43.J	ı h	4.0	÷	7.5	1	2
MO051150	75.0	h	21.2		22.9		41.0	н Б	59.5	h	7.0	-	20.2	1	2
MO050617	75.0	וו ה	31.3		20.2		43.9		56.5	11 b	10.1	-	20.3	2	3
MO050817	70.0	n	37.9		32.0		33.0	-	55.6	n	10.1		10.3	2	2
MO041020	66.7		24.3	I	20.1		23.5		45.8		7.3	1	4.0	3	0
MO050917	77.0	h	35.2		28.1		36.8		55.9	h	11.5	ļ	5.8	2	2
VA06W-553	65.1		29.7		16.9	I	21.5	I	41.4	T	2.9	I	5.3	4	0
VA06W-558	55.3	T	25.4	Ι	15.3	I	19.7	Т	38.8	T	3.9	Ι	43.5	6	0
VA06W-561	59.7	I	20.7	Ι	16.8	I.	39.3		47.6		5.7	I.	28.8	4	0
VA06W-615	70.3	h	36.8		25.5		31.0	I.	48.6		5.1	Т	12.7	2	1
VA06W-622	70.6	h	24.1	T	23.0		44.7	h	49.2		15.1		25.2	1	2
TRIBUTE	63.9		29.5		23.8		36.0	Т	48.8		8.6	Т	30.0	2	0
BDLS, HONEY-6	75.5	h	46.5	h	39.4	h	43.4	h	62.1	h	10.7	1	60.7	1	5
SE98 1083-14	64.8		27.6	I	20.5		32.9	1	44.4	1	8.3	Ì	7.8	4	0
SEKY93 C-1699-14	70.9	h	35.0	•	28.0		31.7	i	47.7	•	12.3	•	70.3	1	1
SE94 C-0480-2-2	73.4	h	40.6	h	32.6		18.2	h	59.3	h	10.5		65.3	1	
SE08 1106-6	73.4	h	35.8		27.2		24.0		45 7		11.3	÷	63	2	1
SE04 1012 25	71.1	n h	45.7	h	27.2	h	24.0 52.0	ו ה	40.7	h	12.2		72.0	2	5
<u>SE94-1012-25</u>	72.0	<u> </u>	40.7	- 11	37.4		00.0		00.3		12.3		12.0	0	0
KY02C-3005-25	52.0	I	18.2		11.8		26.3		39.5	I	2.3		4.0	6	0
KY02C-3005-44	64.6		25.7	I	18.3	I	35.8	I	47.9		9.5	1	4.0	4	0
KY02C-3008-05	68.4		22.3	I	16.0	I	41.4	h	50.7		9.4	I	3.0	3	1
KY02C-3004-04	63.6		24.6	I	16.7	I	24.6	I	42.1	I	3.3	I	7.7	5	0
KY01C-1542-07	82.4	h	51.8	h	43.6	h	44.7	h	60.9	h	20.9	h	44.3	0	6
KY99C-1205-06-1	75.0	h	36.1		32.2		55.5	h	59.4	h	13.7		28.5	0	3
M04-4566	74.9	h	45.0	h	34.5	h	50.6	h	64.3	h	13.0		65.5	0	5
M04-4715	70.1	h	41.5	h	34.7	h	42.1	h	52.7	h	14.6		76.3	0	5
M05-1172	72.1	h	33.1		26.3		43.1	h	56.7	h	10.1	Т	29.8	1	3
M05*1589	67.6		31.1		25.3		23.7	1	44.1	1	9.1	Т	21.5	3	0
M05-1531	64.4		24.5	T	24.0		43.0	h	52.4	h	7.0	Т	22.7	2	2
OH04-213-39	73.2	h	40.6	h	34.8	h	56.5	h	65.6	h	97	1	36.5	1	5
OH04-264-59	77.0	h	38.3		31.0		55.0	h	62.0	h	12.2	•	22.8		2 2
OH04-204-30	82.0	h	30.0		33.6		52.0	h	60.6	h	12.2		53.0		ა ა
	60.3	11	38.Z		27.2		JZ.1	11	50.0	11	12.3		20.3	2	3
	09.3		34.0		21.3		29.4	1	30.4		0.3		29.2	2	0
OH03-41-45	07.0		35.3		29.7		30.5	<u> </u>	47.1		9.6	<u> </u>	∠4.5	2	0
DH ACF112103 -8T	73.8	h	35.7		31.2		46.9	h	60.7	h	10.1	1	7.2	1	3
RCUOGF110202D/4	59.5	I	31.1		23.2		27.9	I	44.3	I	10.1	I	7.0	4	0
CUOGDHACF1109O2D	58.3	I	32.4		23.7		51.2	h	53.5	h	11.0	Ι	4.5	2	2
RCATTF174/1C	74.0	h	37.3		29.3		44.7	h	55.9	h	16.7		4.0	0	3
RCATTF203/2	83.3	h	37.6		29.3		30.5	Т	51.2	h	17.9		11.0	1	2
RCATL31	71.0	h	38.4		30.1		33.7		44.8		20.0	h	61.2	1	2
IL01-34159	45.9	Ī	16.2	Ī	10.6	1	16.4	I	31.0	I	1.7	1	3.7	6	0
IL79-002T-B-B	60.2	Т	26.3	Т	17.6	I	26.4	T	42.1	I	3.9	Т	4.5	6	0
II 04-7874	65.9		30.0		21.9		28.4	Т	44.5	1	6.5	I.	19.8	3	0
II 04-8445	59.8	1	30.5		21.7		28.7	i	43.8	i	6.0	Ì	28.2	4	0
II 04-17204	60.4	i	33.0		21.2		20.2	ì	12 1	÷	0.0 8 2	÷	20.2	1	0
	60.4	1	20.2		21.2		20.2	I	+2.1	1	0.2	1	30.5	4	U
AVERAGE	08.3		32.7		∠5.6 0 0		30.8		50.5		10.1		∠5.3		
LSD(0.05)	15.6		12.3		9.9		24.5		15.1		9.9				
			<u>^</u>		0				2				1		

NAME INC SEV IND FDK ISK DON GH III IIII IIII IIII IIIII IIIII IIIIIII IIIIIIIIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII		.1 y 01	1050		un un	0 200	7-0		••••	1011.							
ERNIE 67.4 31.7 22.7 25.6 h 48.7 h 11.0 1 13.7 1 2 2 TRUMMA 47.4 34.9 23.1 20.3 1 48.7 h 18.2 1 3 0 5 5 10.7 1 6.83 h 0.8 1 10.7 1 2.8 1 0.6 1 0.7 1 7.0 1 7 0 MSU Line E0001 64.5 1 16.5 1 40.2 2.8.1 1 0.7.0 1 1 2.5 1 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 1.5 1 <td>NAME</td> <td>INC</td> <td></td> <td>SEV</td> <td></td> <td>IND</td> <td></td> <td>FDK</td> <td></td> <td>ISK</td> <td></td> <td>DON</td> <td></td> <td>GH</td> <td></td> <td>#I</td> <td>#h</td>	NAME	INC		SEV		IND		FDK		ISK		DON		GH		#I	#h
TRUMAN Y7.4 I 21.4 10.9 I 31.8 I 65.6 98.8 I 45.2 I 30 0 PIONEER 2545 74.9 h 52.2 h 42.8 30.7 h 85.7 h 19.0 18.3 I 30.5 0 5 MSU Line E6001 61.2 30.7 10.5 I 11 12.85 I 3.0 26.5 11.6 10.5 I 11.1 12.85 I 2 2 MSU Line E6011 66.1 h 40.7 10.3 10.25 I 3.0 26.5 h 14.1 11.4 11.	ERNIE	57.4		31.7		22.7		25.6	h	48.7	h	11.0	I	13.7	I	2	2
FREEDOM 57.4 36.4 9.7.4 20.3 1 45.6 9.8 1 18.2 1 0 5 MSU Line E6000 65.2 35.7 25.6 21.8 1 42.5 1 10.7 1 28.8 1 10.7 1 28.8 1 10.7 1 28.8 1 10.7 1 28.8 1 10.7 1 12.3 1 1 23.3 1 <	TRUMAN	47.4	1	21.6	Т	14.5	I.	10.9	1	31.8	Т	6.7	I	4.5	I.	7	0
PIONEER 245 74.9 h 52.2 h 42.8 30.7 h 68.7 h 19.0 48.3 h 0 5 MSU Line E6002 56.2 30.7 25.6 21.8 I 13.1 22.8 I 3 2 2 1 1 20.8 I 1.0 1 2 2 1 1 2 1 1 2 2 1 1 2 2 1 1 1 1.0 1 1 1 1 2 2 1 1 1 2 1 3 1 1.0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 3 1	FREEDOM	57.4		34.9		23.1		20.3	I.	45.6		9.8	I.	18.2	1	3	0
MSU Line E6002 65.2 35.1 25.6 21.8 I 42.5 I 13.1 26.8 I 16.7 I 2 2 MSU Line E6003 64.5 I 17.6 I 10.7 I 13.0 15.3 I 1 3 PerofFact 66.6 h 36.1 33.0 26.5 h 14.4 I 14.5 I 15.3 I 1 3 3 PerofFact h 45.2 h 40.0 13.7 I 15.5 I 3<.3 1 3 3 3 PerofFact h 39.3 24.1 I 15.5 I 43.3 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1 10.8 1	PIONEER 2545	74.9	h	52.2	h	42.8		30.7	h	58.7	h	19.0		48.3	h	0	5
MSU Line E6001 61:2 35.1 25.7 h 42.2 h 10.7 I 6.7 I 7.0 I 7.1 8.1 h 4.5.1 4.0.2 2.6.1 h 5.5.5 h 1.4 h 1.4 1.5.5 I 1.3 3.3 P.03112A1-7.3 60.7 .44.3 22.6 21.5 I 48.2 h 1.0.8 I 5.6 I 4.1 2.1 KS06M01443 71.9 H 3.3.3 1.0.8 1.4.2 1.1 1.6.2 H 4.8.1 1.4 1.0.2 1 1.4.3 1.1 1.5 1.3.3 I 1.4.4 1.4.2 1.4.1 1.5 1.4.1 1.5 1.4.1 1.5 1.4.1 1.5 1.4.1 1.5 1.4.1<	MSU Line E6002	55.2		30.7		25.6		21.8	I.	42.5	Т	13.1		25.8	T	3	0
MSU Line E0003 48.5 I 10.7 I 0.7 I 7 0 7 0 7 0 7 0 7 0 7 1 3 1 33 1 23.6 1 20.0 15.3 1 <	MSU Line E6001	61.2		35.1		25.7		25.7	h	48.2	h	10.7	T	6.7	T	2	2
MSU Line E5011 68.1 h 46.1 40.2 26.1 h 50.5 h 13.0 15.3 1 23.3 P.98600A2-433 66.6 h 38.1 33.0 26.5 h 51.4 h 11.4 1 16.6 1 1 2 3 P.011241-73 60.7 34.3 22.6 21.5 1 43.4 1 10.8 1 1.6 1 3 3 3 1 10.8 1 1.6 1 3 1 1.8 1 1.8 1 1.8 1 1.8 1 2.8 1 1.6 1 3.0 1 3.0 1 1.8 1 3.3 1 3.0 1 1.1 3.3 1 1.1	MSU Line E6003	48.5	T	17.6	Т	10.5	Т	9.1	T	28.8	T	6.7	T	7.0	T	7	0
P.9600A2-4-93 66.6 h 38.1 33.0 26.5 h 51.4 h 11.4 l 26.5 l 2.0 h 15.7 h 11.5 l 15.5 l 3 3 3 P.011010A1-15 67.2 h 40.7 33.8 22.6 21.5 l 43.2 l 10.8 l 55.6 l 3 3 P.011010A1-15 00.7 34.3 22.6 l 17.5 l 42.1 h 17.6 l	MSU Line E5011	68.1	h	45.1		40.2		26.1	h	50.5	h	13.0		15.3	1	1	3
P.0170A1-17 63.6 36.3 26.3 20.2 h 40.0 h 11.7 18.6 I 1 2 P.011010A1-15 67.2 h 40.7 33.8 22.7 h 51.7 h 11.5 I 15.7 h 15.7 h 15.6 I 4.4 0 KS065HV14-3 71.9 h 32.8 I 22.6 14.2.8 h 7.7 h 15.0 16.4 I 3.0.2 h 4.8.0 h 1.7 I 6.7 1.4 0 KS065HV14-3 71.9 h 13.5 25.6 I 22.6 h 4.2.8 h 9.7 1.5 I 1.4 1 3.8 I 7.9 1.5 I 1.4 1 5.0 1.4 1.4 1.5 1.5 I 1.6 1.4 1.5 1.4 1.5 1.4 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5	P.99600A2-4-93	66.6	h	38.1		33.0		26.5	h	51.4	h	11.4	1	26.5	1	2	3
P.011010A1-16 67.2 h 40.7 33.8 22.7 h 51.7 h 11.5 I 15.5 I 3 3 P.03112A1-7.3 60.7 34.3 22.6 21.5 I 43.3 I 10.8 I 15.6 I 2.1 KS9804722-2 60.9 40.2 27.9 21.5 I 43.1 I 0.8 I 6.1 I 2.1 MO050101 35.6 28.7 19.5 14.1 I 33.8 I 7.9 I 8.7 I 5.0 I 4.4 1 4.5 I 0.9 I 0.1 1.4 1 5.5 I 0.0 NO NO 10.3 I 4.7 I 4.8 I 1.4 I 1.4 I 1.5 I	P 0179A1-17	63.6		36.3		26.3		30.2	h	49.0	h	13.7	•	18.6	i	1	2
P.03112A17-3 60.2 Cl. 1 Cl. 5 I B.33 I TO.3 I I TO.3 I I TO.3 TO.3 <thto.3< th=""> <th< td=""><td>P 011010A1-15</td><td>67.2</td><td>h</td><td>40.7</td><td></td><td>33.8</td><td></td><td>22.7</td><td>hl</td><td>51 7</td><td>h</td><td>11.5</td><td>1</td><td>15.5</td><td>÷</td><td>3</td><td>3</td></th<></thto.3<>	P 011010A1-15	67.2	h	40.7		33.8		22.7	hl	51 7	h	11.5	1	15.5	÷	3	3
ISSN 012-22 00.7 34.3 27.9 21.5 1 43.2 1 10.3 1 10.3 1 2 1 KS096112-22 00.9 40.2 27.9 21.5 1 48.7 h 15.0 30.2 I 1 3 MO050613 52.6 1 28.7 19.4 1 15.1 I 4.4 1 1 4.7 1 6.0 1 4.1 1 6.0 1 4.1 1 6.0 1 4.1 1 1.4 1.5 1 1.4 1.5 1 1.4 1 4.9 1 7 0 W0050101 45.6 1 23.1 15.5 1 1.6 1 1.4 3.8 1 1.5.3 1 1.7.9 1 5.7 1 4.9 1 5.0 0 0 0.0 0.0 1.0.3 1 1.0.3 1 1.0.3 1 1.0.3	P 0311201-7-3	60.7		2/ 2		22.6		21.5		12.2		10.8	÷	5.6	÷	1	0
K800H/21-2 01.9 H 10.2 21.9 11.6 11.6 11.6 1 1 1 1 1 1 1 2 1 3 1 1 2 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1 1 1 3 1	KS090512.2.2	60.0		40.2		22.0		21.5	- <u>-</u> -	40.0	ь г	17.6		16.1	<u> </u>	7 2	1
NB00PW145 17.3 17.3 19.4 11.5 13.7 1 1.6 1.3 1.7 1 6.7 1 4.9 1 7 0 W0050021 45.6 1 23.1 13.4 1 1 4.3 1 1.3 1.3 1.4 1.3 1 1.5.3 1 1.1 5.3 1 1.0 1 3.3 1 1.4 1.3 1.4 1.0 1 3.3 1 1.4 1 1.3 1.1 1.3 1.3 1.3 1.3 </td <td>KS960312-2-2</td> <td>71.0</td> <td>h</td> <td>40.2</td> <td></td> <td>27.9</td> <td></td> <td>21.5</td> <td>ו ה</td> <td>40.2</td> <td>11 b</td> <td>17.0</td> <td></td> <td>20.2</td> <td>-</td> <td>4</td> <td>2</td>	KS960312-2-2	71.0	h	40.2		27.9		21.5	ו ה	40.2	11 b	17.0		20.2	-	4	2
MOUGUIA S2.5 I S2.5 Z2.6 I Z2.8 I G.1 I A.7 I S.0 I A I T I D.0 D.0 D.0 D.0 I Z.3 I T I D.0 I Z.3 I J J J J<1 J<1 <thj<1< th=""> J<1 J<1</thj<1<>	KSUSHW14-3	71.9	<u>n</u>	39.3		34.1		29	<u>n</u>	40.7	<u>n</u>	15.0		30.2	<u> </u>		3
MOUSUBUB 61.1 35.5 25.9 22.6 ni 42.8 i 61.1 i 4.9 1 7 0 MOOSD101 49.9 I 27.3 19.5 14.1 I 33.8 I 7.9 I 4.7 I 5.0 I 4.7 I 5.0 I 4.7 I 5.0 I 4.7 I 5.7 I 4.7 I 5.0 I 4.7 I 5.7 I 4.8 I 5.0 I 7.0 I 1.4 I 38.7 I 5.7 I 4.8 I 5.0 I 7.0 I 1.4 I 4.1 I 6.3 I 2.0 I 1.1 I 1.2 I 1.2 I 1.2 I 1.2 I 1.3 I 1.2 I I I 1.3 I I I I I I I I <td>MO050143</td> <td>52.6</td> <td>1</td> <td>28.7</td> <td></td> <td>19.4</td> <td>I</td> <td>11.5</td> <td></td> <td>33.7</td> <td></td> <td>7.1</td> <td></td> <td>6.7</td> <td></td> <td>6</td> <td>0</td>	MO050143	52.6	1	28.7		19.4	I	11.5		33.7		7.1		6.7		6	0
MO050921 49.6 I 24.1 I 16.4 I 29.8 I 6.1 I 4.9 I 7 0 VA05W-425 60.0 33.1 23.4 22.9 NI 40.9 I 6.9 I 10.3 I 4 1 VA05W-775 55.4 28.8 17.7 I 19.9 I 6.9 I 1.4 1 38.1 I 5.7 I 4.9 I 5 0 VA05W-775 55.2 26.1 18.8 I 1.7 I 4.8 I 1.7 I 4.8 I 1.6 1.6 3.0 I 0.0 1.0 1.4 0 0.0 1.0 1 3.3 1.4 1.0 1.4 1.3 1.0 1.4 0.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	MO050699	61.1		35.5		25.9		22.6	hl	42.8	I	9.7	I	5.0	I	4	1
MO050101 49.9 I 27.3 19.5 14.1 I 33.8 7.9 I 8.7 I 5 0 VA05W-775 55.4 28.8 17.7 I 19.9 I 40.9 I 6.9 I 1.1 I 5 0 VA05W-775 55.4 28.1 18.5 I 16.9 I 38.7 I 5.7 I 4.9 I 7 0 7 0 7 0 7 0 7 1 1 4 44.0 4.7 I 4.8 I 5.5 0 MD01W233-06-1 56.6 28.5 20.3 19.8 I 41.7 I 6.0 I 4.3 10.7 14.8 1 40.9 I 22.5 11.2 1 2 1 1 3.0 NY403-1-9100 61.6 37.0 33.1 16.8 I 40.9 I 22.5 14.5	MO050921	49.6	I	24.1	I	16.4	I	13.4	I	29.8	I	6.1	I	4.9	I	7	0
VA05W-425 60.0 33.1 23.4 22.9 hI 40.9 I 6.9 I 10.3 I 4 1 VA05W-775 57.2 28.1 18.5 I 16.9 I 38.7 I 5.7 I 4.4 I 4.9 I 5.0 I 7.7 57.2 28.1 11.8 I 1.4 I 3.8.7 I 5.7 I 4.8 I 5.7 I 4.8 I 5.7 I 4.8 I 5.7 I 4.8 I 1.7 I 4.8 I 5.7 I 4.8 I 1.7 I 4.8 I 1.7 I 3.8 I 1.2 I 7.0 3.3 I 1.2 I 1.8 3.1 I 1.2 I 1.8 1.1 I 1.8 3.1 I 2.2 I 1.8 1.8 I 1.8 1.8 I I	MO050101	49.9		27.3		19.5		14.1		33.8		7.9		8.7		5	0
VA05W-777 S5.4 28.8 17.7 I 19.9 I 40.9 I 5.7 I 4.9 I 5.0 VA05W-534 52.6 I 24.9 I 17.0 I 14 I 38.7 I 5.3 I 1.7.9 I 7 0 MD01W233-06-1 56.6 28.5 20.3 19.8 I 1.4 I 48.0 I 6.3 I 2.0.4 I 4 0 MD01W233-06-11 63.3 38.8 27.9 33.6 h 49.0 h 8.0 1 10.1 1 1.8 1 3.0 10.8 1 2.0 1 1.4 1.0 3.1 16.8 14.3.7 12.5 11.2 1 2 1 1 3.2 1 1.3 1 2 1 1.4 2 1 1.3 1 2.5 1.33.1 1.4 2 1 1.4 1.5	VA05W-425	60.0		33.1		23.4		22.9	hl	40.9	I	6.9	I	10.3	I	4	1
VA05W-77 57.2 28.1 18.5 1 6.9 1 8.7 1 5.3 1 7.0 0 MD01W233-06-1 53.5 1 24.9 1 17.0 1 14 1 38.1 1 5.3 1 2.6 0 0 1 44.0 1 44.0 1 4.7 1 4.8 1 5.0 MD01W233-06-11 63.3 38.8 27.9 33.6 4.90.0 1 4.8 51.1 h 0 3 NYCalresel-L 60.0 40.2 33.1 16.8 4.0.9 1 2.6 11.4.8 51.1 h 0 3 0 NYW103-1-9100 61.6 37.0 33.1 16.8 4.0.9 1 1.4.2 1 3 0 1 34.2 1 2 1 N 3 1 5 1 0.1 34.2 1 1 2 1 1 3 </td <td>VA05W-775</td> <td>55.4</td> <td></td> <td>28.8</td> <td></td> <td>17.7</td> <td>I</td> <td>19.9</td> <td>I.</td> <td>40.9</td> <td>I</td> <td>5.1</td> <td>I.</td> <td>4.1</td> <td>I.</td> <td>5</td> <td>0</td>	VA05W-775	55.4		28.8		17.7	I	19.9	I.	40.9	I	5.1	I.	4.1	I.	5	0
VA05W-534 52.6 I 24.9 I 17.0 I I I 15.3 I 7 0 MD01W233-06-16 55.6 26.5 20.3 19.8 I 41.7 I 6.3 I 20.4 I 4 0 MD01W233-06-16 56.6 26.5 20.3 13.8 I 41.7 I 6.3 I 20.4 I 4 0 MVGatesel-L 00 40.2 33.1 16.8 I 40.9 I 12.5 11.2 I 2 1 NY94052-9340 59.1 31.7 26.2 23.3 16.8 I 43.7 12.5 11.8 I 2 1 NYW103-70-9232 66.0 h 42.3 38.1 22.5 I 45.6 h 11.0 I 8.8 I 2 1 3 SE89-1089-34 75.2 h 36.7 24.7 23.3 I	VA05W-777	57.2		28.1		18.5	Т	16.9	I.	38.7	I	5.7	I.	4.9	I.	5	0
MD01W233-06-16 55.5 2 2 1 18.8 1 9.4 1 4.0 - 4.7 1 6.3 1 8.0 1 41.7 1 6.3 1 2.04 1 3.0 MD99W483-06-11 63.3 38.8 27.9 33.6 h 49.0 h 1.8 1 40.0 1 1.2.5 1.1.2 1 2 1 3.3 1 1.6.8 1 40.9 1 1.2.6 1.7.8 1 3 0 NYW103-70-9232 66.0 h 42.3 38.1 2.2.5 1 48.5 h 1.0.1 18.8 1 2 2 SE911492-4 64.1 33.2 25.5 2.3.3 h1 44.2 9.0 1 34.7 1 25.6 h 3.7 1 5.6 1.0.7 h 3.7 1 5 1 3.5 1 1.5 1 1.5 1 <	VA05W-534	52.6	I	24.9	Ι	17.0	I	14	Ι	38.1	Ι	5.3	I	17.9	I	7	0
MD01W233-06-16 56.6 28.5 20.3 19.8 I 41.7 I 6.3 I 20.4 I 4 0 MD99W483-06-11 63.3 38.8 27.9 33.6 h 49.0 h 8.3 I 20.4 h 53.7 h 14.8 T 10.0 3 NY94052-9340 59.1 31.7 26.2 24.1 h 43.7 12.5 11.2 I 2 1 NYW103-70-9232 66.0 h 42.3 38.1 22.5 145.6 17.5 13.4 I 2 2 SE891402-4 64.1 33.2 25.5 23.3 h 48.5 h 10.0 I 18.4 1 2 2 2 SE93-1089-34 78.5 h 60.7 h 52.1 h 38.9 h 63.5 h 2.7 40.7 h 3 3 1 23.7 1 5 1 <	MD01W233-06-1	53.5	I	26.1		18.8	Ι	19.4	Ι	44.0		4.7	I	4.8	I	5	0
MD99W483-06-11 63.3 38.8 27.9 33.6 h 49.0 h 8.0 I 70.0 h I 3 NY4032-0340 50.1 31.7 26.2 24.1 h 43.7 12.5 I 14.5 I 1.0 I 2.5 I 46.6 I 17.8 I 2.5 I 45.6 I 17.8 I 2.2 I 145.6 I 10.0 I 34.2 I 3 I I 1.6 I 14.2 I 3.4 I 2.2 I I 1.6 I 1.4 I 2.2 I I I 3.1 I 2.5 I I 43.5 I I 3.1 I 2.7 I </td <td>MD01W233-06-16</td> <td>56.6</td> <td></td> <td>28.5</td> <td></td> <td>20.3</td> <td></td> <td>19.8</td> <td>T</td> <td>41.7</td> <td>Т</td> <td>6.3</td> <td>T</td> <td>20.4</td> <td>T</td> <td>4</td> <td>0</td>	MD01W233-06-16	56.6		28.5		20.3		19.8	T	41.7	Т	6.3	T	20.4	T	4	0
NYCalresel-L 60.0 40.2 33.1 33.2 h 53.7 h 14.8 51.1 h 0 3 NY94052-9340 59.1 31.7 26.2 24.1 h 43.7 12.6 11.2 1 2 1 NYW103-70-922 66.0 h 42.3 38.1 22.5 1 46.6 17.5 34.1 1 2 2 SE911492-4 64.1 33.2 25.5 23.3 h 44.2 9.0 1 34.2 1 3 1 SE98-1089-34 78.5 h 60.7 h 52.1 h 38.9 h 63.5 h 90.7 40.7 h 0 6 SE98-108-48 50.0 126.3 19.7 24.5 h 41.5 1 8.3 1 23.7 1 2 3 NE05549 68.2 h 46.6 41.0 23.1 h 55.1 13.3 <td>MD99W483-06-11</td> <td>63.3</td> <td></td> <td>38.8</td> <td></td> <td>27.9</td> <td></td> <td>33.6</td> <td>h</td> <td>49.0</td> <td>h</td> <td>8.0</td> <td>T</td> <td>70.0</td> <td>h</td> <td>1</td> <td>3</td>	MD99W483-06-11	63.3		38.8		27.9		33.6	h	49.0	h	8.0	T	70.0	h	1	3
NY94052-9340 59.1 31.7 26.2 24.1 hI 43.7 12.5 11.2 I 2 1 NYW103-1-9100 61.6 37.0 33.1 16.8 I 40.9 I 12.6 17.5 34.1 2 1 NY93246SP-9070 61.9 34.9 32.7 29.1 h 48.5 h 11.0 I 18.8 I 2 2 SE911492-4 64.1 33.2 25.5 23.3 hI 44.2 9.0 I 34.2 I 3 1 5 5 34.1 1 8.8 h 62.5 h 1.6 1 1.2 3 1 2 3 1 3 1 2 3 1 3 1 2 3 1 1 3 1 1 3 1 3 1 2 3 1 1 3 1 2 1 3 1 <	NYCalresel-L	60.0		40.2		33.1		33.2	h	53.7	h	14.8		51.1	h	0	3
NYW103-1-9100 61.6 37.0 33.1 16.8 I 40.9 I 12.6 17.8 I 3 0 NYW103-70-9232 66.0 h 42.3 38.1 22.5 I 45.6 17.5 34.1 I 2 1 NY93246SP-9070 61.9 34.2 25.5 23.3 hI 44.2 9.0 I 34.2 I 3 1 SE981487-2 65.5 39.4 31.9 23.6 hI 46.8 13.7 62.2 h 1 2 SE98-1089-34 78.5 h 60.7 h 52.1 h 48.5 h 1.5 h 1.5 1 1 3.1 2.2 h 1 2.9 1 4.3 1 2.3 1 41.0 31.3 1 2.2 1 4.0 1 1.5 1 1.5 1 1.5 1 1.5 1.3 1.5 1.5 1.3 <td>NY94052-9340</td> <td>59.1</td> <td></td> <td>31.7</td> <td></td> <td>26.2</td> <td></td> <td>24.1</td> <td>hl</td> <td>43.7</td> <td></td> <td>12.5</td> <td></td> <td>11.2</td> <td>1</td> <td>2</td> <td>1</td>	NY94052-9340	59.1		31.7		26.2		24.1	hl	43.7		12.5		11.2	1	2	1
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	NYW103-1-9100	61.6		37.0		33.1		16.8	1	40.9	1	12.0		17.8	÷	3	0
NY93246SP-9070 61.9 34.9 32.7 22.1 h 46.5 h 11.0 I 18.8 I 2 2 SE9114924 64.1 33.2 25.5 23.3 hI 44.2 9.0 I 34.2 I 3 1 SE89-1873-2 65.5 39.4 31.9 23.6 hI 46.8 h 13.7 40.7 h 0 6 SE98-1089-34 67.2 h 39.9 32.8 31.7 h 53.6 h 19.8 29.9 I 1 3 NE05418 62.2 h 46.6 10.0 23.1 hI 50.9 h 14.0 31.3 I 2 3 N NE05439 66.4 37.7 34.0 20.2 I 41.5 I 11.5 I 32.2 I 46.3 h 2 1 KY00C-2059-16 58.0 36.6 24.8 21 I 40.7 I 15.3 46.3 h 2 1 KY00C-2755-03	NVW/103-70-0232	66.0	h	12.3		28.1		22.5	÷	40.0	•	17.5		3/ 1	÷	2	1
NE32403 01.3	NV03246SP-0070	61.0		34.0		32.7		22.0	r h	49.5	Ь	11.0		19.9	÷	2	2
SE91r492-4 64.1 33.2 23.3 11 44.2 30.0 1 34.2 1 3 1 SE98-1873-2 65.5 39.4 31.9 23.6 h1 44.8 13.7 62.2 h 1 2 SE98-1089-34 78.5 h 60.7 h 52.1 h 38.9 h 63.5 h 20.7 40.7 h 0 6 SE93-1094-8 67.2 h 36.6 11.0 23.1 h1 50.9 h 14.0 31.3 I 2 3 NE05537 54.8 36.7 24.7 22.3 I 41.5 I 11.5 I 32.2 I 4 0 NE05537 54.8 36.7 24.7 22.3 I 41.5 I 11.5 I 32.2 I 4 0 NE05418 66.4 37.7 24.1 h 42.5 I 1.40.7 I 15.3 46.3 h 2 1 NC00C-2755-0 61.2	N1932403F-9070	64.4		24.9		32.1 05.5		29.1		40.5		0.0	<u> </u>	24.2	<u> </u>	2	2
SE99-18/3-2 65.5 39.4 31.9 23.6 n1 46.8 13.7 62.2 n 1 2 SE99-1093-34 67.2 h 39.9 32.8 31.7 h 53.6 h 19.8 29.9 I 1 3 NE05418 52.0 I 26.6 41.0 23.1 hI 50.9 h 14.0 31.3 I 2.7 I 5 1 NE05537 54.8 36.7 27.7 22.3 I 41.5 I 13.1 58.8 h 2 2 NE01643 66.4 h 37.7 27.3 24.1 hI 42.4 I 13.1 58.8 h 2 2 NE01643 66.4 h 37.7 34.0 20.2 I 45.7 15.7 33.7 2 1 KY00C-2143-08 59.2 32.8 21.2 18.2 I 45.7 1 1.2 1 KY00C-2145-05 61.2 h 66.7 41.6 35.9 h <td>SE911492-4</td> <td>64.1</td> <td></td> <td>33.2</td> <td></td> <td>25.5</td> <td></td> <td>23.3</td> <td>ni</td> <td>44.2</td> <td></td> <td>9.0</td> <td>I</td> <td>34.2</td> <td>1</td> <td>3</td> <td>1</td>	SE911492-4	64.1		33.2		25.5		23.3	ni	44.2		9.0	I	34.2	1	3	1
SE98-1089-34 78.5 n 60.7 n 52.1 n 38.9 n 63.6 n 19.8 20.9 1 1 3 NE05418 52.0 l 26.3 19.7 24.5 h 41.5 l 8.3 l 27.7 l 5.6 h 14.0 31.3 l 2 3 NE05549 68.2 h 46.6 41.0 23.1 h 50.9 h 14.0 31.3 l 2 3 NE05537 54.8 36.7 24.7 22.3 l 41.5 l 15.7 33.7 l 2 1 NE05637 54.8 36.6 24.8 21 46.7 15.7 33.7 l 2 1 KY00C-2143-08 59.2 32.8 21.2 18.2 1 40.7 l 15.3 46.3 h 2 1 KY00C-2143-08 59.5 33.5 26.6 25.9 h 49.4 h 12.0 1 44.6 h 3	SE89-1873-2	65.5		39.4		31.9		23.6	nı	46.8		13.7		62.2	n	1	2
SE93-1094-8 67.2 h 39.9 32.8 31.7 h 53.6 h 19.7 24.5 hI 41.5 h 8.3 i 23.7 i 5 1 NE05549 68.2 h 46.6 41.0 23.1 hI 15.0 h 14.0 31.3 i 2 3 NE05537 54.8 36.7 24.7 22.3 i 41.5 i 15.7 33.7 1 2 3 NE05649 66.4 h 37.7 24.1 hI 40.7 i 15.7 33.7 2 1 KY00C-2059-16 58.0 36.6 24.8 21 i 40.7 i 15.3 46.3 h 2 1 KY00C-2755-03 61.2 39.0 30.1 23.9 hI 49.4 h 12.0 i 44.6 h 1 3 M04-4802 65.5 41.0 32.8 32.3 h 53.1 h 44.6 h 1 3 6 1	SE98-1089-34	78.5	n	60.7	h	52.1	h	38.9	n	63.5	h	20.7		40.7	h	0	6
NE05418 52.0 1 26.3 19.7 24.5 hl 41.5 l 8.3 l 23.7 l 5 1 NE05537 54.8 36.7 24.7 22.3 l 41.5 l 11.5 l 31.3 l 2 3 NE0537 54.8 36.7 27.3 24.1 hl 42.7 l 13.1 58.8 h 2 2 NE01643 66.4 h 37.7 34.0 20.2 l 45.7 l 15.7 33.7 l 2 1 KY00C-2059-16 58.0 36.6 24.8 21 l 41.2 l 8.9 l 39.5 h 3 1 2 1 KY00C-2143-08 59.2 32.8 21.2 18.2 141.2 l 8.9 l 39.5 h 3 1 2 46.5 h 0 4 4 46.6 h 1 3 1 46.4 1 3 30.5 1 1 2.6	SE93-1094-8	67.2	h	39.9		32.8		31.7	h	53.6	h	19.8		29.9	1	1	3
NE05549 68.2 h 46.6 41.0 23.1 hI 50.9 h 14.0 31.3 I 2 3 NE05537 54.8 36.7 24.7 22.3 I 41.5 I 11.5 I 32.2 I 4 0 NE05438 62.5 33.7 27.3 24.1 hI 42.4 I 13.1 58.8 h 2 2 NE01643 66.4 h 37.7 34.0 20.2 I 45.7 15.7 33.7 I 2 1 KY00C-2059-16 58.0 36.6 24.8 21 I 40.7 I 15.3 46.3 h 2 1 KY00C-2755-03 61.2 39.0 30.1 23.9 hI 49.1 h 12.6 36.5 1 2 3 1 49.5 h 0 4 4 6 1 3 1 1 3 1 3 1 2 1 1 3 1 2 1 1	NE05418	52.0	I	26.3		19.7		24.5	hl	41.5	I	8.3	I	23.7	I	5	1
NE05537 54.8 36.7 24.7 22.3 I 41.5 I 11.5 I 32.2 I 4 0 NE03488 62.5 33.7 27.3 24.1 hI 41.5 I 13.1 58.8 h 2 1 NE01643 66.4 h 37.7 34.0 20.2 45.7 15.7 33.7 I 2 1 KY00C-2059-16 58.0 36.6 24.8 21 I 40.7 I 15.3 46.3 h 2 1 KY00C-2755-03 61.2 39.0 30.1 23.9 hI 49.1 h 12.0 I 44.6 h 0 4 M04*5109 59.5 33.5 26.6 25.9 h 49.4 h 14.0 I 13.8 I 6 0 M04*5109 59.5 33.5 26.6 17.3 1 14.1.3 I 9.1 I 13.8 I 6 0 M03*3616-C10 51.9 1 25.4	NE05549	68.2	h	46.6		41.0		23.1	hl	50.9	h	14.0		31.3	T	2	3
NE03488 62.5 33.7 27.3 24.1 hI 42.4 I 13.1 58.8 h 2 2 NE01643 66.4 h 37.7 34.0 20.2 I 45.7 15.7 33.7 I 2 1 KY00C-2059-16 58.0 36.6 24.8 21 I 45.7 15.3 33.7 I 2 1 KY00C-2143-08 59.2 32.8 21.2 18.2 I 41.2 I 8.9 I 39.5 h 3 1 KY00C-0321-05-2 67.2 h 46.7 41.6 35.9 h 57.8 h 18.0 49.5 h 0 4 M04*5109 59.5 33.5 26.6 25.9 h 49.4 h 12.0 1 44.6 h 1 3 M03-3616-B11 53.4 26.6 19.3 I 19.4 41.3 19.1 1 13.8 I 62 1 7 1 OH02-13567 54.1 25.4 <td>NE05537</td> <td>54.8</td> <td></td> <td>36.7</td> <td></td> <td>24.7</td> <td></td> <td>22.3</td> <td>I.</td> <td>41.5</td> <td>I</td> <td>11.5</td> <td>I</td> <td>32.2</td> <td>T</td> <td>4</td> <td>0</td>	NE05537	54.8		36.7		24.7		22.3	I.	41.5	I	11.5	I	32.2	T	4	0
NE01643 66.4 h 37.7 34.0 20.2 I 45.7 15.7 33.7 I 2 1 KY00C-2059-16 58.0 36.6 24.8 21 I 40.7 I 15.3 46.3 h 2 1 KY00C-2143-08 59.2 32.8 21.2 18.2 I 41.2 I 8.9 I 39.5 h 3 1 KY00C-2755-03 61.2 39.0 30.1 23.9 h 49.1 h 12.6 39.5 h 3 1 2 45.5 h 0 4 M04*5109 59.5 33.5 26.6 25.9 h 49.4 h 12.0 1 44.5 h 0 3 M03-3616-C10 51.9 I 26.4 17.3 I 24.4 h 9.1 1 17.9 I 7 1 OH02-13567 54.1 I 28.7 18	NE03488	62.5		33.7		27.3		24.1	hl	42.4	I	13.1		58.8	h	2	2
KY00C-2059-16 58.0 36.6 24.8 21 I 40.7 I 15.3 46.3 h 2 1 KY00C-2143-08 59.2 32.8 21.2 18.2 I 41.2 I 8.9 I 39.5 h 3 1 KY00C-2143-08 59.2 32.8 21.2 18.2 I 41.2 I 8.9 I 39.5 h 3 1 KY00C-2755-03 61.2 39.0 30.1 23.9 hI 49.4 h 12.6 44.6 h 1 3 M04*5109 59.5 33.5 26.6 25.9 h 49.4 h 12.0 I 44.6 h 1 3 M03-3616-B11 53.4 I 26.6 19.3 I 19 I 41.3 I 10.7 I 17.9 I 7 1 OH02-13567 54.1 I 28.7 18.3 I 22.8 hI 42.2 I 15.5 I 2 2 O	NE01643	66.4	h	37.7		34.0		20.2	1	45.7		15.7		33.7	1	2	1
KY00C-2143-08 59.2 32.8 21.2 18.2 I 41.2 I 8.9 I 39.5 h 3 1 KY00C-2755-03 61.2 39.0 30.1 23.9 hI 49.1 h 12.6 36.4 I 2 KY97C-0321-05-2 67.2 h 46.7 41.6 35.9 h 57.8 h 18.0 49.5 h 0 4 M04*5109 59.5 33.5 26.6 25.9 h 49.4 h 12.0 1 44.6 h 1 3 M04-4802 65.5 41.0 32.8 32.3 h 53.1 h 14.5 45.5 h 0 3 M03-3616-C10 51.9 I 25.4 I 17.3 I 24.4 hI 49.2 I 8.2 I 6.2 I 6 1 0 0 3 1 2 2 2 2 0 0 13.8 24.5 I 2 1 0 1 1	KY00C-2059-16	58.0		36.6		24.8		21	I.	40.7	Т	15.3		46.3	h	2	1
KY00C-2755-03 61.2 39.0 30.1 23.9 h 49.1 h 12.6 36.4 1 2 KY97C-0321-05-2 67.2 h 46.7 41.6 35.9 h 57.8 h 18.0 49.5 h 0 4 M04*5109 59.5 33.5 26.6 25.9 h 49.4 h 12.0 I 44.6 h 1 3 M04*4802 65.5 41.0 32.8 32.3 h 53.1 h 12.0 I 44.6 h 0 3 M03-3616-B11 53.4 I 26.6 19.3 I 19.4 I 10.7 I 17.9 I 7 1 OH02-13667 54.1 I 28.7 18.3 I 22.8 hI 49.0 I 6.2 I 6.2 I 6.1 1 0 0 1 15.7 I 10.7 I 15.8 I 2 2 2 0 OH02-13667 54.4 I	KY00C-2143-08	59.2		32.8		21.2		18.2	T	41.2	Т	8.9	T	39.5	h	3	1
KY97C-0321-05-2 67.2 h 46.7 41.6 35.9 h 57.8 h 18.0 49.5 h 0 4 M04*5109 59.5 33.5 26.6 25.9 h 49.4 h 12.0 I 44.6 h 1 3 M04-4802 65.5 41.0 32.8 32.3 h 53.1 h 14.5 I 44.6 h 0 3 M03-3616-B11 53.4 I 26.6 19.3 I 19 I 41.3 I 9.1 I 13.8 I 6 0 M03-3616-C10 51.9 I 25.4 I 17.3 I 24.4 hI 39.1 I 10.7 I 13.8 I 6.2 I 6.2 I 6 1 OH02-12675 54.1 I 28.7 18.3 I 22.8 I 16.3 I 41.9 I 8.1 I 2.5 I 1 OH02-12678 54.4 I 27.0 <t< td=""><td>KY00C-2755-03</td><td>61.2</td><td></td><td>39.0</td><td></td><td>30.1</td><td></td><td>23.9</td><td>hl</td><td>49.1</td><td>h</td><td>12.6</td><td></td><td>36.4</td><td></td><td>1</td><td>2</td></t<>	KY00C-2755-03	61.2		39.0		30.1		23.9	hl	49.1	h	12.6		36.4		1	2
M04*5109 59.5 33.5 26.6 25.9 h 49.4 h 12.0 I 44.6 h 1 3 M04-4802 65.5 41.0 32.8 32.3 h 53.1 h 14.5 45.5 h 0 3 M03-3616-B11 53.4 I 26.6 19.3 I 19 I 41.3 I 9.1 I 13.8 I 6 0 M03-3616-C10 51.9 I 25.4 I 17.3 I 24.4 hI 39.1 I 10.7 I 17.9 I 7 1 OH02-13567 54.1 I 28.7 18.3 I 22.8 hI 42.2 I 8.2 I 6.2 I 6 1 OH02-12678 54.4 I 27.0 20.3 16.2 I 41.9 I 8.1 I 23.3 I 5 0 DH22	KY97C-0321-05-2	67.2	h	46.7		41.6		35.9	h	57.8	h	18.0		49.5	h	0	4
M04-4802 65.5 41.0 32.8 32.3 h 53.1 h 14.5 45.5 h 0 3 M03-3616-B11 53.4 1 26.6 19.3 1 19 1 13.8 1 17.9 1 13.8 1 6 0 M03-3616-C10 51.9 1 25.4 1 17.3 1 24.4 hl 39.1 1 10.7 1 17.9 1 7 1 OH02-13567 54.1 1 28.7 18.3 1 22.8 hl 42.2 1 8.2 1 6.2 1 6 1 OH02-13567 54.4 1 27.0 20.3 16.2 1 41.9 1 8.1 1 23.3 1 5 0 OH02-7217 59.4 28.3 21.2 16.3 1 39.6 1 11.5 1 4 0 DH 22/8 58.4 36.8 28.5 21.9 1 50.6 h 14.4 30.1 1 <td< td=""><td>M04*5109</td><td>59.5</td><td></td><td>33.5</td><td></td><td>26.6</td><td></td><td>25.9</td><td>h</td><td>49.4</td><td>h</td><td>12.0</td><td>1</td><td>44.6</td><td>h</td><td>1</td><td>3</td></td<>	M04*5109	59.5		33.5		26.6		25.9	h	49.4	h	12.0	1	44.6	h	1	3
M03-3616-B11 53.4 i 26.6 19.3 i 19 i 41.3 i 9.1 i 13.8 i 6 0 M03-3616-B11 51.9 i 25.4 i 17.3 i 24.4 hi 39.1 i 10.7 i 17.9 i 7 1 OH02-13567 54.1 i 28.7 18.3 i 22.8 hi 42.2 i 8.2 i 6.2 i 6 1 OH03-235-2 59.0 40.7 31.4 24.6 hi 50.3 h 13.8 24.5 i 2 2 OH02-12678 54.4 i 27.0 20.3 16.2 i 41.9 i 8.1 i 23.3 i 5 0 OH02-7217 59.4 28.3 21.2 16.3 39.6 i 11.4 30.1 i 2 1 DH 22/2 42.4 1 19.5 i 12.5 i 17 i 31.6 i 14	M04-4802	65.5		41.0		32.8		32.3	h	53.1	h	14.5	•	45.5	h	0	3
M03-3616-C10 51.9 1 25.4 1 17.3 1 24.4 hl 39.1 1 10.7 1 17.9 1 7 1 OH02-13567 54.1 1 28.7 18.3 1 22.8 hl 42.2 1 8.2 1 6.2 1 6 1 OH03-235-2 59.0 40.7 31.4 24.6 hl 50.3 h 13.8 24.5 1 2 2 OH02-12678 54.4 1 27.0 20.3 16.2 1 41.9 1 8.1 1 23.3 1 5 0 OH02-7217 59.4 28.3 21.2 16.3 1 39.6 1 11.5 1 12 1 DH 22/8 58.4 36.8 28.5 21.9 1 50.6 h 14.4 30.1 1 2 1 DH 22/24 42.4 1 19.5 1 12.5 1 17 1 31.6 1 7.8 1 9.0 1	M03-3616-B11	53.4	1	26.6		10.3	Т	19	1	41 3	1	9.1	1	13.8	1	6	0
Mido-Gold-Old 51.5 1 25.4 1 11.5 1 24.4 1 35.1 1 11.5 1 11.5 1 11.5 1 11.5 1 11.5 1 11.5 1 11.5 1 11.5 1 11.5 1 12.2 2 2 0 0 0.2 1 6.2 1 6 1 2 2 0 0 13.8 24.5 1 2 2 2 0 0 0 13.8 24.5 1 2 2 2 0 0 0 15.5 1 2 2 2 0 0 0 15.5 1 2 1 15.5 1 2 1 0 0 11.5 1 15.5 1 4 0 0 0 11.5 1 15.5 1	M03-3616-C10	51 0	÷	25.0		17.3	÷	24.4	, Ы	30.1	÷	10.7	÷	17.0	÷	7	1
OH02-13307 54.1 1 28.7 18.3 1 22.5 11 42.2 1 6.2 1 2.3 1 5 0 OH02-12678 54.4 1 27.0 20.3 16.2 1 41.9 1 8.1 1 2.3 1 5 0 OH02-7217 59.4 28.3 21.2 16.3 1 39.6 1 14.4 30.1 1 2 1 DH 22/8 58.4 36.8 28.5 21.9 1 50.6 h 14.4 30.1 1 2 3 DH 22/8 42.4 36.1 12		51.5	- <u>-</u> -	20.4		10.2	<u> </u>	27.7	ы	42.2	+	0.7		6.2		6	1
OH03-235-2 59.0 40.7 31.4 24.6 fill 50.3 fill 13.6 24.3 fill 2 2 OH02-12678 54.4 i 27.0 20.3 16.2 i 41.9 i 8.1 i 23.3 i 5 0 OH02-7217 59.4 28.3 21.2 16.3 i 39.6 i 11.5 i 15.5 i 4 0 DH 22/8 58.4 36.8 28.5 21.9 i 50.6 h 14.4 30.1 i 2 1 DH 22/24 42.4 i 19.5 i 12.5 i 17 i 31.6 i 7.8 i 9.0 i 7 0 DH 19/176B 67.4 h 42.4 36.1 23.6 hi 48.4 h 19.8 8.1 i 2 3 DH F/SF, 23 64.7 50.1 h 39.1 37.6 h 55.7 h 33.8 h 43.1 h 0	OH02-13307	54.1	1	20.7		10.3		22.0	111 61	42.Z	ו ה	12.0	1	0.2	-	0	2
OH02-12678 54.4 1 27.0 20.3 16.2 1 41.9 1 8.1 1 23.3 1 5 0 OH02-7217 59.4 28.3 21.2 16.3 1 39.6 1 11.5 1 15.5 1 4 0 DH 22/8 58.4 36.8 28.5 21.9 1 50.6 h 14.4 30.1 1 2 1 DH 22/8 58.4 1 19.5 1 12.5 1 17 1 31.6 1 7.8 1 9.0 1 7 0 DH 19/176B 67.4 h 42.4 36.1 23.6 hl 48.4 h 19.8 8.1 1 2 3 DH F/SF,23 64.7 50.1 h 39.1 37.6 h 55.7 h 33.8 h 43.1 h 0 5 IL02-18228 41.9 1 15.3 1 13.0 1 10.7 1 28.1 1 16.3 1	OH03-235-2	59.0		40.7		31.4		24.0		50.5	n	13.0		24.5	-	2	2
OH02-7217 59.4 28.3 21.2 16.3 1 39.6 1 11.5 1 15.5 1 4 0 DH 22/8 58.4 36.8 28.5 21.9 1 50.6 h 14.4 30.1 1 2 1 DH 22/24 42.4 1 19.5 1 12.5 1 17 1 31.6 1 7.8 1 9.0 1 7 0 DH 19/176B 67.4 h 42.4 36.1 23.6 hl 48.4 h 19.8 8.1 1 2 3 DH F/SF,23 64.7 50.1 h 39.1 37.6 h 55.7 h 33.8 h 43.1 h 0 5 IL02-18228 41.9 1 15.3 1 13.0 1 10.7 1 28.1 1 5.5 1 24.1 1 7 0 IL02-18228 41.9 1 15.3 1 10.5 1 35.7 1 8.2 1	OH02-12678	54.4	1	27.0		20.3		16.2		41.9		8.1		23.3		5	0
DH 22/8 58.4 36.8 28.5 21.9 I 50.6 h 14.4 30.1 I 2 1 DH 22/24 42.4 I 19.5 I 12.5 I 17 I 31.6 I 7.8 I 9.0 I 7 0 DH 19/176B 67.4 h 42.4 36.1 23.6 hI 48.4 h 19.8 8.1 I 2 3 DH F/SF, 23 64.7 50.1 h 39.1 37.6 h 55.7 h 33.8 h 43.1 h 0 5 IL02-18228 41.9 I 15.3 I 13.0 I 10.7 28.1 I 5.5 I 24.1 I 7 0 IL02-18228 41.9 I 15.3 I 13.0 I 10.7 I 28.1 I 16.3 I 4 0 IL04-10118 55.9 24.7 I 18.9 I 10.5 I 38.8 I 9.8	OH02-7217	59.4		28.3		21.2		16.3	1	39.6		11.5		15.5		4	0
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	DH 22/8	58.4		36.8		28.5		21.9	I	50.6	h	14.4		30.1	I	2	1
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	DH 22/24	42.4	I	19.5	I	12.5	I	17	I	31.6	I	7.8	I	9.0	I	7	0
DH F/SF, 23 64.7 50.1 h 39.1 37.6 h 55.7 h 33.8 h 43.1 h 0 5 IL02-18228 41.9 I 15.3 I 13.0 I 10.7 I 28.1 I 5.5 I 24.1 I 7 0 IL02-19463 57.4 31.9 22.0 12.8 I 35.9 I 7.7 I 16.3 I 4 0 IL04-10118 55.9 24.7 I 18.9 I 10.5 I 35.7 I 8.2 I 14.5 I 6 0 IL04-10721 61.1 28.1 21.0 15 I 38.8 I 9.8 I 7.6 I 4 0 IL04-10721 61.1 28.1 21.0 15 I 38.5 I 7.0 I 29.8 I 7 0 AVERAGE 59.4 34.0 26.2 22.3 44.2 11.6 23.9 I I I </td <td>DH 19/176B</td> <td>67.4</td> <td>h</td> <td>42.4</td> <td></td> <td>36.1</td> <td></td> <td>23.6</td> <td>hl</td> <td>48.4</td> <td>h</td> <td>19.8</td> <td></td> <td>8.1</td> <td>I</td> <td>2</td> <td>3</td>	DH 19/176B	67.4	h	42.4		36.1		23.6	hl	48.4	h	19.8		8.1	I	2	3
IL02-18228 41.9 I 15.3 I 13.0 I 10.7 I 28.1 I 5.5 I 24.1 I 7 0 IL02-19463 57.4 31.9 22.0 12.8 I 35.9 I 7.7 I 16.3 I 4 0 IL04-10118 55.9 24.7 I 18.9 I 10.5 I 35.7 I 8.2 I 14.5 I 6 0 IL04-10721 61.1 28.1 21.0 15 I 38.8 I 9.8 I 7.6 I 4 0 IL04-10741 48.7 24.1 1 18.9 I 21.6 I 38.5 I 7.0 I 29.8 I 7 0 AVERAGE 59.4 34.0 26.2 22.3 44.2 11.6 23.9 I I I I I I I I I I I I I I I I I I	DH F/SF, 23	64.7		50.1	h	39.1		37.6	h	55.7	h	33.8	h	43.1	h	0	5
IL02-19463 57.4 31.9 22.0 12.8 1 35.9 1 7.7 1 16.3 1 4 0 IL04-10118 55.9 24.7 1 18.9 1 10.5 1 35.7 1 8.2 1 14.5 1 6 0 IL04-10721 61.1 28.1 21.0 15 1 38.8 1 9.8 1 7.6 1 4 0 IL04-10721 61.1 28.1 21.0 15 1 38.8 1 9.8 1 7.6 1 4 0 IL04-10741 48.7 24.1 1 18.9 1 21.6 38.5 1 7.0 1 29.8 1 7 0 AVERAGE 59.4 34.0 26.2 22.3 44.2 11.6 23.9 - </td <td>IL02-18228</td> <td>41.9</td> <td>1</td> <td>15.3</td> <td>1</td> <td>13.0</td> <td>1</td> <td>10.7</td> <td>1</td> <td>28.1</td> <td>- I</td> <td>5.5</td> <td>1</td> <td>24.1</td> <td>1</td> <td>7</td> <td>0</td>	IL02-18228	41.9	1	15.3	1	13.0	1	10.7	1	28.1	- I	5.5	1	24.1	1	7	0
IL04-10118 55.9 24.7 I 18.9 I 10.5 I 35.7 I 8.2 I 14.5 I 6 0 IL04-10721 61.1 28.1 21.0 15 I 38.8 I 9.8 I 7.6 I 4 0 IL04-10741 48.7 I 24.1 I 18.9 I 21.6 I 38.5 I 7.0 I 29.8 I 7 0 AVERAGE 59.4 34.0 26.2 22.3 44.2 11.6 23.9 I	IL02-19463	57.4		31.9		22.0		12.8	I.	35.9	Т	7.7	T	16.3	I.	4	0
IL04-10721 61.1 28.1 21.0 15 I 38.8 I 9.8 I 7.6 I 4 0 IL04-10741 48.7 I 24.1 I 18.9 I 21.6 I 38.5 I 7.0 I 29.8 I 7 0 AVERAGE 59.4 34.0 26.2 22.3 44.2 11.6 23.9 I I 1	IL04-10118	55.9		24.7	Т	18.9	Т	10.5	Т	35.7	Ι	8.2	T	14.5	T	6	0
IL04-10741 48.7 I 24.1 I 18.9 I 21.6 I 38.5 I 7.0 I 29.8 I 7 0 AVERAGE 59.4 34.0 26.2 22.3 44.2 11.6 23.9 I 7 0 LSD(0.05) 12.8 10.6 9.0 16.3 15.5 7.3 31.0 I # Environments 10.0 11.0 12.0 5 4.0 7 2.0 I	IL04-10721	61.1		28.1		21.0		15	Т	38.8	I	9.8	T	7.6	T	4	0
AVERAGE 59.4 34.0 26.2 22.3 44.2 11.6 23.9 LSD(0.05) 12.8 10.6 9.0 16.3 15.5 7.3 31.0 # Environments 10.0 11.0 12.0 5 4.0 7 2.0	IL04-10741	48.7	I	24.1	I	18.9	T	21.6	I	38.5	I	7.0	I	29.8	I	7	0
LSD(0.05) 12.8 10.6 9.0 16.3 15.5 7.3 31.0 # Environments 10.0 11.0 12.0 5 4.0 7 2.0		59.4	•	34 0	•	26.2		22.3	•	44.2	•	11.6	•	23.9	•		
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π Environments 10.0 11.0 12.0 0 4.0 1 2.0	# Environmente	10.0		11.0		12.0		5		⊿ ∩		7.5		20			
in indicate a mean that is not significantly unreferr than the lowest (1) or highest (n) mean in that column	1.h indicate a mean that is	s not sig	nifica	ntly diff	eren	t than the	e low	vest (1) or	high:	$\frac{-1.0}{\text{est (h) n}}$	nean	in that co	olum	1			

Table 6. Summary of results of the 2007-08 NUWWSN.

TEN YEARS OF UNIFORM FHB TESTING OF SOFT WINTER WHEAT FROM THE NORTHERN U.S. C. Sneller^{1*}, P. Paul² and M. Guttieri¹

¹Dept. of Horticulture and Crop Science, and ²Dept. Plant Pathology, The Ohio State University, Wooster, Ohio 44691 *Corresponding Author: PH: (330)263-3944; E-mail: sneller.5@osu.edu

OBJECTIVES

Soft winter wheat germplasm adapted to the northern part of the eastern US has been evaluated for resistance to Fusarium Head Blight (FHB) in two uniform trials: the Northern Uniform Winter Wheat Nursery (NUWWSN) and a preliminary version (PNUWWSN). Each breeder has different criteria objectives for the placing entries in the tests. Most entries are candidates for release as cultivars while others are being considered for release to meet other objectives. Some entries have gone through prior selection for FHB resistance while others have not. Our objectives were to summarize FHB testing from 1998 to 2007 and to assess trends over years.

MATERIALS AND METHODS

Each year the test are sent to multiple cooperators. Most grow the tests in inoculated nurseries and collect data on multiple traits. The number of locations reporting data on each trait varies by year and disease severity varies by location and year. From 1998-2007, three checks have been grown at each location and each year: Ernie (MR), Freedom (MR) and Pioneer 2545 (S). We analyzed incidence (INC, % of heads with at least one symptomatic spikelet), severity (SEV, % of symptomatic spikelets on heads showing symptoms), Index (IND, % = (INC*SEV)/100), deoxynivalenol content (DON, ppm), and percentage of seeds showing symptoms (FDK, %).

We standardized the trait value of the ith genotype in the jth environment (Y_{ij}) relative to the mean of the MR checks $(Y'_{ij}=Y_{ij}-(mean of Ernie and Freedom))$ to adjust for year and location effects. A second standardized trait value was obtained by dividing Y'_{ij} by the standard deviation from a particular environment

and test $(Y''_{ij}=Y'_{ij}/stdev of jth environment and test)$: Y, Y', and Y'' are the means of Y_{ii} , Y'_{ii} , Y'_{ii} , for a genotype over all environments. We obtained a best linear unbiased prediction (BLUP) of Y, Y', and Y" for each genotype using a mixed model where test, year, and location were considered fixed effects and genotype was considered random. The precision of the estimate of each effect varies as the number of testing environments varied by genotype. The variation in precision is incorporated in the BLUPs. For example, say genotypes A and B have equal means, but A was tested for one year while B was tested for five years. The value of A will regress towards the mean more than the value of B when obtaining BLUPs so their BLUPs will not be equal. The average BLUP for all genotypes in an analysis will be zero. To evaluate trends over years we regressed Y, Y', Y" and the BLUPs of each for all non-check genotype tested in a year on year of testing.

A principal component analysis using the correlation matrix among traits was conducted using BLUPS of Y' for all traits and all 462 genotypes.

RESULTS

Over the ten years, 462 unique genotypes were evaluated for FHB resistance. Significant genetic variation was found for each trait using Y, Y', or Y". There was a significant positive correlation among all traits for Y with the strongest correlations being among the three spike traits (INC, SEV, IND: all r > 0.7) and the weakest correlations being between the spike traits and DON (all r < 0.47). The principal component analysis of the BLUPs of Y' captured 64% of the variation in PC1 and 13% in PC2 (Fig. 1). PC1 modeled variation from all traits, but was dominated by the spike traits and FDK. PC2 primarily modeled a component of DON that is independent of the first axis. The BLUPs of Y' for the genotypes with low PC1 and PC2 scores are shown in Table 1. Table 1 also has BLUPs of Y' for several other genotypes that had either low IND or low DON values. Only four genotypes were among the best 31 genotypes for IND, DON, FDK, and GH: IL97-6755, MO980829, Bess, NY87048W-7388, and OH904 (Table 1).

Using BLUPs of Y', genotypes that were superior to Truman were rare for except DON where 16.3% of the genotypes had lower DON values than Truman (Figs. 2 and 3). Genotype worse that the susceptible check Pioneer 2545 were also rare. More than 28% of all genotypes were superior to Freedom for all traits except GH.

There was no significant linear trend for increased resistance over time for any trait or measure (Y, Y', Y", or their BLUPs) except for Y" for Index (Table 2, Fig, 4). The greatest or second greatest Y' for all traits occurred in 2002 and this may have prevented finding a significant linear trend from 1998-2007 (Fig. 5). Performing regression using data from 2002 to 1998 decreased the slope of nearly all measures and traits and produced significant slopes for DON (Y), GH (Y', Y") and INC (Y") (Table 2). A trend for improved resistance from 2002-2007 was suggested for all traits.

DISCUSSION

A significant number of genotypes in the NUWWSN and PNUWWSN displayed a high level of FHB resistance as assessed by multiple traits. In general, resistance that is superior to that of Freedom was fairly common, while strong moderate resistance such as that displayed by Truman was rare. Seventeen (41%) of the 41 non-check genotypes in Table 1 have an exotic source of FHB resistance in their pedigree. This percentage is likely much higher than would be found among all 462 entries. Of the 10 best genotypes for IND, five had exotic parentage and putatively have some Asian QTL alleles that are known to improve IND (Table 1). Two of the10 best for DON had exotic parentage (Table1). The use of native or exotic parentage can lead to strong moderate resistance.

The data and tests are not well suited to investigate the effect of selection over time. The entries come from multiple breeders, each using different populations, different FHB selection pressure prior to submission, and have different objectives they are trying to attain with their entries. Entries are not necessarily the most FHB resistance material from each breeder. Rather they are their genotype most likely to meet their individual objectives such as improved yield, quality, or resistance to other diseases, as well as resistance to FHB. Despite these issues that would minimize directional selection for FHB resistance, trends for increased resistance for all traits over time were evident. This was most notable for IND from 1998 to 2007, and for DON and GH from 2002-2007.

	NAME		IND		INC		SEV		DON		FDK		GH
PCA	*0128A1-36	194	-1.6	84	-4.6	57	-5.5	64	-2.6	17	-9.8	92	-8.1
PCA	*01931A1-5	134	-3.0	149	-2.6	53	-5.6	46	-3.0	11	-11.0	66	-10.0
PCA	*97395B1-4-2-7	240	-0.1	202	-0.5	118	-3.4	12	-4.5	47	-6.7	155	-4.8
PCA	*97417A1-3-4	83	-4.3	138	-3.1	111	-3.8	13	-4.3	96	-4.7	108	-7.0
PCA	9793A1-5	51	-5.3	69	-5.3	84	-4.4	24	-3.8	43	-6.9	201	-2.2
PCA	HONDO	7	-8.7	83	-4.7	16	-7.6	18	-4.1	113	-4.2	134	-5.8
PCA	IL00-8061	25	-6.6	18	-9.8	24	-7.0	33	-3.3	3	-13.0	84	-8.6
PCA	IL00-8530	210	-1.1	188	-1.0	188	-1.4	52	-2.9	9	-11.2	93	-8.1
PCA	IL01-11934	162	-2.4	53	-6.2	127	-3.1	77	-2.3	31	-8.0	102	-7.7
PCA	*IL01-34159	49	-5.4	72	-5.1	80	-4.6	71	-2.4	32	-8.0	31	-12.0
PCA	IL01-5943	43	-5.6	76	-4.9	38	-6.1	67	-2.6	58	-6.3	123	-6.4
PCA	IL02-7735	59	-4.9	27	-8.8	104	-4.0	117	-1.6	40	-7.2	295	4.4
PCA	IL95-4162	93	-3.9	23	-9.2	150	-2.3	60	-2.7	29	-8.1	138	-5.7
PCA	*IL96-24851-1	19	-6.8	100	-4.0	29	-6.5	14	-4.2	119	-4.0	18	-14.0
PCA	IL96-3073	24	-6.6	9	-12.2	43	-6.0	19	-4.1	15	-10.2	44	-11.3
PCA	IL96-6472	189	-1.6	90	-4.3	203	-1.0	7	-5.4	6	-11.7	110	-6.8
PCA	IL97-1828	16	-7.2	16	-9.9	17	-7.6	3	-6.9	7	-11.7	167	-4.1
PCA	IL97-2945	39	-5.8	11	-11.3	148	-2.3	25	-3.8	19	-9.2	189	-2.9
PCA	IL97-4228	228	-0.3	48	-6.4	262	0.7	11	-4.5	74	-5.7	98	-7.8
PCA	*3 IL97-6755	4	-9.6	6	-14.1	1	-13.0	2	-7.3	4	-13.0	13	-14.8
PCA	IL99-20756	173	-2.1	117	-3.5	109	-3.9	101	-1.9	1	-14.3	49	-10.9
PCA	IL99-27048	94	-3.9	55	-6.1	64	-5.2	27	-3.6	10	-11.2	135	-5.8
PCA	MO980829	1	-11.8	10	-11.6	11	-8.7	5	-6.3	28	-8.1	3	-17.4
PCA	Bess = MO981020	12	-8.2	25	-8.9	13	-7.9	10	-4.9	20	-9.0	7	-15.8
PCA	*3,6 NY87048W-7388	10	-8.4	42	-6.8	9	-9.0	31	-3.4	12	-10.9	14	-14.8
PCA	NY89064SP-7139	18	-6.8	87	-4.5	205	-1.0	1	-8.2	55	-6.4	141	-5.5
PCA	*2,3,5 OH902	23	-6.6	14	-10.3	128	-3.1	49	-2.9	42	-6.9	60	-10.3
PCA	*2,3,5 OH903	5	-8.9	2	-17.4	3	-10.1	23	-3.8	24	-8.8	130	-5.8
PCA	*2,3,5 OH904	6	-8.8	4	-16.3	7	-9.1	16	-4.1	25	-8.6	17	-14.2
PCA	*VA02W708	15	-7.7	46	-6.5	51	-5.7	15	-4.1	16	-10.2	80	-8.9
PCA	*3 VA04W-563	193	-1.6	141	-3.0	166	-1.9	37	-3.2	13	-10.6	53	-10.7
PCA	*VA05W-417	92	-3.9	62	-5.7	85	-4.4	59	-2.7	81	-5.3	70	-9.8
IND	89118RC1-X-9-3-3	11	-8.4	109	-3.7	25	-6.9	222	-0.7	78	-5.5		
IND	*981359C1-4	8	-8.6	130	-3.3	74	-4.8	232	-0.6	5	-11.7	121	-6.5
IND	*2,3,5 E6003	2	-10.5	1	-18.9	6	-9.3	213	-0.7	23	-8.8	39	-11.6
IND	MO011174	14	-7.9	37	-7.3	66	-5.1	53	-2.8	51	-6.5	16	-14.5
IND	OH618	9	-8.5	47	-6.5	8	-9.1	181	-1.0	138	-3.4		
DON	IL96-3514	272	0.7	147	-2.6	274	0.9	8	-5.1	65	-6.2	150	-5.0
DON	IL98-6718	243	-0.1	215	0.0	218	-0.7	4	-6.8	50	-6.5	299	4.5
DON	NY89082-7159	152	-2.6	213	-0.1	323	2.6	9	-5.0	201	-0.8	341	8.8
DON	*VA02W694	291	1.1	64	-5.6	191	-1.3	6	-6.2	177	-2.1	388	14.1
	TRUMAN	3	-10.0	7	-13.6	2	-11.9	75	-2.3	14	-10.3	1	-18.8
	ERNIE	68	-4.6	49	-6.4	32	-6.4	265	-0.2	48	-6.7	65	-10.1
	FREEDOM	138	-2.9	285	1.6	129	-3.1	228	-0.6	331	3.1	34	-11.8
	PIO2545	457	11 4	461	12.9	459	11 7	437	54	461	15.6	359	11.2

Table 1. BLUPs of Y' values and rank of the best genotypes based on principal component analysis (PCA, Fig. 1) or ranking by Index (IND) or DON values.

* indicates genotype with parentage from exotic sources of FHB resistance. 2, 3, 5, 6 indicate a genotype that likely has an exotic FHB resistance allele for QTL on 2DL, 3BS, 5AS, or 6BS based on haplotype.

	All Year	Ś		2002-2007					
	Y	Y'	Y"	Y	Y'	Y"			
INC	0.19	026	0.10	-0.95	-0.96	-0.08*			
SEV	-0.29	-0.64	-0.05	-2.25	-1.81	-0.15			
IND	-0.19	-0.32	-0.04*	-1.55	-0.16	-0.03			
FDK	-0.45	0.62	0.08	-1.86	-0.42	-0.05			
DON	-0.36	-0.01	0.02	-2.43*	-0.68	-0.07			
GH	-0.98	-0.38	-0.02	-3.10	-1.92*	-0.11*			

Table 2. Regression coefficients (*b*) from regressing mean trait values (Y, Y', Y") of all non-check genotypes tested in a year on the year of test.



Figure 1. First two principal components from analysis of 462 genotypes and six traits. T (Truman), E (Ernie) ,F (Freedom), and P (PIO 2545) indicate position of checks.



Figure 2. Distribution of the BLUPs of standardized Index values (Y') of all genotypes tested from 1998 to 2007.



Figure 3. Distribution of the BLUPs of standardized DON values (Y') of all genotypes tested from 1998 to 2007.



Figure 4. Regression of mean Y" of Index for non-check genotypes on year of test.



Figure 5. Mean Y' for each trait of non-check genotypes for each year.

WHEAT QUALITY EVALUATION OF FUSARIUM HEAD BLIGHT (FUSARIUM GRAMINEARUM) RESISTANT SOFT WHEATS AND THE EFFECT OF FUNGICIDE MANAGEMENT ON WHEAT QUALITY. E. Souza^{1*}, C. Sneller², P. Paul², L. Sweets³ and M.J. Guttieri²

¹USDA-ARS, Wooster, OH; ²Ohio State University, Wooster, OH; and ³University of Missouri, Columbia, MO ^{*}Corresponding Author: PH: (330) 263-3891; E-mail: edward.souza@ars.usda.gov

ABSTRACT

Wheat quality was evaluated for Fusarium head blight resistant germplasm entered into regional FHB nurseries. A sub-set of lines were identified in the evaluation that had good soft wheat quality and significant resistance to Fusarium head blight. Results will be summarized in the poster. In a second study, wheat quality was evaluated for Fusarium head blight resistant cultivars and for the effect of fungicide vs. non-fungicide treatments on soft white wheats. To test the effect of fungicide treatment, milling and baking quality were compared in wheat grown at four locations in paired plots of five varieties with or without fungicide treatment applied at flowering. Contrary to previous European studies, we found that the application of fungicide did not change grain falling number values and had no significant implication in quality. Treatment with Prosaro reduced the FHB index by approximately 40%. Yet, even for susceptible varieties, the fungicide did not significantly improve milling and baking quality. We also tested the historical relationship between FHB resistance and quality. An examination of FHB resistance scores publicized by cooperating researchers against quality data generated at the USDA-ARS Soft wheat Quality Lab and compiled for 15 trials across the eastern US and comprising 377 entries, revealed no consistent trend between resistance and quality. The best sources of FHB resistance, such as Bess and Truman, are moderate in quality. Using inoculated trials, we also evaluated FHB resistance against milling and baking quality for matched uninoculated plots among 38 commercial cultivars in Wooster in 2007. In these trials at Wooster, several lines with very good quality were found to have moderate FHB resistance. AGI 401 followed by SC 1348 and SC 1358 fit these criteria and may be good choices for managing FHB infection while maintaining good soft wheat quality.

INTO THE WILD: FHB RESISTANCE IDENTIFIED IN HORDEUM VULGARE SUBSP. SPONTANEUM. B.J. Steffenson^{*} and S.K. Dahl

Department of Plant Pathology, University of Minnesota, St. Paul, MN *Corresponding Author: PH: (612) 625-4735; E-mail: bsteffen@umn.edu

ABSTRACT

Fusarium head blight (FHB), caused by Fusarium graminearum, has devastated the malting barley industry in the Upper Midwest. The deployment of cultivars with resistance to F. graminearum and its associated mycotoxins (i.e. deoxynivalenol or DON) is the best means for combating the disease. Extensive evaluations of cultivated barley germplasm have identified only a few sources of partial resistance to FHB. The objective of this study was to expand the search for FHB resistance in the wild progenitor of cultivated barley, Hordeum vulgare subsp. spontaneum. An ecogeographically diverse collection of wild barley accessions (1,770 in total), primarily from the Fertile Crescent but also Central Asia and North Africa, was evaluated for reaction to FHB in screening nurseries at Zhejiang University in Hangzhou, China from 2003-2008. Accessions were planted in late October to early November, inoculated in March, and scored for FHB severity in May. Inoculations were performed using the "grain-spawn" method, and the nurseries were irrigated daily to promote infection. At the mid-dough stage, disease assessments were made on plants using a 1-5 scale, where 1 is most resistant and 5 is most susceptible. Of the 1,770 accessions tested, only 20 (1.1%) exhibited a resistance level comparable to Chevron, the six-rowed resistant control. Eleven of the 20 accessions were from Israel, suggesting that this country may be a center of concentration for FHB resistance. Other resistant accessions originated from Iran, Iraq, Syria, Jordan, and Azerbaijan. One of the most resistant accessions found (PI 466423) comes from Israel near the Jordan River and has a distinct morphology (a petite spike) compared to other wild barley accessions. In replicated tests conducted in Hangzhou, China in 2005-2007, PI 466423 exhibited FHB severities that were slightly lower (1.4 vs. 1.7) than Chevron. Being a unique wild barley accession, PI 466423 likely possesses alleles for FHB resistance that have not yet been exploited in breeding programs. Our ultimate goal is to reduce the losses caused by FHB, including quality discounts due to DON contamination. This can be best achieved by developing barley cultivars with the highest level of resistance possible. The next objectives for this project are to determine the number and chromosomal position of FHB resistance loci in PI 466423 and transfer them as quickly as possible into cultivated barley. This is now being done using the "advanced backcross QTL" method. The information generated from this study will lead to the development of malting barley cultivars with enhanced FHB resistance and low DON accumulation.

ACKNOWLEDGEMENT AND DISCLAIMER

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AN UPDATE ON THE DEVELOPMENT OF FUSARIUM HEAD BLIGHT (FHB) RESISTANT WHEAT GERMPLASM WITH LOWER DEOXYNIVALENOL (DON) ACCUMULATION AT THE UNIVERSITY OF GUELPH, ONTARIO, CANADA. L.Tamburic-Ilincic^{1*}, D.E. Falk² and A.W. Schaafsma¹

¹Ridgetown Campus, University of Guelph, 120 Main St. E., Ridgetown, Ontario, N0P 2C0, Canada; and ²Department of Plant Agriculture, University of Guelph, 50 Stone Road. E., Guelph, Ontario, N1G 2W1, Canada
*Corresponding Author: PH: (519) 674-1557; E-mail: ltamburi@ridgetownc.uoguelph.ca

INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schwabe), is an important wheat disease. Deoxynivalenol (DON) is the most frequent mycotoxin in wheat grain in Canada produced by *F. graminearum*. Different types of FHB resistance have been reported in wheat, including type I and II resistance (resistance to initial infection and spread of symptoms within the spike, respectively) and type III and IV resistance (resistance and tolerance mechanisms to trichothecenes mycotoxins including DON). The disease is strongly influenced by the environment; multiple locations screening with reliable checks, is needed to identify new FHB resistant breeding material.

MATERIALS AND METHODS

The wheat breeding program at the University of Guelph has participated in the collaborative Northern Uniform Winter Wheat Scab Nursery (NUWWSN) and Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN) with other breeders from USA for the past five and two years, respectively. Each partner has been contributing up to six wheat lines which are tested for different types of *Fusarium* resistance across all locations every year. The winter wheat lines were inoculated in the field with *F. graminearum* at anthesis, and rated two-three weeks later for visual symptoms of FHB infection (FHB index (%)= severity x incidence/100). Number of locations ranged from eight to fourteen from 2002 to 2007. Severity of wheat heads, point-inoculated with

F. graminearum at anthesis in the greenhouse (GH), was also rated for each line included in the test. In mature grain, the DON content and percent of scabby seed (PSS) was estimated from each line from several locations each year. In addition, ISK index- % (resistance based on incidence, severity and percent of scabby seed) was calculated from each line from 2003 to 2007.

RESULTS

- Some lines showed low levels for *Fusarium* head traits recorded, but high for *Fusarium* kernel traits recorded and *vice versa*.
- In 2002-2003, our line RCATL33 was rated amongst some of the most resistant entries in the test (registered as germplasm-Crop Sci. 2006. 46:1399-1400), (Table 1).
- In 2004-2005, line RCAT31 was our most *Fusarium* resistant line tested (Table 1).
- In the 2005-2006 NUWWSN test, RCAT TF203/ 2 was among the best entries for Fusarium resistance (Table 1), in addition to excellent soft wheat quality traits (<u>http://www.scabusa.org</u> -NUWWSN Reports).
- In 2005-2006, line RCATTF 174/1C was among the best entries for all traits recorded across all locations in PNUWWSN test (Table 2).

- In 2006-2007, lines RCUOGF110202D/4 and RCUOGDHACF1109O2D were among the best entries for all traits recorded across all locations in NUWWSN test. Line RCUOGF110202D/4 had low mean DON level of 1.7 ppm (Table 1).
- In 2006-2007, line RCUOG10/18 had the lowest DON level (1.3 ppm- Table 2) among all lines developed in our program to date and was 1 out of 3 lines in PNUWWSN test with DON level <2.0 ppm.

CONCLUSIONS

- The results showed that is necessary to select for all types of FHB Resistance simultaneously.
- Multiple locations tests have been very beneficial for all participants.
- Different sources of FHB resistance have been used in our Breeding program with a goal to pyra-

mid FHB resistance to type I, Il, III and IV in single cultivars.

- Our long term objective is to release winter wheat cultivars, adapted to Ontario, with improved FHB resistance, quality, and yield.

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Year	Line	Source	FHB	GH	DON	PSS	ISK
		of resistance	index	severity	(ppm)	(%)	index %)
			(%)	(%)			
2002-200	3		10 locat.*	5 locat.	4 locat.	4 locat.	
	RCATL33	Frontana	14.6 L**	46.2	13.2 L	39.3	
		+Sumai 3					
	RCATL10	SVP72017-17-5	-27.6	34.7 L	16.3	48.9	
		10-1					
	RCATL13	Frontana	36.0 H**	65.9 H	29.6 H	51.5 H	
	RCATTF19/26	EX9806	25.4	51.9	8.9 L	50.4 H	
	RCATTF2/4	EX9806	19.3 L	56.2 H	14.1 L	49.1	
	RCATTF17/34		39.3 H	61.2 H	28.3 H	53.8 H	
	ERNIE-MR check		19.0 L	27.6 L	17.3	36.3 L	
	FREEDOM-MR check		21.6	37.8 L	14.6	49.9 H	
	PIONEER 2545-S check		34.4 H	43.5	21.2 H	58.6 H	
2003-200	4 Line		14 locat.	4 locat.	6 locat.	5 locat.	5 locat.
	RCATL33	Frontana	23.0	31.2	4.4 L	28.5	25.0
		+Sumai 3					
	RCATL10	SVP72017-17-5	-26.5	51.0 H	11.5	43.0 H	32.7
		10-1					
	RCAT 24	Ena + Frontana	16.2 L	33.9	10.6	30.5	16.4 L
	RCATL12	AC Morley	24.5	26.1	6.7	44.5 H	27.5
	RCATL2	Frontana	28.5	23.6 L	5.4 L	25.1 L	24.0
	ERNIE		24.4	13.9 L	4.4 L	18.6 L	18.6 L
	TRUMAN-MR/R check		13.0 L	8.2 L	3.9 L	22.6 L	14.0 L
	FREEDOM		26.3	12.2 L	4.6 L	35.9	32.2
2004 200	PIONEER 2545		43.5 H	32.7	11.1	48.2 H	42.0 H
2004-200	5 Line	F (11 locat.	3 locat.	4 locat.	3 locat.	4 locat.
	RCAT 13/18	Frontana	23.6	57.9 H	8.9 L	27.6	48.0 H
	RCAT 23/1	Ena	19.2	24.2 L	9.2 L	22.6 L	44.9
	RCAT 29	Ena + Frontana	21.7	12.1 L	1/.0 H	20.1 L	40.1
	RCAT 28	Frontana	22.3	33./ 25.1 I	11.4 L	36.1 H	48.3 H
	RCAT 31	Frontana + $SVD72017, 17.5$	14.2 L	25.1 L	2.7 L	20.3 L	35.0
		SVP/201/-1/-3	-				
	EDNIE	10-1	11 2 I	10 5 I	621	22.0	21.4 I
			11.5 L	12.3 L	0.2 L 2 7 I	23.9	51.4 L 25.0 I
	EDEEDOM		9.9 L 16 1	9.0 L 17 2 I	2.7 L 6 1 I	17.4 L 20.7	23.9 L 40.3
	PIONEEP 2545		10.1 32.5 H	17.2 L 27.4 I	0.1 L 11 3	29.7 41.0 H	40.3 50.2 H
2005 200	6 Line		13 locat	27.4 L 3 locat	7 locat	41.011 4 locat	1 locat
2003-200	0 Elle		15 locat.	5 10cat.	/ 10cat.	4 Iocat.	4 Iocat.
	RCAT 202D/ 1	Freedom	21.3	22.5	7.0	15 O I	42.5
	RCAT 32/157	Frontana	21.5 14 7 I	22.5 55.0 H	7.0	15.0 L 25.0	31.61
	Kerri 52/15/	+Sumai 3	14.7 L	55.011	1.5	25.0	51.0 L
	RCATTF 203/2	Sumai 3	14 9 I	15.8	54	821	27 4 I
	RCAT19/4c	AC Morley	14.9 L 14 8 I	45.5	5.4 5.0	24.8	27.4 L 31.0 I
		+Sumai 3	17.0 L	тэ.э	5.0	27.0	51.0 L
	FRNIE	- Sumar S	20.2	24.3	5 5	74L	2541
	TRUMAN		12.4 L	3.3 L	4.5	5.2 L	24.1 L
	FREEDOM		16.4 L	13.3 L	7.0	17.8 L	38.4
	PIONEER 2545		31.9 H	38.2	10.4 H	44.0 H	57.9 H

Table 1. NUWWSN test-average level for *Fusarium* traits recorded across all locations for RCAT/ RCUOG lines and check entries (2002-2007).

Table 1	(cont).						
Year	Line	Source of resistance	FHB Index (%)	GH severity (%)	DON (ppm)	PSS (%)	ISK index %)
2006-20	07 Line		13 locat.	2 locat.	2 locat.	5 locat.	5 locat.
	RCUOG19/21	Sumai 3	15.8 L	31.2 L	7.8	14.2 L	29.5
	RCUOGF110202D/4	SD07060 + R. Star	14.7 L	10.7 L	1.7 L	11.1 L	23.5 L
	RCUOGF111202A/3	Freedom	21.9	25.5 L	5.9	22.4 H	42.5 H
	RCUOGDHACF1109O2D	SD07060 +Freedom	12.1 L	25.4 L	3.9 L	22.7 H	26.7 L
	RCUOGNS984-1		28.3 H	45.9 H	8.2	26.6 H	42.6 H
	ERNIE		16.8	18.0 L	6.2	4.3 L	30.7
	TRUMAN		6.1 L	3.4 L	3.9 L	12.6 L	16.6 L
	FREEDOM		15.2 L	20.7 L	5.8	7.1 L	34.9
	PIONEER 2545		30.0 H	50.5 H	11.6	18.5 H	50.3 H

*average (number of locations) for each trait; L**, H** indicate a mean that is not significantly different (LSD=0.05) than the

lowest or highest mean.

Table 2. PNUWWSN test-average level for Fusarium traits recorded across all locations for RCAT/RCUOG lines
and check entries (2005-2007).

Year	Line	Source	FHB	GH	DON	PSS	ISK
		of resistance	index (%)	severity (%)	(ppm)	(%)	Index (%)
2005-			9 locat.*	2 locat.	4 locat.	2 locat.	3 locat.
2006							
	RCAT 32/35B	B Frontana +Sumai 3	25.4 H**	35.2 L**	^с 6.4 Н	8.9 L	30.6 H
	RCAT F 13	Maringa	20.1 H	23.0 L	7.3 H	38.5 H	43.2 H
	RCATTF	Sumai 3	9.1 L	8.1 L	4.8 L	21.3	22.6 L
	174/1C						
	ERNIE-MR		14.6 L	26.5 L	5.2 L	3.4 L	20.3 L
	check						
	TRUMAN-		7.1 L	13.7 L	2.2 L	4.8 L	19.9 L
	MR/R check						
	FREEDOM-		14.8 L	14.4 L	4.5 L	7.2 L	29.2 H
	MR check						
	PIONEER		27.7 H	58.4 H	8.9 H	15.4 L	38.8 H
• • • • •	2545-S check						
2006-	Line		8 locat.	1 locat.	3 locat.	2 locat.	3 locat.
2007	DOLLOG		20.011	05 4 11	0.0.11	40.1.11	
	RCUOG		38.9 H	85.4 H	8.3 H	40.1 H	46./H
	Golden Value	E	1701	76 2 11	4 C I	02.1	25 6 11
	RCUUGLIS	Frontana + $SVP/201/-1/-$	17.9 L	/6.2 H	4.6 L	23.1	35.0 H
	RCUOGI 4	5-10-1 EX9806	22.6	40 3 I	60H	13 Q I	31.1
	RCUOGL 17	SVP72017-17-5-10-1	22.0 17.7 I	40.3 L 66 7 H	0.7 II 4 5 I	13.7 L 11 2 I	25.6
	RCUOG10/18	$\frac{5 \sqrt{172017-17-5-10-1}}{5 \sqrt{172017-17-5-10-1}}$	16.8 I	17 <i>4</i> I	13L	68I	25.0 16.9 I
	FRNIF	Trontana (Sunnai S	10.0 L 12 9 I	28.6 I	1.5 L 4 9	12.7 L	23.6 I
	TRUMAN		73I	12 3 I		94I	17 8 I
	FREEDOM		156L	50L	60H	12.5 L	26.2
	PIONEER		309 H	50 5 H	65H	28 6 H	39.8 H
	2545		50.7 11	50.5 11	0.5 11	20.0 11	57.0 11

*average (number of locations) for each trait; L**, H** indicate a mean that is not significantly different (LSD=0.05) than the lowest or highest mean.

INTROGRESSION OF FHB RESISTANCE FROM ALIEN SPECIES-DERIVED LINES INTO SPRING WHEAT. Q. Zhang ¹, R.E. Oliver ⁴, R.I. McArthur¹, S. Chao³, R.W. Stack ², S. Zhong ², S.S. Xu ³ and X. Cai^{1*}

Departments of ¹Plant Sciences and ²Plant Pathology, North Dakota State University, ³USDA-ARS, Northern Crop Science Lab, Fargo, ND 58105; and ⁴USDA-ARS, SGPG Research Unit, Aberdeen, ID 83210 *Corresponding Author: PH: (701) 231-7404; E-mail: xiwen.cai@ndsu.edu

ABSTRACT

We have produced and collected over 300 wheat lines derived from the crosses of wheat with wild species related to wheat. Evaluation of these lines for reaction to Fusarium head blight (FHB) identified 74 lines with resistance comparable to "Sumai 3" in two greenhouse seasons. Most of the resistant lines, however, cannot be utilized directly in wheat breeding because of the linkage drag associated with the alien chromatin from the wild species. We have been eliminating unwanted alien chromatin from the resistant lines by manipulating chromosomes and introgressing resistance into adapted spring wheat backgrounds through backcrossing and disease screening. To date, we have developed 285 alien introgression lines $(BC_{1,2}F_{6,0})$ that have consistently showed resistance in several greenhouse seasons. Some of the lines exhibited a level of resistance comparable to "Sumai 3". The most resistant lines (~150) were evaluated for FHB resistance and agronomic performance in the field at Langdon and Prosper, ND and Jianvang, China. About 20% of the lines maintained resistance under the high disease pressure in the fields. Most of the resistant lines contain minimal amounts of alien chromatin and do not have obvious linkage drag. We will continue improving the resistant lines with undesirable genes from wild species through chromosome manipulation. The resistant introgression lines were haplotyped at the molecular marker loci closely linked to several well-characterized FHB resistance QTLs. Some of the resistant lines were found to have the haplotypes different from those associated with the known QTL at the molecular marker loci investigated, suggesting the difference of the resistance QTL in the introgression lines from those known resistance QTLs. Currently, we have been preparing seed for DON testing and a larger scale of field evaluation to validate resistance of the introgression lines.