# VARIETY DEVELOPMENT AND HOST PLANT RESISTANCE

# TISSUE CULTURE INDUCED VARIABILITY: CRITICAL ISSUES THAT IMPACT THE EVALUATION AND USE OF TRANSGENIC PARENTS

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## OBJECTIVES

To 1) review the results of the performance of tissue-culture-derived non-transgenic and transgenic barley, and transgenic wheat, tested in multi-year, replicated tests in Idaho; and 2) discuss the impact of somaclonal variation on interpretation of results and on the use of transgenic plants as parents of potential new cultivars.

## INTRODUCTION

Transformation of wheat and barley is dependent on the ability regenerate plants from cultured tissues that are amenable to biolistic- or *Agrobacterium*-mediated introduction of DNA. For both crops, tissue culture protocols have been worked out, particularly for Golden Promise barley and Bobwhite wheat, that enable a number of labs to routinely train scientists who successfully achieve transformation of these difficult-to-transform species. This was not always the case, and decades ago there was intense study of the process of regeneration (*e.g.,* embryogenesis or organogenesis) and the characteristics of regenerated plants (*e.g.,* abnormalities in phenotype and genotype, aka "somaclonal variation"). The accumulation of genetic variability was so widespread and striking that it was proposed as a method of generating varieties with unique characteristics, akin to other methods of mutagenesis (Larkin and Scowcroft, 1981). Despite the depth of the literature on somaclonal variation, most current reports of transgenic plants make no mention of its potential impact on the expression of engineered traits. Several sets of experimental lines were evaluated for agronomic performance and in various yield trials consisting of spaced plant, single row, or standard small plot yield trial formats of barley and wheat derived from tissue culture (both non-transgenic and transgenic). The results of these studies documented striking reductions in the agronomic performance and malting quality of almost all barley lines tested. In contrast, many wheat lines showed no or relatively modest reductions in performance and quality. For both crops, but especially barley, these results mean that somaclonal variation is a confounding factor in experiments that must be considered when evaluating plant performance, especially for traits in which incremental changes in performance are the intended result. In addition, breeding schemes—again, especially for barley—should take into account the near-certainty of heritable changes in performance in addition to that contributed by the transgene.

## RESULTS AND DISCUSSION

*Transformation induces variability in barley beyond that induced by tissue culture alone.* Transformation protocols induce stress from chemical selection, osmotic changes, and/or *Agrobacterium* infection in addition to those imposed by the *in vitro* environment *per se*. Whether the transformation process would cause performance changes in addition to those caused by tissue culture was investigated in null-segregant (no transgene) Golden Promise barley plants derived from hemizygous, transgenic parents. They were tested as rows of spaced $T_2$ and $T_4$ plants at two Idaho locations in 1994 (two replicates) and 1996 (four replicates) (Bregitzer et al. 1998). In contrast to the tissue-culture-derived, non-transgenic plants

where no visual abnormalities were detected, these transgenic families contained clearly-mutant plants, including plants with extreme dwarfism (0 to 9%), a semi-prostrate growth habit (0 to 17%), and extremely late maturity (0 to 3%).

In contrast to the performance of tissue culture-derived, non-transgenic Golden Promise families, null-segregant families derived from transgenic, hemizygous plants performed remarkably—shockingly—poorly (Table 3, 4). Somaclonal variation is has been traced in many cases to epigenetic alterations (Kaeppler and Phillips, 1993), and may not be heritable. However, the observation of reduced performance in advanced generations meant that significant determinants of the observed performance must be heritable. Overall, these data suggested that most barley lines derived directly (via self-pollination) from regenerated transgenic plants would perform substantially worse than their non-transgenic parents.

*Tissue culture alone induces significant variability in barley agronomic performance.* A study (Bregitzer and Poulson 1995) was conducted to investigate agronomic performance of plants derived from 10–12-wk-old callus (Table 1). $R_0$ plants were advanced to $R_2$, space-planted in the greenhouse, and phenotypically normal plants (no abnormal plants were detected) were advanced and tested as $R_4$ and $R_5$ and tested in yield trials using a standard, small-plot format (randomized complete block design; four replicates at each of three locations over two years). None of the families were visually different from the controls. However, there was a clear trend towards reduced performance. The degree and frequency of observed alterations was affected by genotype (all Atlas 57-derived lines families were significantly reduced, but none derived from Steptoe were). Subsequently, malting quality was evaluated on grain derived from three of these cultivars (Bregitzer et al. 1995). Again, there was a trend towards reduced performance (Table 2), and the tissue culture-derived families presented malt profiles similar to that associated with stress (increased protein). Overall, the malting and agronomic data suggested that recovering barley lines from tissue culture that were equivalent in performance to their parent would be expected to be uncommon.

Thus, at least in barley, the recovery of performance would require introgression of transgenes into other backgrounds via one or more rounds of crossing. But—assuming that some performance loss was epigenetic in nature—what would be the expectations for heritability? If epigenetic alterations were involved, would they be stable? To answer this question eight lines derived from four transgenic events (containing either *PDR5* or *TRI101*) produced in the background Conlon ($T_4$ and $T_5$) and 35 lines derived from single backcrosses to one of the primary transgenic lines were tested in 2005 and 2006 in Aberdeen, ID, and Langdon, ND (Bregitzer et al. 2008). The backcross-derived lines included both transgenic and null-segregant lines. Each line was tested as a single row, with six (Aberdeen) and five (Langdon) replicates per line. Interestingly, the Conlon lines advanced by self-pollination were agronomically much better than the Golden Promise lines described above, showing again the potential for background genotype to influence the degree of somaclonal variation. The mean yield was 69% of non-transgenic Conlon (range 57 to 84%). The mean yield of the backcross-derived lines was 94% (range of 90 to 97%), and the performance of these lines was correlated with the relative performance of their respective transgenic parents. Thus, the amount of yield recovery was in line with expectations for the expectation that a single backcross would replace 75% of the donor (transgenic) parent genome with the wildtype Conlon genome. Therefore, regardless of the source (genetic or epigenetic), the determinants of reduced performance induced by tissue culture and transformation behaved as stable, heritable factors.

No differences were detected between null segregant and transgene-containing lines, suggesting that yield depression was a result solely of somaclonal variation. This, and the recovery of performance upon backcrossing, provided evidence that the

observed variation was not caused by transgene expression.

Malting quality was assessed also in these lines. The primary transgenic lines showed widespread reductions in malting quality, as seen before for the Golden Promise-derived lines, with substantial recovery of performance seen for the backcross derived lines (data not shown).

*Transgenic wheat lines performed relatively better than transgenic barley lines*. The performance of transgenic wheat was evaluated at one Idaho and two California locations in 2002 and 2003 (Bregitzer et al. 2006). The experimental format was a standard, small-plot yield trial format (randomized complete block design; four replicates at each location). Fifty-four independent transgenic wheat lines (each expressing a variant high-molecular-weight glutenin gene), and ten null segregant lines, were compared to the performance of the non-transgenic parent, Bobwhite. The performance of all of the null segregant lines, and of 33 of the 44 transgenic lines, was not significantly different from that of Bobwhite (data not shown). This suggested that in these wheat lines, expression of the transgenes was primarily responsible for significant reductions in performance, not somaclonal variation.

Since these wheat lines made it through tissue culture and transformation relatively unscathed, should the conclusion be that somaclonal variation can be discounted in wheat transgenics? Given that somaclonal variation has been documented in all plant species studied where it was searched for, it is unlikely that wheat is an exception. If it were, one would expect a population of wheat lines regenerated from transgenic wheat cultures to have mean performance equal to that of Bobwhite, and exhibit a normal distribution around the values for Bobwhite. Examining the performance of the null segregant lines shows this may not be true. Of the 30 data points for null segregants (10 lines x 3 locations; mean values over two years), only 10 were numerically higher for yield compared to the control, and their overall mean was 6200 kg/ha vs. 6301 for Bobwhite (Table 5). Perhaps these differences are indeed insignificant; nevertheless, the point to be made here is that the conservative assumption should be that the performance of transgenic lines developed by self-pollination may be compromised by somaclonal variation.

*The effect of somaclonal variation on interpreting the results of genetic engineering experiments.* It is obvious that various hybridization-based breeding approaches can remove determinants of somaclonal variation that are unlinked to the transgenic locus, and thus somaclonal variation is of no or negligible importance to the final product. Even in the absence of somaclonal variation, it is unlikely that the desired background would be Bobwhite, Golden Promise, or Conlon (the most commonly-transformed barley and wheat cultivars). The problem lies in interpreting the success of your initial experiments.

A transgenic alteration that eliminates susceptibility to FHB is of little value to a producer if it depresses yield or compromises end-use quality. Especially for qualitative traits, somaclonal variation makes interpretations of the potential agricultural utility difficult. Therefore, it is essential to develop proper controls that take somaclonal variation into account. The background parent that has not gone through the transformation process, a commonly-used control in transgenic studies, is nearly useless for comparing qualitative traits because the effects of the transgene and somaclonal variation are confounded. Null segregants, preferably more than one, derived from the same transformation event as the tested transgenic line, can be developed concurrently with the transgenic line and provide a superior control. Perhaps the best control, a near-isogenic line derived by backcrossing, is often impractical because of the time required to develop it before testing can begin.

## ACKNOWLEDGEMENT

This material is in part based upon work supported by the U.S. Department of Agriculture. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative.

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**Table 1**. Selected agronomic characteristics of lines derived from 10–12-wk-old callus, as measured in small-plot yield trials at three Idaho locations, 1992–1993.

| Cultivar | | Yield (# sig. dif. from control) (kg/ha) | Test weight ((kg/m$^3$) | Plump kernels (%) |
|---|---|---|---|---|
| Atlas 57 | Control | 5859 | 595 | 88.9 |
| | 6 $R_2$-derived families | 4547–5128 (6)[a] | 568–584 (6) | 83.3–90.6 (3) |
| Golden Promise | Control | 6165 | 618 | 58.8 |
| | 6 $R_2$-derived families | 5612–6036 (2) | 605–622 (2) | 42.0–54.5 (3) |
| Klages | Control | 5859 | 640 | 62.6 |
| | 4 $R_2$-derived families | 4956–5379 (3) | 609–649 (1) | 45.4–61.2 (2) |
| Morex | Control | 5364 | 632 | 69.3 |
| | 3 $R_2$-derived families | 4929–5128 (0) | 619–628 (2) | 65.3–68.9 (1) |
| Piroline | Control | 6063 | 667 | 78.4 |
| | 5 $R_2$-derived families | 5208–5746 (2) | 631–664 (3) | 45.2–72.3 (3) |
| Steptoe | Control | 6923 | 597 | 82.5 |
| | 6 $R_2$-derived families | 6600–7036 (0) | 588–601 (0) | 80.4–83.5 (0) |

[a]Number of families with means significantly different from the control for the specified trait.

**Table 2.** Selected malting quality characteristics of lines derived from 10–12-wk-old callus, as measured in small-plot yield trials at two Idaho locations, 1992–1993.

| Cultivar | | Barley protein (%) | Malt extract (%) | Soluble/total protein (%) | Diastatic power (°ASBC) | α-amylase (DU) |
|---|---|---|---|---|---|---|
| Klages | Control | 12.7 | 77.0 | 36.2 | 109 | 39.5 |
| | 4 $R_2$-derived families | 13.2–14.0 (2)[a] | 76.0–77.9 (1) | 34.1–40.0 (1) | 104–118 (0) | 36.1–38.0 (1) |
| Morex | Control | 12.8 | 77.4 | 40.0 | 142 | 44.0 |
| | 3 $R_2$-derived families | 13.6–14.0 (2) | 76.2–77.6 (1) | 39.9–40.6 (0) | 167–192 (3) | 39.4–40.5 (2) |
| Piroline | Control | 12.2 | 76.7 | 34.6 | 115 | 34.4 |
| | 5 $R_2$-derived families | 13.2–13.7 (3) | 74.6–76.7 (2) | 28.5–35.0 (2) | 107–139 (1) | 28.7–35.8 (2) |

[a]Number of families with means significantly different from the control for the specified trait.

**Table 3**. Agronomic performances of transgenic barley grown at two locations in 1994.

| Family[a] | # lines in family[b] | Height[c] | Yield[c] | 100-seed-weight[c] |
|---|---|---|---|---|
| GP717B-2 | 1 | 88 (88–88)[d] | 56 (56–56) | 74 (74–74) |
| GP717B-4 | 5 | 98 (94–103) | 85 (69–108) | 84 (82–92) |
| GP717B-11 | 2 | 86 (84–88) | 54 (50–58) | 70 (68–73) |
| GP717B-14 | 2 | 73 (70–76) | 16 (16–16) | 57 (55–58) |
| GP717B-31 | 1 | 79 (79–79) | 47 (47–47) | 77 (77–77) |
| GP717B-32 | 5 | 94 (89–97) | 66 (53–80) | 79 (72–85) |
| GP717B-33 | 4 | 90 (86–93) | 64 (57–73) | 74 (72–75) |
| GP717B-59 | 1 | 87 (87–87) | 64 (64–64) | 81 (81–81) |
| GP717B-189 | 4 | 77 (69–87) | 27 (16–41) | 66 (58–75) |
| GP717B-197 | 5 | 82 (68–96) | 49 (21–81) | 72 (57–85) |
| GP724B-1 | 1 | 87 (87–87) | 45 (45–45) | 74 (74–74) |
| GP724B-4 | 4 | 87 (82–90) | 60 (42–68) | 93 (80–118) |
| GP724B-47 | 1 | 92 (92–92) | 79 (79–79) | 88 (88–88) |
| GP724B-96 | 4 | 80 (76–88) | 50 (40–65) | 75 (72–83) |

[a]Each family represents an individual transformation event
[b]Each line derives from an individual regenerated plant
[c]Data are expressed as percentages of the non-transgenic GP control performance
[d]Data presented as: family mean (range of line means)

**Table 4** Agronomic performances of Golden Promise and transgenic-derived null-segregant barley lines in 1996

| Line | Traits | | | |
|---|---|---|---|---|
| | Heading date (d after Jan. 1) | Height (cm) | Yield per plant (g) | 100-seed weight (g) |
| Golden Promise | 193.9 | 49.6 | 30.2/1.2[a] | 3.7/0.27 |
| GP717B-14-8 | 196.2* | 41.7* | 5.2*/2.4* | 2.3*/0.61 |
| GP717B-14-12 | 198.3* | 39.6* | 4.4*/2.4* | 2.3*/0.77* |
| GP717B-31-3 | 195.3 | 47.2* | 15.9*/1.8* | 3.2*/0.47 |
| GP717B-32-6 | 195.2 | 46.3* | 15.9*/1.8* | 3.0*/0.50 |
| GP717B-32-11 | 194.3 | 47.4* | 18.2*/1.7* | 3.0*/0.43 |
| GP717B-33-3 | 196.7* | 42.7* | 12.1*/1.9* | 2.8*/0.54 |
| GP717B-33-3 | 193.6 | 46.2* | 18.0*/1.6 | 3.4/0.26 |

* SigniÞcantly di□erent from G.P. as determined by Dunnett's multiple comparison procedure ($P$=0.05).
[a]Data presented as: trait mean/estimated deviation. Estimated deviation = range / trait mean, calculated on a per-plot basis.

**Table 5.** Agronomic performance of transgenic wheat lines, 2002 and 2003.

| Location | Aberdeen, ID | | | Davis, CA | | El Centro, CA | |
|---|---|---|---|---|---|---|---|
| Line | Yield (kg/ha) | Protein (%) | Test weight (kg/m$^3$) | Yield (kg/ha) | 100-seed-weight (g) | Yield (kg/ha) | Test weight (kg/m$^3$) |
| Bobwhite | 6486 | 12.5 | 792 | 2481 | 3.34 | 9936 | 746 |
| Dx51Dy10-C null | 6346 | 12.6 | 788 | 2547 | 3.25 | 11006 | 766 |
| Hybrid-B null1 | 6023 | 13 | 790 | 2281 | 3.35 | 9133 | 763 |
| Hybrid-B null2 | 5974 | 13.1 | 802 | 2333 | 3.1 | 9323 | 766 |
| LongDx5-B null | 6476 | 12.9 | 796 | 1780 | 3.2 | 10126 | 766 |
| LongDx5-F null | 6226 | 13 | 795 | 1927 | 3.19 | 10095 | 775 |
| LongDx5-H null | 6217 | 13 | 793 | 2064 | 3.41 | 9983 | 759 |
| LongDx5-I null | 5982 | 12.9 | 792 | 1905 | 3.34 | 10735 | 775 |
| ShortDx5-C null | 6113 | 13.8 | 792 | 2599 | 3.31 | 9771 | 766 |
| ShortDx5-D null | 6597 | 13 | 787 | 2399 | 3.09 | 11078 | 756 |
| ShortDx5-H null | 6082 | 13.9 | 792 | 1928 | 3.21 | 10960 | 788* |

*, **, ***: significant at $P$=0.05, 0.01, and 0.001, respectively.

# GENOMIC SELECTION FOR FHB RESISTANCE USING THE UNIFORM SCAB SCREENING NURSERIES

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**ABSTRACT**

Genomic selection (GS) involves use of genome-wide marker data in combination with phenotypic data to develop models for predicting performance of untested lines using only genotypic data. Wheat breeders in the eastern winter wheat region have collaborated to evaluate FHB resistance in adapted breeding lines across years and locations. Disease evaluation data from the 2011 through 2015 Uniform Southern Soft Red Winter Wheat Scab Nursery were analyzed using mixed model in SAS 9.3 to obtain BLUES for each genotype. Entries in the 2011 through 2016 USSRWWSN were genotyped using genotyping-by-sequencing and data were obtained for 15,013 markers distributed throughout the genome. In addition, KASP evaluations were performed for markers linked to FHB resistance QTL, the *Fhb1* locus, and the *Rht-B1* and *Rht-D1* loci. GS models were implemented with the R-package RR-BLUP and accuracy evaluated by correlation between Genomic Estimate Breeding Values (GEBVs) and BLUES for each line. The mean observed accuracies (r) from 100 cycles of five-fold cross validation were 0.46 for incidence, 0.66 for severity, 0.61 for Index, 0.59 for FDK, 0.59 for ISK and 0.53 for DON. Addition of markers for the *Rht1* and *Fhb1* loci as fixed effects in the model resulted in small increases in prediction accuracy. In particular, incidence accuracies increased with the addition of the *Rht-D1* marker (r = 0.50). DON accuracies were slightly increased with the addition of the *Fhb1* marker (r = 0.57). Based on GS models using the 2011-2015 nurseries as a training population, GEBVs were determined and reported for entries in the 2016 Uniform Southern Soft Red Winter Wheat Scab Nursery report. Results of genome-wide association mapping using this genotypic and phenotypic dataset will also be reported. Overall, our results suggest that GS for FHB resistance can be utilized to streamline variety selection and evaluation.

# IMPLEMENTATION OF GENOMIC SELECTION FOR RESISTANCE TO FUSARIUM HEAD BLIGHT INTO A TRADITIONAL WHEAT BREEDING PROGRAM

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## ABSTRACT

Genomic selection is a new method applied in plant breeding that uses high density genotyping associated with observed phenotypes to predict unobserved phenotypes via a marker-trait model. Our objective was to implement this method in a traditional soft winter wheat breeding program to increase overall genetic gains in developing wheat cultivars resistant to *Fusarium graminearum* (teleomorph: *Gibberella zea*). Phenotyping for Fusarium Head Blight (FHB) was conducted on a set of 417 lines from which a training population was derived. FHB incidence, severity, *Fusarium* damaged kernels (FDK), and deoxnivalenol levels were assessed on the lines grown in an inoculated and mist-irrigated scab nursery in Virginia. Genotyping was done using double digest rad-seq or often referred to as GBS using the enzymes *PstI* and *MseI*. SNPs were aligned using the International Wheat Genome Sequencing Consortium's whole genome assembly v0.4. Prior to imputation of missing genotypes, the genotypic dataset was filtered to remove SNPs with missing data frequencies >20%, heterozygous call frequencies >15%, and minor allele frequency < 5%. In addition, all unaligned SNPs were removed. Imputations were achieved using the R package *LinkImpute*. This package implements a nearest-neighbor algorithm using both the *k* nearest individuals and the *l* SNPs in highest LD with the specific missing SNP genotype that must be imputed. Genomic selection (GS) accuracies were assessed using best linear unbiased prediction (*rr-Blup*) using the *kin.blup* function. This function estimates genomic values in which performance of individuals are predicted based upon kinship to other lines in the training population. Average accuracies for Grain Yield, FHB Severity, Incidence, Index, and FDK were 60%, 38%, 47%, 45%, and 43%, respectively after 1000 permutation cycles for each trait. The R package *PopVar* was also used to predict parents to cross to generate high genetic variance for resistance to FHB.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-4-032. 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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

# ASSOCIATION MAPPING IN A PANEL OF MINNESOTA SPRING WHEAT BREEDING LINES REVEALS QTL MAINTAINED OVER DECADES OF PHENOTYPIC SELECTION

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## ABSTRACT

Developing varieties with improved resistance to Fusarium head blight (FHB) has been a major goal in the University of Minnesota's wheat improvement program since the scab epidemics of the 1990s. At that time, a number of diverse lines, particularly from Asian germplasm, were crossed into the program to introduce sources of genetic resistance. Early lines developed from resistance sources had poor agronomic and quality characteristics. In the intervening years, the focus has been on improving agronomic and quality traits while maintaining FHB resistance. We have selected for two major QTL, *Fhb1* (since 2001) and *Fhb5* (2005), using DNA markers and phenotype all $F_5$ and more advanced lines, about 3,500 per year, in controlled disease nurseries. To assess the number and locations of resistance QTL currently present in the UMN wheat breeding program, a panel of 383 $F_7$-derived lines in advanced yield testing were phenotyped for several FHB resistance-associated traits in a minimum of five environments between 2009 and 2013. The panel was genotyped at high density using the 90K Illumina Infinium iSelect Assay, resulting in 14,221 mapped SNP markers for association analysis. Association mapping revealed the presence of *Fhb1*, *Fhb2*, *Fhb5*, and over two dozen additional significant regions ($p<0.001$) across the genome, many corresponding to the locations of previously reported QTL. Analysis of the pedigrees confirms the presence of reported source lines in several cases. KASP markers based on significant Illumina 90K iSelect markers are being designed to facilitate the tracking of these QTL in the program. This study demonstrates the efficacy of phenotypic selection for long-term maintenance of favorable FHB resistance alleles.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-4-019. 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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

# EVALUATION OF GERMPLASM RESISTANCE TO FUSARIUM HEAD BLIGHT DISEASE

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**ABSTRACT**

Fusarium Head blight (FHB) disease of wheat reduces yield and deteriorates quality. Breeding for FHB resistance requires identification of sources of resistance. This poster summarizes one year of FHB screening effort at Purdue University. The germplasm includes 80 advanced breeding lines developed at Purdue, 17 doubled haploid lines developed from the cross between Indiana wheats INW0412 and INW0411, 200 recombinant inbreeding lines from the cross between INW0412 and 992060G1-1-5, and lastly, 33 accessions introduced from the European FHB resistance breeding program in Austria. Plant materials were phenotyped in greenhouse and field conditions. Type II resistance for FHB was recorded following artificial infection. Germplasm showing type II FHB response of less than 20% were screened for further validation studies. Leveraging marker data for known loci indicated that a select number of lines carrying FHB resistance alleles also harbored important disease resistance genes such as stem rust resistance *Sr36* (effective against the race Ug99), height reducing loci (*Rht1* and *Rht2*), and *Bdv2/3* for barley yellow dwarf virus disease. After validation phenotyping, lines harboring multiple resistance alleles will be used in crossing schemes. Besides germplasm enhancement through phenotypic assessment, one future direction is to develop genome-wide markers to enable genomic prediction for FHB resistance and other traits of agronomic importance.

# RESPONSE OF A COLLECTION OF *WAXY* (REDUCED AMYLOSE) WHEAT BREEDING LINES TO *FUSARIUM GRAMINEARUM*

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**ABSTRACT**

Loss of function mutations in the *Waxy* (*Wx*) gene encoding granule bound starch synthase I (GBSSI) that synthesizes amylose, results in starch granules containing mostly amylopectin. Wheat grain with this trait has increased functionality as an optimal substrate for production of modified food starches and for increased nutritional value in livestock and poultry feed. However, impaired GBSSI activity may alter grain structure and composition and, consequently, responses to pathogens. There are no published reports on response of *waxy* wheats to Fusarium head blight (FHB). A screen of colonization by *Fusarium graminearum* of *waxy* breeding lines and wild-type and *waxy* checks was conducted on grain grown at Mead, NE during 2013 and 2014. Grain was either surface disinfested before plating, or directly plated, onto medium semi-selective for *Fusarium* spp., indicating internal or both internal and superficial fungal infections, respectively. Fungi were identified using morphological characteristics. Chi-square analysis showed that internal and superficial total *Fusarium* infection rates (directly plated grain) were significantly higher among the *waxy* breeding lines and the waxy cultivar Mattern (55.1%) as compared with wild-type checks (45.6%) ($P < 0.01$). However, there were no significant differences in the proportion of these fungi that were *F. graminearum* in *waxy* (4.3%) *versus* wild-type (3.4%) grain ($P = 0.11$). Percent of internal infections (disinfested grain) of *waxy* (4.4%) and wild-type (3.9%) grains were not significantly different ($P = 0.45$). However, chi-square analyses indicated that the proportion of these fungi in *waxy* grain that were *F. graminearum* (4.7%) was significantly less than that of wild-type (17.5%) ($P = 0.03$). When grain was analyzed using GC-MS for four trichothecene mycotoxins, only deoxynivalenol (DON) was detected. In spite of internal and superficial levels of *F. graminearum* colonization, *waxy* breeding lines and Mattern combined had significantly higher levels of DON (0.58 ppm) than wild-type checks (0.52ppm) (SE= 0.02; $P = 0.03$). However, waxy breeding line NX12Y8213 (PI 677877) had mean rates of internal infection (0.00%) and DON levels (0.49 ± 0.05 ppm) which were the same as the FHB tolerant wild-type lines McGill and Freeman. The proportion of superficial and internal infection of NX12Y8213 by *F. graminearum* (1.7%) was not significantly different from that of the wild-type checks (3.4%) ($P = 0.47$). Therefore, NX12Y8213 is promising for breeding for *waxy* lines with tolerance to FHB.

# SIMULTANEOUS MAPPING AND PYRAMIDING LOCI IN WHEAT BREEDING POPULATIONS: IDENTITY BY DESCENT MAPPING APPROACHES

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**ABSTRACT**

Pyramiding QTL from multiple sources for FHB resistance presents an opportunity to enhance the FHB resistance of elite wheat germplasm. Conventionally, pyramiding QTL using a marker assisted-selection approach would require preliminary mapping studies to identify the resistance QTL from each parental line and validation studies to assess the QTL effects in multiple genetic backgrounds. Mapping FHB resistance QTL directly in wheat breeding populations would eliminate the need for purpose built mapping populations, and thus accelerate marker-assisted pyramiding efforts. This presentation will discuss our recent studies showing how multiple QTL for FHB resistance can be mapped directly in early generation breeding populations by application of identical-by-descent (IBD)-based linkage mapping. We used IBD-based linkage analysis in spring and winter wheat segregating $F_1$ progeny derived from 43 and 28 four-way crosses respectively, among Fhb1 donor lines and multiple native sources of resistance including plant introductions, SDSU and UMN breeding lines in spring wheat; and Lyman, Overland, Ernie and Freedom in winter wheat. In the spring wheat experiment QTL for FHB resistance were identified on chromosomes 2A, 2B, 3B and 7B, explaining between 18 to 21% of the variance for FHB severity in different evaluations. The QTL detected on chromosome 2A appears to have a resistance allele conferred by MN99126 in the same region detected by QTL-meta analysis from Ning8026, Wangshuibai, Spark and Rubens. In the winter wheat experiment a total of 15 QTL for FHB resistance were mapped on chromosomes 1A, 1B, 2A, 3A, 3B, 4A, 4B, 4D, 5A, 6A, 6D and 7D, including known loci Fhb1, Fhb5, and Rht-B1. QTL conferring native resistance in the cultivars Lyman and Overland are mapped for the first time in this study, including a QTL on chromosome 1AS (Qfhb.sdsu-1A) explaining between 4.5 to 9.9% of the phenotypic variance in all evaluations. Marker haplotypes for these QTL regions can be used to conduct marker assisted selection and fixation of resistance alleles in subsequent generations of these breeding populations.

Subsequent efforts with double haploid derived from these breeding populations are validating the reported results.

# EVALUATION OF SOUTHERN SOFT RED WINTER WHEAT LINES FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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## ABSTRACT

Fusarium head blight (FHB) is a disease of small grains caused by the fungal pathogen *Fusarium graminearum*. FHB poses potential economic losses and health risks due to the accumulation of the mycotoxin deoxynivalenol (DON) on infected seed heads. The objectives of this study are; 1) evaluate soft red winter wheat (SRWW) lines for resistance to FHB in terms of resistance to initial inoculum (incidence); resistance to spread within the head (severity); resistance to DON accumulation; and resistance to *Fusarium* damaged kernels (FDK), 2) determine the frequency and effect of known FHB resistance genes and quantitative trait loci (QTL), and 3) Identify novel resistance loci using a genome wide association (GWAS) approach. In 2015-2016, 360 SRWW breeding lines were evaluated in inoculated misted FHB nurseries in Fayetteville and Newport, AR in a randomized complete block design. At both locations, lines were sown in two row plots, inoculated with *F. graminearum* infected corn (*Zea mays* L.) and overhead misted for a total of 480 and 520 minutes, for Fayetteville and Newport, respectively, throughout the months of April and May to provide optimal conditions for FHB infection. In addition to visual ratings and DON analysis, lines are currently being screened with molecular markers linked to known FHB resistance genes, including *Fhb1*, 3BSc from Massey and recently identified QTL for native resistance from Jamestown (1B, 6A) and Bess (2B, 3B). Future work will use markers generated through genotype by sequence to perform GWAS. The overall goal of this research is to produce marketable wheat cultivars with improved resistance to FHB using a combination of both traditional and molecular breeding methods.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0200-3-007.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.

# EPIGENETIC CONTROL OF FHB IN DURUM WHEAT

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## ABSTRACT

Fusarium head blight (FHB) continues to be a serious problem for wheat production in the U.S. and elsewhere in the world. Economic losses associated with FHB occur due to low grain yield and contamination of grain with mycotoxins. *Fusarium graminearum* is the major causative agents of FHB in the U.S. Durum wheat (*Triticum turgidum* L. var. *durum*) is particularly susceptible to FHB and breeding for resistance has been impeded by low genetic variation. Thus, it is important to identify other means of enhancing resistance to FHB in durum wheat. DNA methylation and demethylation have been documented to be involved in immunity against the plant pathogens by regulating transcriptional and co-transcriptional immune-responsive genes.

We treated eight advanced durum breeding homozygous lines with 5-Methyl-azacytedine that removes CG methylation. The treated lines were selected for resistance at each generation and advanced to the $M_4$ generation, resulting in 32 selected lines that were further analyzed, along with the eight parental controls. All 40 lines were tested for FHB resistance under greenhouse and field conditions. Five of the 32 demethylated lines tested showed promise, having less than 30% disease severity as compared with a range of 50-100% for the parental lines and FHB susceptible lines included as checks. The proportion of *Fusarium*-damaged kernels (FDK) of the five lines identified ranged from 10 to 30%, whereas the parental and other treated lines showed values between 30 and 60%. The range of deoxynivalenol (DON) concentrations of grain harvested from the five lines was from 2.46 to 5.60 ppm, whereas the parental lines, checks and the remaining 27 treated lines had DON concentrations from 5.10 to 18.27 ppm. The FDK and DON analyses supported the findings of the disease development assessed in the field and the greenhouse. These five lines, together with their respective parental lines and some highly susceptible checks are being further analyzed to determine the specific epigenetic changes that are responsible for the enhanced resistance observed. We have advanced these lines by backcrossing them to the parental cultivars with the aim of testing the stability and inheritance of the resistance.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture. 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.

# THE 2016 UNIFORM SOUTHERN SOFT RED WINTER WHEAT SCAB NURSERY

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## ABSTRACT

The Uniform Southern Soft Red Winter Wheat Scab Nursery provides breeders in the public and private sectors the opportunity for multi-environment, independent evaluations of FHB resistance in advanced generation breeding lines compared with the current most resistant check varieties 'Ernie', 'Bess' and 'Jamestown'. Valuable data are provided on resistance to other important fungal and viral diseases, milling and baking quality and agronomic characteristics. In addition, genotypic analyses identify alleles present at numerous important loci. For the first time we provide Genomic Estimated Breeding Values (GEBV) for nursery entries. These were estimated from a training population of nursery entries from 2011 to 2015. A combined mixed model analysis of the phenotypic data from 2011 to 2015 was performed using SAS 9.3 and BLUEs for each genotype were recorded. The number of SNP markers utilized was 70,081. The Genotypic Selection model utilized Ridge Regression BLUP through the R-package RR-BLUP to predict GEBVs for individuals in the 2016 nursery. GS model accuracy is evaluated by Pearson correlation between GEBVs and best linear unbiased estimate (BLUE) for the 2016 lines. Correlation varied between 0.55 for FHB Severity to 0.13 for FHB Index.

The 2015-16 nursery comprised 51 advanced generation breeding lines and four check cultivars, Ernie, Bess, Jamestown (partially resistant) and 'Coker 9835' (susceptible). Six U.S. public programs (Arkansas, Georgia, Louisiana, North Carolina, Virginia, and USDA-ARS), and two private companies (KWS and Limagrain) submitted entries. Data were returned from up to eight locations in the US and one in Hungary. In addition, three USDA-ARS laboratories conducted evaluations for Hessian fly resistance, milling and baking quality and marker genotypes.

Copies of the full report will be available at the 2016 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-064. 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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

**Table 1.** Phenotypic means across locations, correlations between GEBV and phenotypic means and genotypic content of regions associated with FHB resistance.

| | Cultivar/ Designation | FHB Incidence | RANK | FHB Severity | RANK | FHB Index | RANK | FDK | RANK | ISK | RANK | DON | RANK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ERNIE | 49 | 40 | 25 | 33 | 14 | 29 | 32 | 38 | 31 | 32 | 3 | 10 |
| 2 | COKER9835 | 84 | 55 | 56 | 55 | 49 | 55 | 56 | 54 | 57 | 55 | 7 | 52 |
| 3 | BESS | 31 | 6 | 17 | 12 | 7 | 5 | 15 | 3 | 19 | 1 | 2 | 1 |
| 4 | JAMESTOWN | 35 | 14 | 19 | 15 | 10 | 18 | 23 | 16 | 26 | 16 | 4 | 23 |
| 5 | AR06024-7-2 | 33 | 8 | 13 | 3 | 8 | 8 | 20 | 10 | 25 | 14 | 2 | 1 |
| 6 | ARS10-389 | 34 | 11 | 14 | 5 | 6 | 3 | 26 | 21 | 21 | 5 | 2 | 1 |
| 7 | AR07010-7-1 | 30 | 4 | 19 | 15 | 9 | 15 | 25 | 20 | 24 | 10 | 5 | 35 |
| 8 | AR07053-13-1 | 33 | 8 | 19 | 15 | 8 | 8 | 20 | 8 | 24 | 10 | 6 | 45 |
| 9 | AR07078-7-4 | 44 | 28 | 27 | 36 | 17 | 38 | 24 | 17 | 33 | 36 | 6 | 45 |
| 10 | AR07108-6-1 | 28 | 2 | 17 | 12 | 8 | 8 | 17 | 5 | 22 | 8 | 4 | 23 |
| 11 | ARLA06146E-20-1 | 39 | 19 | 19 | 15 | 12 | 24 | 24 | 19 | 29 | 23 | 4 | 23 |
| 12 | ARLA07084C-10-1 | 26 | 1 | 13 | 3 | 5 | 2 | 23 | 14 | 21 | 5 | 5 | 35 |
| 13 | ARS11-2086 | 65 | 54 | 34 | 50 | 27 | 51 | 58 | 55 | 45 | 52 | 5 | 35 |
| 14 | ARS12-201 | 59 | 51 | 31 | 47 | 24 | 50 | 50 | 50 | 40 | 47 | 5 | 35 |
| 15 | ARS13-159 | 51 | 42 | 29 | 42 | 19 | 42 | 46 | 47 | 40 | 47 | 3 | 10 |
| 16 | ARS13-215 | 50 | 41 | 28 | 41 | 19 | 42 | 27 | 26 | 32 | 34 | 15 | 55 |
| 17 | ARS14W0539 | 32 | 7 | 16 | 8 | 6 | 3 | 34 | 40 | 29 | 23 | 5 | 35 |
| 18 | ARS14W0623 | 40 | 23 | 24 | 30 | 10 | 18 | 47 | 48 | 32 | 34 | 11 | 54 |
| 19 | ARS14W1012 | 47 | 33 | 27 | 36 | 19 | 42 | 40 | 46 | 40 | 47 | 4 | 23 |
| 20 | ES14-0057 | 39 | 19 | 19 | 15 | 9 | 15 | 22 | 13 | 25 | 14 | 3 | 10 |
| 21 | ES14-0528 | 58 | 50 | 21 | 22 | 15 | 32 | 30 | 34 | 30 | 27 | 3 | 10 |
| 22 | ES14-1293 | 29 | 3 | 12 | 1 | 4 | 1 | 18 | 7 | 20 | 2 | 3 | 10 |
| 23 | ES14-1350 | 54 | 46 | 29 | 42 | 21 | 47 | 48 | 49 | 42 | 50 | 2 | 1 |
| 24 | GA08250-15ES14 | 35 | 14 | 16 | 8 | 8 | 8 | 12 | 1 | 21 | 5 | 3 | 10 |
| 25 | GA08293-15ES3 | 46 | 31 | 29 | 42 | 19 | 42 | 35 | 42 | 34 | 39 | 5 | 35 |
| 26 | GA09361-15ES38 | 47 | 33 | 31 | 47 | 19 | 42 | 27 | 27 | 34 | 39 | 6 | 45 |
| 27 | GA091252-15ES35 | 52 | 44 | 25 | 33 | 15 | 32 | 34 | 41 | 34 | 39 | 6 | 45 |
| 28 | GA08281-15ES1 | 46 | 31 | 24 | 30 | 14 | 29 | 29 | 31 | 30 | 27 | 4 | 23 |
| 29 | GANC9337-15ES27 | 44 | 28 | 20 | 20 | 13 | 28 | 15 | 4 | 24 | 10 | 3 | 10 |
| 30 | GA09343-15ES33 | 56 | 48 | 32 | 49 | 23 | 48 | 30 | 33 | 33 | 36 | 6 | 45 |
| 31 | GANC 10014-15ES24 | 44 | 28 | 22 | 26 | 14 | 29 | 36 | 43 | 35 | 43 | 5 | 35 |
| 32 | KWS 053 | 39 | 19 | 12 | 1 | 8 | 8 | 17 | 6 | 20 | 2 | 3 | 10 |
| 33 | KWS 060 | 52 | 44 | 27 | 36 | 16 | 37 | 21 | 11 | 28 | 20 | 2 | 1 |
| 34 | KWS 074 | 36 | 16 | 16 | 8 | 8 | 8 | 28 | 30 | 28 | 20 | 4 | 23 |
| 35 | KWS 081 | 33 | 8 | 16 | 8 | 8 | 8 | 26 | 23 | 26 | 16 | 2 | 1 |
| 36 | KWS 083 | 34 | 11 | 15 | 6 | 7 | 5 | 20 | 9 | 23 | 9 | 4 | 23 |
| 37 | KWS 087 | 38 | 17 | 22 | 26 | 12 | 24 | 28 | 29 | 28 | 20 | 3 | 10 |
| 38 | LA06146E-P4 | 38 | 17 | 21 | 22 | 12 | 24 | 30 | 35 | 30 | 27 | 5 | 35 |
| 39 | LA08090C-9-2 | 34 | 11 | 24 | 30 | 12 | 24 | 32 | 36 | 31 | 32 | 6 | 45 |
| 40 | LA08265C-50 | 40 | 23 | 27 | 36 | 15 | 32 | 30 | 32 | 30 | 27 | 4 | 23 |
| 41 | LA09011UB-2 | 47 | 33 | 29 | 42 | 18 | 40 | 38 | 45 | 35 | 43 | 6 | 45 |
| 42 | LA09225C-33 | 40 | 23 | 29 | 42 | 15 | 32 | 28 | 28 | 34 | 39 | 5 | 35 |
| 43 | NC10435-11 | 51 | 42 | 27 | 36 | 18 | 40 | 22 | 12 | 30 | 27 | 3 | 10 |
| 44 | NC12-22225 | 43 | 27 | 21 | 22 | 10 | 18 | 26 | 25 | 29 | 23 | 3 | 10 |
| 45 | NC13-20076 | 30 | 4 | 15 | 6 | 7 | 5 | 15 | 2 | 20 | 2 | 2 | 1 |
| 46 | NC13-22350 | 40 | 23 | 18 | 14 | 9 | 15 | 24 | 18 | 24 | 10 | 2 | 1 |
| 47 | NC13-23449 | 56 | 48 | 34 | 50 | 27 | 51 | 37 | 44 | 38 | 46 | 4 | 23 |
| 48 | VA12W-68 | 39 | 19 | 20 | 20 | 10 | 18 | 26 | 24 | 27 | 18 | 8 | 53 |
| 49 | VA13W-38 | 48 | 39 | 21 | 22 | 11 | 23 | 26 | 22 | 27 | 18 | 2 | 1 |
| 50 | VA09MAS6-122-7-1 | 47 | 33 | 23 | 28 | 17 | 38 | 23 | 15 | 29 | 23 | 4 | 23 |
| 51 | VA08MAS1-188-6-4-1 | 47 | 33 | 23 | 28 | 15 | 32 | 32 | 37 | 33 | 36 | 3 | 10 |
| 52 | VA13FHB-26 | 47 | 33 | 25 | 33 | 12 | 24 | 50 | 51 | 43 | 51 | 4 | 23 |
| 53 | VA14FHB-14 | 62 | 52 | 39 | 53 | 30 | 53 | 55 | 53 | 48 | 54 | 3 | 10 |
| 54 | VA14FHB-13 | 64 | 53 | 39 | 54 | 31 | 54 | 54 | 52 | 45 | 52 | 4 | 23 |
| 55 | VA14FHB-28 | 55 | 47 | 35 | 52 | 23 | 48 | 33 | 39 | 36 | 45 | 5 | 35 |
| | Mean | 44 | | 24 | | 14 | | 30 | | 31 | | 4 | |
| | LSD (0.05) | 37 | | 28 | | 26 | | 34 | | 23 | | 4 | |
| | CV% | 43.0 | | 59.6 | | 90.1 | | 56.4 | | 37.3 | | 50.1 | |
| | Mean v GEBV Correlation | 0.44 | | 0.55 | | 0.13 | | 0.30 | | 0.49 | | 0.44 | |

**Table 1.** Continued

| | Cultivar/ Designation | Heading Date | RANK | Plant Height | RANK | Flour Yield % | RANK | Softness Equivalent % | RANK | Hessian Fly Biotype L | Fhb1 | Fhb Massey 3B | Fhb 5A | Fhb 2DL-Wuhan1/W14 | Bess 2B | Bess 3B | Jamestown 1B | Jamestown 6A | NC-Neuse 1A | NC-Neuse 6A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ERNIE | 123 | 12 | 33 | 12 | 63 | 50 | 52 | 42 | 0-19 | no | yes | yes | no | no | no | no | no | yes | yes |
| 2 | COKER9835 | 126 | 27 | 32 | 3 | 66 | 30 | 60 | 4 | 0-17 | no | no | no | no | no | no | no | no | no | no |
| 3 | BESS | 127 | 33 | 35 | 31 | 65 | 41 | 54 | 24 | 0-19 | no | no | no | no | yes | yes | yes | no | yes | no |
| 4 | JAMESTOWN | 121 | 1 | 33 | 12 | 65 | 41 | 54 | 24 | 0-18 | no | no | no | no | no | no | yes | yes | yes | no |
| 5 | AR06024-7-2 | 125 | 22 | 36 | 37 | 64 | 49 | 55 | 17 | 0-19 | het | no | no | no | no | no | yes | no | yes | no |
| 6 | ARS10-389 | 121 | 1 | 35 | 31 | 71 | 1 | 39 | 53 | 0-16 | no | no | no | no | no | no | no | no | no | no |
| 7 | AR07010-7-1 | 129 | 51 | 39 | 50 | 66 | 30 | 52 | 42 | 0-18 | no | no | no | no | no | no | no | no | no | no |
| 8 | AR07053-13-1 | 128 | 39 | 39 | 50 | 66 | 30 | 54 | 24 | 0-13 | no | no | no | no | no | no | no | no | no | no |
| 9 | AR07078-7-4 | 130 | 53 | 39 | 50 | 68 | 11 | 54 | 24 | 0-16 | no | no | no | no | no | no | no | het | no | no |
| 10 | AR07108-6-1 | 128 | 39 | 40 | 53 | 67 | 21 | 55 | 17 | 0-18 | no | no | no | no | no | yes | het | no | yes | no |
| 11 | ARLA06146E-20-1 | 126 | 27 | 40 | 53 | 65 | 41 | 56 | 10 | 0-14 | no | no | no | no | no | no | yes | yes | yes | no |
| 12 | ARLA07084C-10-1 | 128 | 39 | 37 | 45 | 67 | 21 | 60 | 4 | 0-12 | no | no | no | no | no | no | no | no | no | no |
| 13 | ARS11-2086 | 128 | 39 | 32 | 3 | 68 | 11 | 53 | 35 | 14-6 | no | no | yes | no | no | no | yes? | no | yes | yes |
| 14 | ARS12-201 | 128 | 39 | 33 | 12 | 69 | 5 | 51 | 45 | 4-13 | no | no | yes | no | no | no | yes | no | yes | yes |
| 15 | ARS13-159 | 127 | 33 | 38 | 48 | 68 | 11 | 61 | 1 | 0-18 | no | yes | no | no | no | no | yes | no | no | no |
| 16 | ARS13-215 | 128 | 39 | 34 | 20 | 69 | 5 | 47 | 49 | 0-16 | no | no | no | no | no | no | no | no | yes | no |
| 17 | ARS14W0539 | 131 | 54 | 32 | 3 | . | . | . | . | 0-20 | no | yes | no | no | no | no | no | no | no | no |
| 18 | ARS14W0623 | 133 | 55 | 37 | 45 | . | . | . | . | 0-17 | no | yes | no | no | no | no | no | no | no | no |
| 19 | ARS14W1012 | 127 | 33 | 33 | 12 | 66 | 30 | 48 | 47 | 0-13 | no | no | no | no | no | no | no | no | no | yes |
| 20 | ES14-0057 | 129 | 51 | 36 | 37 | 65 | 41 | 58 | 7 | 0-18 | no | no | no | no | no | no | het | no | yes | no |
| 21 | ES14-0528 | 124 | 16 | 36 | 37 | 70 | 2 | 54 | 24 | 0-19 | no | yes | ND | no | no | no | yes | no | no | no |
| 22 | ES14-1293 | 128 | 39 | 40 | 53 | 67 | 21 | 54 | 24 | 18-0 | no | no | no | no | no | no | no | no | yes | no |
| 23 | ES14-1350 | 126 | 27 | 36 | 37 | 66 | 30 | 47 | 49 | 0-19 | no | yes | no | no | no | no | yes | no | yes | no |
| 24 | GA08250-15ES14 | 125 | 22 | 36 | 37 | 69 | 5 | 55 | 17 | 0-16 | no | no | no | no | no | no | no | yes | yes | no |
| 25 | GA08293-15ES3 | 122 | 7 | 34 | 20 | 62 | 53 | 46 | 52 | 0-18 | no | no | no | no | no | no | no | yes | no | no |
| 26 | GA09361-15ES38 | 125 | 22 | 34 | 20 | 70 | 2 | 52 | 42 | 0-20 | no | no | yes | no | no | no | het | no | no | no |
| 27 | GA091252-15ES35 | 125 | 22 | 34 | 20 | 70 | 2 | 53 | 35 | 0-19 | no | no | no | no | no | no | no | no | no | no |
| 28 | GA08281-15ES1 | 124 | 16 | 32 | 3 | 67 | 21 | 55 | 17 | 0-19 | no | no | no | no | no | no | no | no | no | no |
| 29 | GANC9337-15ES27 | 122 | 7 | 33 | 12 | 66 | 30 | 56 | 10 | 0-18 | no | no | no | no | no | no | yes | no | yes | no |
| 30 | GA09343-15ES33 | 121 | 1 | 32 | 3 | 68 | 11 | 56 | 10 | 0-20 | no | no | no | no | no | no | no | no | no | no |
| 31 | GANC 10014-15ES24 | 128 | 39 | 33 | 12 | 63 | 50 | 54 | 24 | 0-15 | no | no | yes | no | no | no | yes | no | no | yes |
| 32 | KWS 053 | 122 | 7 | 34 | 20 | 68 | 11 | 54 | 24 | 0-18 | no | no | no | no | no | no | yes | no | yes | no |
| 33 | KWS 060 | 126 | 27 | 37 | 45 | 68 | 11 | 61 | 1 | 0-20 | no | yes | no | no | no | no | no | no | yes | yes |
| 34 | KWS 074 | 126 | 27 | 34 | 20 | 65 | 41 | 61 | 1 | 0-23 | no | no | no | no | no | no | no | no | het | no |
| 35 | KWS 081 | 127 | 33 | 38 | 48 | 67 | 21 | 60 | 4 | 0-16 | no | no | no | no | yes | no | yes | no | no | no |
| 36 | KWS 083 | 128 | 39 | 35 | 31 | 63 | 50 | 55 | 17 | 0-19 | no | no | no | no | no | no | no | no | yes | no |
| 37 | KWS 087 | 126 | 27 | 34 | 20 | 67 | 21 | 56 | 10 | 21-0 | no | no | no | no | no | no | no | no | yes | no |
| 38 | LA06146E-P4 | 121 | 1 | 32 | 3 | 65 | 41 | 47 | 49 | 0-17 | no | no | no | no | no | no | yes | yes | yes | no |
| 39 | LA08090C-9-2 | 128 | 39 | 35 | 31 | 67 | 21 | 48 | 47 | 0-20 | no | no | no | no | no | no | no | no | no | no |
| 40 | LA08265C-50 | 124 | 16 | 35 | 31 | 68 | 11 | 54 | 24 | 0-17 | no | no | no | no | no | no | yes | no | yes | no |
| 41 | LA09011UB-2 | 121 | 1 | 30 | 1 | 68 | 11 | 51 | 45 | 15-2 | no | no | no | ND | no | no | no | no | yes | no |
| 42 | LA09225C-33 | 128 | 39 | 36 | 37 | 69 | 5 | 54 | 24 | 0-14 | no | no | no | no | no | no | no | no | no | no |
| 43 | NC10435-11 | 123 | 12 | 34 | 20 | 68 | 11 | 53 | 35 | 13-1 | no | no | no | no | no | no | yes | no | yes | yes |
| 44 | NC12-22225 | 128 | 39 | 33 | 12 | 65 | 41 | 53 | 35 | 17-5 | Fhb1 | yes | no | no | no | no | no | no | yes | no |
| 45 | NC13-20076 | 124 | 16 | 36 | 37 | 66 | 30 | 56 | 10 | 1-19 | no | no | no | no | no | no | no | no | no | no |
| 46 | NC13-22350 | 127 | 33 | 34 | 20 | 66 | 30 | 56 | 10 | 13-4 | Fhb1 | yes? | Ning | no | no | no | no | no | no | yes |
| 47 | NC13-23449 | 127 | 33 | 36 | 37 | 69 | 5 | 56 | 10 | 0-16 | no | no | no | no | no | no | yes | no | no | no |
| 48 | VA12W-68 | 123 | 12 | 32 | 3 | 66 | 30 | 53 | 35 | 21-0 | no | yes? | no | no | ND | no | no | no | no | no |
| 49 | VA13W-38 | 122 | 7 | 32 | 3 | 67 | 21 | 53 | 35 | 0-18 | no | no | no | no | no | no | yes | yes | no | no |
| 50 | VA09MAS6-122-7-1 | 124 | 16 | 31 | 2 | 68 | 11 | 57 | 9 | 0-20 | no | no | no | no | no | no | no | no | no | no |
| 51 | VA08MAS1-188-6-4-1 | 124 | 16 | 32 | 3 | 66 | 30 | 53 | 35 | 0-19 | het | no | yes | no | no | no | no | no | no | no |
| 52 | VA13FHB-26 | 125 | 22 | 35 | 31 | 65 | 41 | 55 | 17 | 0-19 | no | no | het | no | no | no | no | no | no | no |
| 53 | VA14FHB-14 | 123 | 12 | 34 | 20 | 67 | 21 | 58 | 7 | 0-18 | no | no | no | no | no | no | no | no | yes? | no |
| 54 | VA14FHB-13 | 122 | 7 | 33 | 12 | 66 | 30 | 55 | 17 | 0-17 | no | no | no | no | no | no | no | no | yes | no |
| 55 | VA14FHB-28 | 121 | 1 | 34 | 20 | 69 | 5 | 54 | 24 | 0-20 | no | no | no | no | no | no | no | no | no | no |
| | Mean | 125 | | 34 | | 67 | | 54 | | . | | | | | | | | | | |
| | LSD (0.05) | 4 | | 4 | | . | | . | | . | | | | | | | | | | |
| | CV% | 1.7 | | 5.6 | | . | | . | | . | | | | | | | | | | |
| | Mean v GEBV Correlation | 0.20 | | 0.30 | | 0.43 | | 0.44 | | | | | | | | | | | | |

# EVALUATING METHODS OF UPDATING TRAINING DATA IN LONG-TERM GENOMIC SELECTION FOR FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

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## OBJECTIVE

To examine prediction accuracy and response to selection when updating the training population each cycle with the best predicted lines, the worst predicted lines, random lines, criterion-selected lines, or no lines.

## INTRODUCTION

The improvement of populations in plant breeding through recurrent selection may benefit tremendously from genomic selection. Of particular worth are the high accuracies and shortened breeding cycles of genomic selection, which allow for greater genetic gains per unit time (Bernardo and Yu 2007; Heffner *et al.* 2009; Lorenz *et al.* 2011; 2012). Genomic selection has already been employed in established oat and barley breeding programs (Asoro *et al.* 2013; Sallam *et al.* 2015). The advantages of genomic selection depend on maintaining sufficient genetic gain over time. This requires accurate predictions, based on markers located throughout the genome, of the genotypic value of candidates within a selection population (Meuwissen *et al.* 2001). Training the statistical model necessitates reliable phenotypic data on a training population and sufficient marker data such that many or all quantitative trait loci (QTL) are in linkage disequilibrium (LD) with at least one marker (Lorenz *et al.* 2011). If such requirements are fulfilled, the training data will capture the effects of alleles at QTL. Selection can then act to increase the frequency of favorable alleles in a population and shift the mean of a population in a desirable direction.

Maintaining selection accuracy over time will likely require updating the training population with new genotypes and there are practical considerations in how a breeder selects lines to fulfill this need (Lorenz and Smith, 2015). All breeding programs will advance their "best" lines to the next stage of evaluation and this data could be used to update the training population. An important question is whether it is necessary to include other lines for phenotyping strictly for the purpose of building prediction models. If so, then then how to do this will be an important consideration when allocating resources for expensive traits like FHB resistance and DON concentration.

The objective of this study was to investigate various methods of updating a training population and their impact on genomewide recurrent selection. Using simulations, we envisioned a breeding program implementing genomewide recurrent selection for FHB resistance in barley. Six different training population update methods were compared, along with two scenarios of training population composition. Over 15 cycles of selection, we tracked prediction accuracy and response to selection.

## METHODS AND MATERIALS

We simulated a barley breeding program selecting for FHB resistance using genomic selection and a one-year breeding cycle (Figure 1). To incorporate the observed LD structure in barley breeding populations into our simulations, we used empirical marker data from the University of Minnesota (UMN) and North Dakota State University (NDSU) breeding programs. Marker

genotypes from 768 six-row spring inbred lines at 3,072 bi-allelic SNP loci were obtained from the Triticeae Toolbox (T3) database (Close *et al.* 2009). Markers missing more than 10% data and lines missing more than 10% data were excluded. We set all heterzyogous genotype calls to missing and imputed missing genotypes using the mean genotype call across all samples, rounded to the nearest homozygote. This left a set of 764 breeding lines and 1,590 homozygous markers spanning 1,137 cM.

*Genetic Model to Simulate QTL*
Each iteration of the simulation was initiated by randomly selecting 100 SNP loci to become causal QTL. Genotypic values for QTL were drawn from a geometric series, as suggested by Lande and Thompson (1990). At the *k*th QTL, the value of the favorable homozygote was $a^k$, the value of the heterozygote was 0, and the value of the unfavorable homozygote was $-a^k$, where $a = (1-L)/(1+L)$. The value of the first allele of a QTL was randomly assigned to be favorable or unfavorable. The genotypic value of a given individual was calculated as the sum of the effects of QTL alleles carried by that individual. Phenotypic values were simulated by adding nongenetic effects to the genotypic values.

Phenotyping was assumed to take place in three environments with one replication. The variance of environmental effects and the variance of residual effects remained unchanged over cycles of selection, allowing the heritability to vary. The mean phenotypic value of each individual over the three environments was used in genomewide prediction.

*Base Population and Cycle 1 of Genomic Selection*
The base population (i.e. cycle 0 training population) consisted of genotypic and phenotypic data on the 764 breeding lines. Based on these simulated phenotypes, the top fifty (most resistant) UMN lines and the top fifty NDSU lines were intermated between breeding programs to generate the cycle 1 population. Specifically, fifty crosses were simulated, using each parent once, and twenty $F_3$-derived lines were generated per cross. Gametes were generated following Mendelian laws of segregation, with recombination events simulated according to the genetic map positions of all loci (Muñoz-Amatriaín *et al.* 2011) and assuming no cross-over interference or mutation. Population development resulted in a pool of 1,000 $F_3$ selection candidates.

The marker data for the training population and selection candidates comprised genotypes at all loci except the 100 QTL. This essentially simulated "genotyping" with complete accuracy. Monomorphic markers and those with a minor allele frequency less than 0.03 were removed prior to genomewide prediction. Marker effects were predicted using ridge-regression best linear unbiased prediction (RR-BLUP) according to the model $y = lu + Z_{TP}u + e$, where y was an Nx1 vector of the phenotypic means of N training population lines, *l* was a Nx1vector of ones, u was the grand mean, $Z_{TP}$ was a Nxm incidence matrix of training population genotypes for m markers, u was a mx1 vector of marker effects, and e was a Nx1 vector of residuals. Elements of $Z_{TP}$ were 1 if homozygous for the first allele, -1 if homozygous for the second allele, and 0 if heterozygous. Genotypic values of the $F_3$ selection candidates were predicted as $g=Z_{SC}u$, where g was a 1,000x1 vector of predicted genotypic values, $Z_{SC}$ was a 1,000x *m* matrix of selection candidate genotypes, and u was a mx1 vector of predicted marker effects. Elements of $Z_{SC}$ were the same as those in $Z_{TP}$.

*Cycles 2 Through 15 of Genomic Selection*
Subsequent cycles of the simulation consisted of three steps: 1) crossing and population development, 2) prediction and selection, and 3) training population updating. These are outlined in the diagram presented in Figure 1. Parents selected in the previous cycle were randomly intermated to form a pool of selection candidates. Again, fifty crosses were simulated and 1,000 $F_3$-derived selection candidates were generated. Prior to predictions, we removed monomorphic markers and those with a minor allele frequency less than 0.03 in both the pool of selection candidates and

in the training population. Since markers could become monomorphic due to selection or drift, the number of markers used for prediction decreased over breeding cycles. We predicted marker effects using the above linear model and phenotypic and genotypic data on the training population. These marker effects were then used to predict genotypic values of the 1,000 selection candidates, and those with the top 100 predicted genotypic values were designated as parents for the next cycle. A subset of all selection candidates were then designated as new additions to the training population according to one of the updating methods described below. We simulated phenotypes for these additions and merged the phenotypic and genotypic data to the pool of training population data.

*Methods of updating the training population*
Six different methods ("Top," "Bottom," "Random," "PEVmean," "CDmean," and "No Change") of updating the training population were explored in the simulations. Each method constituted an independent simulation experiment, and in each case 150 selection candidates from each cycle were chosen and added to the training population. For "Top" and "Bottom," selection candidates with the best ("Top") or worse ("Bottom") values were added to the training population. For "Random," a random sample of selection candidates were added to the training population, and for "No Change," the training population was not updated over breeding cycles. The other two methods were "PEVmean" and "CDmean" as described by Rincent et al. (2012). Using only genotypic data on all individuals, these algorithms aim to create a training population by optimally sampling individuals for which phenotypic data is available to predict the value of individuals for which no phenotypic data is available.

## RESULTS AND DISCUSSION

Prediction accuracy (Figure 2) consistently decreased over cycles of selection for all methods of updating the training population and in both updating scenarios. Within and between scenarios, we observed differences among the update methods in the decay rate of prediction accuracy. A prominent observation was the precipitous decline in accuracy when not updating the training population (i.e. "No Change"). Early in breeding cycles, prediction accuracy for this method was similar to the remaining methods, but by cycle five had decayed beyond the remaining methods. As expected, identical trends were observed for "No Change" in both updating scenarios.

Among methods of actively updating the training population (i.e. excluding "No Change"), differences in prediction accuracy were observed in early cycles, but became increasingly similar in later cycles. The "Top" method resulted in a small, but noticeable accuracy advantage early on that persisted for several cycles. On the other hand, the "Bottom" method displayed a noticeable disadvantage that persisted for a similar length of time. The "Random," "PEVmean," and "CDmean" methods were highly comparable and yielded accuracies intermediate of the "Top" and "Bottom" methods. By cycle ten, the differences between active methods of updating were negligible. These patterns were observed in both the "Cumulative" and "Window" scenarios. Accuracy decay was slightly greater in the "Cumulative" scenario (Figure 2A) compared to the "Window" scenario (Figure 2B). By the fifteenth breeding cycle, the difference in these decay rates amounted to a difference in prediction accuracy of roughly 0.02 – 0.04.

In our simulation experiment of recurrent genomic selection, we confirmed the need to update the training population over breeding cycles. Among the tested methods of updating the training population, adding the lines predicted to have the greatest genotypic value (i.e. the "Top" method) is the most attractive. The desirability of this method stems not only from the resulting prediction accuracy and response to selection, but also from its simplicity and practicality. This means that a breeder can rely primarily on data from typical trials that include the best performing breeding lines to update training population data sets.

## ACKNOWLEDGEMENTS AND DISCLAIMER

We thank Celeste Falcon and Ahmad Sallam for their contributions to the discussions from which this research originated. We also thank the Minnesota Supercomputing Institute for the high performance computing resources used to conduct the experiments. This research was partially supported by funding from the Minnesota Department of Agriculture, Rahr Malting Company, the U.S. Department of Agriculture under Agreement No. 59-0206-4-020 which is a cooperative project with the U.S. Wheat & Barley Scab Initiative, and the National Research Initiative or Agriculture and Food Research Initiative Competitive Grants Program (grant no. 2012-67013-19460) from the USDA National Institute of Food and Agriculture.

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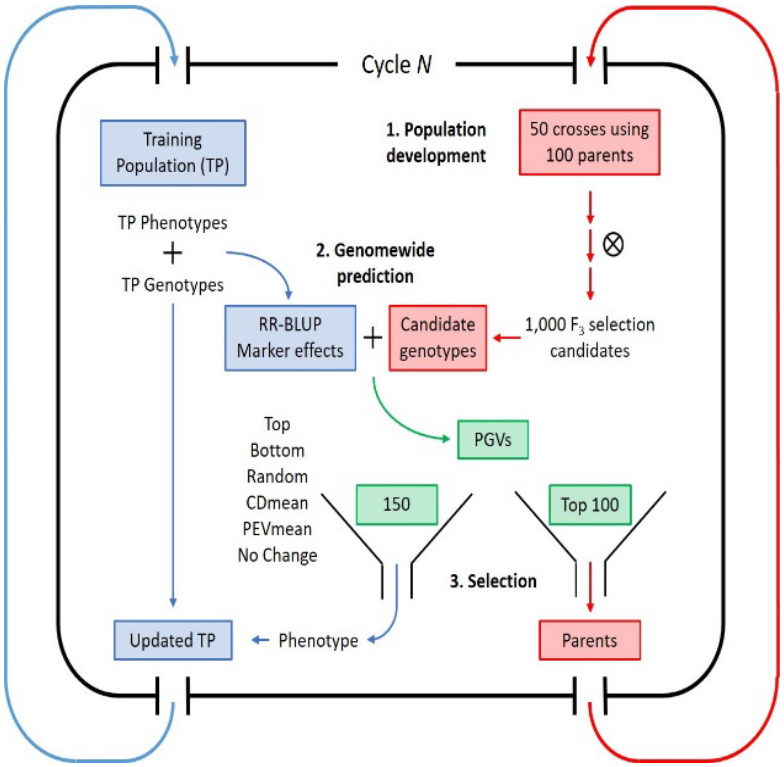


**Figure 1**. A single breeding cycle is broken down into two main streams. Blue indicates steps involving the training population, and red indicates steps involving crossing and population development. Green indicates the intermediate step of selection. 1) Fifty crosses are made using 100 randomly intermated parents from the previous cycle. Population development follows and 1,000 selection candidates are genotyped at the F3 stage. Marker effects are estimated using genotypic and phenotypic data from the training population (TP). 2) The predicted genotypic values of the selection candidates (PGVs) are used in decision-making. 3) The 100 best selection candidates are selected as

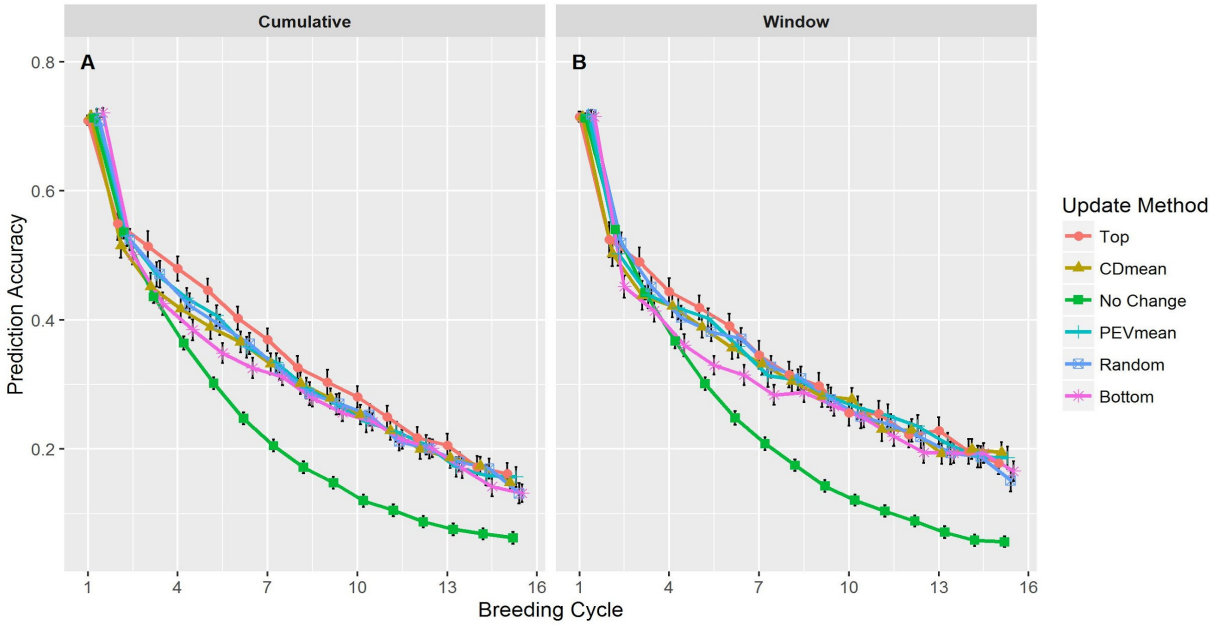


**Figure 2.** Prediction accuracy over breeding cycles of the simulation. Accuracy was measured as the correlation between the predicted and true genotypic values of the selection candidates. Line colors and point shapes delineate the different methods of updating the training population. Plots are separated into the "Cumulative" (A and C) and "Window" (B and D) updating scenarios. Average values are shown with 95% confidence intervals. To help reduce plot clutter, points for each update method are given a small, consistent jitter along the x-axis.

# DISCOVERY OF *FUSARIUM GRAMINEARUM* RESISTANCE IN *AEGILOPS TAUSCHII* GERMPLASM AND INTROGRESSION INTO WHEAT

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**ABSTRACT**

Discovery of genetic resistance to Fusarium head blight (FHB), caused by *Fusarium graminearum*, is vital for the continued improvement of modern wheat. In this study, 109 accessions of the wheat D-genome progenitor species *Aegilops tauschii* Coss. were screened for FHB resistance. Greenhouse grown *Ae. tauschii* were infected with *F. graminearum* by single-floret inoculation (SFI) and disease severity was rated as the percentage of infected spikelets at 21 day post-inoculation. An apparent relationship was identified between the geographical origins of *Ae. tauschii* accessions and FHB resistance. Higher levels of FHB resistance were observed in accessions collected from regions bordering the Caspian Sea that receive higher levels of annual rainfall. In total, 12 resistant to moderately resistant *Ae. tauschii* accessions were identified. One accession, TA1662, with moderate resistance to FHB was crossed directly with the hexaploid wheat line KS05HW14 and backcrossed to restore typical D genome segregation. A population of 141 $BC_2F_{4:7}$ introgression lines (ILs) was planted in a replicated headrow nursery in Mason, MI, in 2015, and FHB incidence and severity were recorded. DNA was isolated from the ILs, genotyping-by-sequencing was performed, and linkage maps of introgressed loci were constructed. QTL analysis identified a QTL for FHB severity on the proximal portion of 7DL. Lines fixed for the *Ae. tauschii* allele at the 7DL QTL had lower average FHB severity than those fixed for the wheat allele. Then, using SFI under greenhouse conditions, three lines fixed for the *Ae. tauschii* allele at the 7DL QTL were compared to three lines fixed for the wheat allele. Those fixed for the *Ae. tauschii* allele had lower disease severity and fewer *Fusarium* damaged kernels ($P$-value < 0.05). The resistant germplasm identified and developed in this study will support long-term Fhb resistance breeding efforts.

# DEVELOPMENT OF NEW WHEAT VARIETIES RESISTANT TO FHB THROUGH MICROSPORE *IN VITRO* SELECTION TECHNOLOGY

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**ABSTRACT**

Germplasm resistant to Fusarium Head Blight (FHB) is the most important and powerful tool to manage the disease. Based on natural selection, microspore culture offers a method to develop new germ lines with a varying degree of resistance to FHB. An *in vitro* selection in presence of *Fusarium* trichothecenes was used to develop homozygous doubled haploid lines of various genotypes showing resistance against FHB. Microspores from $F_1$ of 21 crosses of spring and winter wheat (*Triticum aestivum* L.) were subjected to selection against mixtures of *Fusarium* toxins (DON, 3-ADON, 15-ADON, NIV, T-2) in culture media. So far a total of 3232 doubled haploid lines were produced that have been incorporated into Canadian wheat breeding towards development of FHB resistant germplasm. The presence of trichothecenes in media had deleterious effects on the viability of microspores, formation of embryo like structures and regeneration rate of plants from embryo like structures. The response of different crosses was different to mycotoxins and as well as to media components and growth regulators. We found a novel epigenetic modifier Trichostatin A to be very efficient in stimulating embryogenesis and improving regeneration of plantlets. We were able to regenerate doubled haploids for crosses very recalcitrant to tissue culture. The doubled haploids lines produced through microspore culture technique will be screened for FHB resistance and DON accumulation in growth chamber followed by field trials in different regions of Canada.

# TRENDS IN FHB RESISTANCE IN THE NORTHERN UNIFORM FHB NURSERY

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## ABSTRACT

Wheat breeders in the Northeastern USA have been entering breeding lines in two uniform Fusarium Head Blight (FHB) nurseries (termed the PNUWWSN and NUWWSN) for many years. Each breeder has different criteria for entering lines into these tests, though most lines are elite lines and are candidates for release as new cultivars. Thus tracking trends over years in these trials is a way to assess changes in FHB resistance. The trials are conducted in multiple locations each year and data is collected on multiple FHB traits. The same checks have been used since 1998: Ernie [MR], Freedom [MR] and Pioneer 2545 [S]. In this study, we will assess the change in FHB trait values, the incidence of lines superior to the moderate resistant checks, and the incidence of QTL associated with FHB resistance over time.

A total of 1,212 lines from 18 breeding programs from 1998 to 2016 were evaluated. We determined the mean of lines first tested in a particular year and then regressed that mean on that year for each of seven FHB traits. Principal component analysis of the seven traits was conducted and the first PC score for each line was used as an integrative trait. Trends over time were clearer when using standardized data than when using simple Best Linear Unbiased Predictions (BLUPs). Overall years, there was a significant ($p < 0.05$) reduction in field severity ($r^2$=0.577), index ($r^2$=0.74), and ISK ($r^2$=0.29). There was considerable noise in the data from 1998 to 2002, and so we performed a second set of regressions using only data from 2003 to 2016. In these analyses, there was a significant reduction in severity ($r^2$=0.62), index ($r^2$=0.72), ISK ($r^2$=0.47), DON ($r^2$=0.43), and PC1 score ($r^2$=0.59). There has been a significant increase in the percentage of lines that have lower FHB trait values than the MR checks for all traits except for DON. In 1998 & 1999, just 19.8% of the lines were numerically lower than the MR checks for index. In 2015 & 2016, 68% of the lines had lower index values than the MR checks. On average 66% of the entries are better than the MR checks today, versus just 38% in 1998 & 1999. While no trend was noted for DON, in 2015 and 2016, 54.7% of the lines had lower DON than the MR checks and 33% had lower DON than Truman (the "R" check since 2004).

These analyses revealed a significant reduction in FHB trait values for lines entered in the USWBSI tests and especially since 2003. This is accompanied by a significant increase in lines with trait values that are lower than those of the MR checks.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, underAgreement No. 59-0206-9-064. This is a cooperative project with the U.S. Wheat & Barley ScabInitiative. 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 ofAgriculture.

# A META-ANALYSIS OF THE GENETICS OF FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

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**ABSTRACT**

Fusarium head blight (FHB) devastated the once thriving malting barley industry in the Upper Midwest region of the USA after a series of epidemics starting in 1993. Due to the severe losses caused by these epidemics, breeding for resistance to FHB and the accumulation of mycotoxins produced by causal *Fusarium* pathogens became a priority for many barley improvement programs across North America. Extensive screening efforts of over 30,000 *Hordeum* accessions to FHB revealed very few sources of resistance. Early classical genetic studies revealed the quantitative nature of FHB resistance in barley. Subsequently, a number of molecular mapping studies were initiated to elucidate the number, chromosomal location and effect of resistance loci in these sources. To summarize this body of research, we conducted a meta-analysis of quantitative trait loci (QTL) reported for reduced FHB severity and DON accumulation, along with various agro-morphological traits thought to affect them, based on a single consensus map. This consensus map was constructed using marker data from eight mapping populations, plus two previously developed consensus maps based on simple sequence repeat and single nucleotide polymorphism markers. Consensus map construction was done using linear programming implemented in the LPmerge package of R. Marker order in the consensus map displayed high collinearity with other genetic maps for all chromosomes with an average correlation of 0.97. Sixty-seven and forty unique QTL were detected for low FHB severity and DON accumulation, respectively. These QTL were found across each of the seven barley chromosomes with most explaining just a small portion of the total phenotypic variation. Additionally, many of these QTL were not robust because they were detected in only one of several trials conducted at various locations over multiple years. Agro-morphological traits are thought to influence the level of FHB severity developing on barley. This aspect was investigated by considering these traits together with FHB severity and DON concentration on the consensus map. In chromosome 2H, several major effect genes such as *Ppd-H1* and *Eam6* for heading date, *Vrs1/vrs1* for two-rowed vs. six-rowed spike type and *Cly1*/*cly1* for chasmogamous vs. cleistogamous florets map to locations coincident for QTL controlling low FHB severity and DON. The same was true for the *Nud*/*nud* gene controlling the hulled vs. hulless character in chromosome 7H. These results suggest that some genes controlling agro-morphological traits may have a pleiotropic effect on FHB severity and the subsequent accumulation of mycotoxins. Although cultivars with moderate FHB resistance have been developed (e.g. Quest), the rate of progress has been relatively slow due to a lack of good resistance sources, the complex genetics underlying the trait, the variability associated with screening and selecting for FHB resistance in the field and the pleiotropic effect of various agro-morphological traits. Genomic selection offers a promising new approach for breeding for low FHB severity and DON accumulation in barley--whether the underlying selected loci represent true active resistance genes or the pleiotropic effect of an agro-morphological trait.

# DEVELOPMENT OF HIGH-THROUGHPUT DIAGNOSTIC MARKERS FOR *FHB1*, A MAJOR GENE FOR FHB RESISTANCE IN WHEAT

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## ABSTRACT

Fusarium Head Blight (FHB), mainly caused by *Fusarium graminearum*, is one of the most devastating wheat diseases worldwide. FHB not only significantly reduces grain yield but also affects grain quality due to *Fusarium* damaged kernels and mycotoxin contamination. Although FHB resistance is controlled by quantitative trait loci (QTLs), *Fhb1*, a QTL located on the short arm of chromosome 3B, shows a consistent major effect on reducing the disease spread within a spike in different genetic backgrounds. *Fhb1* has been widely used in wheat FHB resistance breeding programs worldwide. However, FHB resistance evaluation is laborious, time-consuming, and significantly influenced by the environments, which has significantly affected effective transfer of *Fhb1* into locally adapted wheat cultivars in breeding programs. Marker-assisted-selection (MAS) can increase the precision and efficiency of selection for a specific gene in breeding. Previously several markers tightly linked to *Fhb1* have been used for MAS, including the *Fhb1* flanking makers *Gwm533* and *Gwm493*, and tightly linked STS markers *STS256* and *UMN10*, and SNP markers *SNP8* and *SNP319*. However, none of them are functional markers, increased false positives reduces selection efficiency. Therefore, development of user-friendly and high-throughput diagnostic markers for *Fhb1* becomes critical for success in use of the gene in wheat breeding. More recently, we have cloned an *Fhb1* candidate by map-based cloning and found that lose-of-function of the gene confers FHB resistance. Two functional markers, *Fhb1-STS* and *Fhb1-KASP*, are developed based on the causal variation in the gene. *Fhb1-STS* is gel based marker that can be used in breeding programs with simple setup, whereas *Fhb1-KASP* is designed for medium throughput in these breeding programs that set up to run KASP markers. Both markers are codominance and feasible to genotyping segregating breeding populations, and are suitable for MAS to pyramid *Fhb1* with other resistance genes. Using thee markers, we screened a worldwide wheat collection and found that the *Fhb1* resistance allele is present only in some Chinese and Japanese accessions, not in the accessions from other areas. Among those with *Fhb1* resistance allele, many accessions including Sumai3, Ning7840, Huangcandou, Huangfangzhu, Baisanyuehuan, Wangshuibai and Nynbai have been reported to carry *Fhb1* in previous QTL mapping studies. Therefore, both *Fhb1-STS* and *Fhb1-KASP* can be used as diagnostic markers for *Fhb1* in wheat breeding programs.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture. 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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

# BORN, BRED AND BREWED IN NEW YORK

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**ABSTRACT**

The rapidly expanding craft brewing industry in New York has spurred interest in a self-sustaining local brewing economy from ground to glass. Barley production in New York is increasing with demand from craft malthouses but large scale production is still challenged by Fusarium head blight (FHB) and foliar pathogens. Variety testing over the past three years has not identified any cultivars with adequate FHB resistance and agronomics for New York. The Cornell Small Grains breeding program has begun a two-row spring malting barley breeding program to address these needs. High-throughput seed phenotyping and genomic selection are popular plant breeding buzzwords but their implementation in brand new breeding programs is can be challenging. We are using a single kernel near-infrared spectroscopy machine to phenotype large quantities of seed for malt quality traits and will be implementing multivariate genomic selection for disease traits, including FHB and deoxynivalenol, to rapidly advance superior breeding material to the evaluation stage.

# ASSOCIATION MAPPING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SYNTHETIC HEXAPLOID WHEAT

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## ABSTRACT

Synthetic hexaploid wheat (SHW) (×*Aegilotriticum* spp., 2n = 6x = 42, AABBDD) possess genetic diversity for resistance to several biotic and abiotic stresses. In order to investigate the prospects of transferring useful genes from wild and domesticated progenitors into hexaploid bread wheat (*Triticum aestivum* L.), we developed 150 SHW lines using durum wheat (*T. turgidum* L. var. *durum* Desf.) and other five tetraploid subspecies (*T. turgidum* spp. *carthlicum*, *dicoccum*, *polonicum*, *turgidum* and *turanicum*, 2n = 4x = 28, AABB) in crosses with *Aegilops tauschii* Coss. (2n = 2x = 14, DD). The goals of this project were to identify SHW lines carrying Fusarium head blight (FHB) resistance and to map putative novel FHB-resistant QTLs in the resistant SHW lines. In the evaluation experiments 150 SHW lines and their 73 tetraploid wheat parents have been tested in two greenhouse seasons and in the field nurseries at two locations (Fargo and Prosper, ND) for two years (2015 and 2016). The experiments were performed using a randomized complete block design (RCBD) with three replications. The common wheat cultivars 'Sumai 3' and 'Grandin' were used in all the experiments as resistant and susceptible checks, respectively. The statistical analyses of disease severity in the greenhouse and field nurseries showed a significant correlation among the experiments. According to the ANOVA and homogeneity tests, the FHB disease severity data from the two greenhouse seasons were pooled. For field experiments, the FHB data from the two locations were combined for each year. All the SHW lines and their tetraploid parents were genotyped using the Illumina wheat 9K-SNP array. When the mixed linear model (MLM) including both kinship and population structure was used for association mapping analysis, no significant associations were detected between marker data and disease severity. However, based on the general linear model (GLM) including population structure only, a number of marker loci showed significant association with disease severity both in the tetraploid and SHW lines. Several markers on chromosomes 1A, 2D, 3D, 6B and 7B of the SHW lines were verified in different environments including field and greenhouse seasons. By analyzing the FHB severity data, we found several resistant SHW lines having susceptible tetraploid parent, which supports the mapping results that the D genome has genomic regions associated with FHB resistance. These loci originated from *Ae. tauschii* may represent a source of novel resistance genes. Several SHW lines having resistant tetraploid parent showed as low FHB severity as the resistant check Sumai 3, indicating that they may be the useful base germplasm for improving wheat for FHB resistance in wheat breeding.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture. 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.

# MORPHOLOGICAL AND FHB TRAIT VARIATION IN THE ELITE EASTERN MAPPING PANEL

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## ABSTRACT

Previous studies have suggested that morphological traits are related to FHB resistance. The objectives of this study were to evaluate morphological traits in the TCAP elite eastern mapping panel and use phenotypic and genotypic data to conduct a genome wide association study (GWAS). Two hundred sixty two wheat cultivars and breeding lines from the mapping panel were used in two experiments each conducted over two years (2015-2016) at Lexington, KY. In the first study, anther extrusion, plant height, spike length, spike density, number of florets, peduncle length and spike inclination were measured. Evaluation of FHB traits was carried out in an inoculated, irrigated nursery; heading date, plant height, disease incidence and severity, FHB rating, FHB index, *Fusarium* damaged kernels (FDK) and DON were measured. There were significant differences among the mapping panel entries for all traits evaluated. Significant genotype x year interaction for all morphological traits was observed; broad sense heritabilities ranged from 0.39 to 0.61. High heritabilities of all scab traits were recorded, though genotype x year interaction was significant. Correlations between morphological and scab traits varied by heading date. Eighteen of the panel entries had the R alleles at *Fhb1* though the average severity, FDK and DON was not lower in those lines than in the remainder of the entries. All FHB traits were significantly ($P < 0.05$) higher in lines with the height reducing alleles at the *Rht D-1* locus.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-4-002. 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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

# DEVELOPMENT OF FUSARIUM HEAD BLIGHT RESISTANCE GERMPLASM IN HIGHLY ADAPTED SPRING WHEAT BACKGROUND

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**ABSTRACT**

Fusarium head blight (FHB or scab) caused by primarily by *Fusarium graminearum,* is one of the most devastating plant diseases to effect wheat (*Triticum* aestivum L.) and barley (*Hordeum vulgare* L.) worldwide. Despite the fact that FHB may decrease grain yield and quality, it may also lead to serious mycotoxin contamination in the infected grains, which is harmful to the health of human beings and livestock. Breeding for resistance to FHB in wheat is growing considering the scarce availability of varieties conveying adequate resistance to FHB. However, it has been demonstrated that pyramiding other resistance QTLs with *Fhb1* provides enhanced resistance to FHB. Therefore, our research here at SDSU was to screen for FHB severity using double haploid (DH) spring wheat lines derived from selected four-way crosses combining several sources of resistance, to validate *Fhb1* and putative QTLs (Xmc758, Gwm33, xbacr176, Xgm120, Xwmc317, Xwmc332, Xwmc522 and Xwmc296) that could minimize the threat of FHB including the reduction of mycotoxins, to the producers, processors, and consumers of wheat. A total of 225 spring wheat were initially screened in replicated field evaluation nurseries in 2014 and 2015 in three northern plains locations. Lines with low FHB severity were selected as putative resistant materials and were tested in for agronomical traits, in replicated trials, and fungicide application trials. We used molecular techniques to validate DH lines and their corresponding parents. In this study, we report on our finding that support recent discoveries of pyramiding different sources of FHB resistance with *Fhb1* as an opportunity to further enhance FHB resistance of adapted wheat germplasm.

# DEVELOPMENT OF FUSARIUM HEAD BLIGHT RESISTANCE GERMPLASM IN HIGHLY ADAPTED WINTER WHEAT BACKGROUND

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**ABSTRACT**

Wheat is the third most important cereal in United States. However, production is severely constrained by many biotic stresses but the fungal pathogens *Fusarium graminearum* is the major causes of Fusarium head blight (FHB) which is a problematic disease for wheat and barley. FHB has seriously affected the production of wheat due to yield loss, low seed germination, and contamination of grain with mycotoxins. To date, no sources of resistance conferring complete resistance to FHB have been identified in wheat. We are using double haploid (DH) wheat lines derived from selected four-way crosses combining several sources of resistance to validate putative QTLs (Xmc758. Gwm33, xbacr176, Xgm120, Xwmc317, Xwmc332, Xwmc522 and Xwmc296) that could minimize the threat of FHB for the producers, processors, and consumers of wheat. This study attempted to develop and validate wheat lines that should display resistant characteristics to FHB given the materials genetic background. We report that over 50% of our lines had reduction to FHB which builds upon evidence accumulated from multiple studies in which pyramiding multiple sources and components of resistance with *Fhb1* serves to increase resistance to FHB.

# EVALUATION OF WINTER BARLEY CULTIVAR EVE FOR QUANTITATIVE RESISTANCE TO FUSARIUM HEAD BLIGHT

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## ABSTRACT

Fusarium head blight (FHB), caused by the pathogen *Fusarium graminearum* Schwabe, can result in severe yield and quality losses for barley (*Hordeum vulgare*) producers in the Mid-Atlantic region via kernel damage and production of mycotoxins. The demand for cultivars with enhanced resistance to prevalent diseases is essential to barley producers in order to meet the current and future market demands for winter barley in the production health foods, livestock feed, and malt products. The objectives of this study are to identify the FHB resistance QTL in the hulless winter barley cultivar Eve and to develop diagnostic markers for use in marker-assisted selection. Two mapping populations, comprised of recombinant inbred lines (RIL), were derived from crosses of 'Eve' to FHB susceptible parents (Eve/'Doyce' and Eve/VA07H-35WS) for use in mapping resistance to FHB. In 2015-2016 growing season, 180 RILs from each population were evaluated for FHB incidence and severity with the assistance from cooperators in KY and VA. In the 2014-2015 growing season both Eve RIL populations were evaluated in KY, VA, NC, and China for severity and incidence. Grain samples from both growing seasons were evaluated for *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) levels. In the Eve/Doyce (E/D) population a significant ($P$ = <0.0001) correlations were observed between heading date and FHB incidence (r = -0.67376 and FHB severity (r = -0.61233) for only the 2016 Blacksburg, VA data. Eve populations were genotyped using a 9K SNP chip analysis. A putative QTL associated with higher FHB severity, FDK, and DON was identified in the E/D population with a logarithm of odds (LOD) of 5.57 and explaining 43.3% of the phenotypic variation. FHB resistant QTL identified in this population will be validated in the Eve/VA07H-35WS population and diagnostic markers will be identified for use in marker-assisted selection.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-4-032. 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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

# EFFECTS OF ELEVATED [$CO_2$] ON THE DEFENSE RESPONSE OF WHEAT AGAINST *FUSARIUM GRAMINEARUM* INFECTION

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**ABSTRACT**

Fusarium head blight (FHB) is one of the world's most devastating wheat diseases, and results in significant yield loss and contamination of grain with harmful mycotoxins called trichothecenes. Despite emerging risks of increased mycotoxin contamination in food and feed associated with climate change, little is known about how rising [$CO_2$] will influence natural wheat resistance mechanisms against *Fusarium graminearum,* the primary etiological agent of FHB. In this study the defense response of wheat plants grown at ambient (400 ppm) [$CO_2$] and elevated (800 ppm) [$CO_2$] was evaluated and compared. The timing and magnitude of the phytohormone defense response was different at elevated [$CO_2$]. Additionally, pathogenesis-related (PR) and lipoxygenase (LOX) gene transcript levels and metabolite concentrations were altered. Our results suggest that elevated [$CO_2$] reconfigures the defense response of wheat leading to changes in susceptibility to FHB and mycotoxin contamination.

# GENOME-WIDE ASSOCIATION MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT LINES GROWN IN PACIFIC NORTHWEST AND CIMMYT

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## ABSTRACT

Fusarium head blight is one of the destructive diseases of wheat in humid and semi-humid areas of the world. It has emerged in the Pacific Northwest (PNW) in recent years because of changing climate and rotation practice. The objectives of the present study were to characterize FHB resistance in spring wheat lines grown in PNW and CIMMYT and identify QTL associated with FHB resistance. A total of 170 spring wheat lines were evaluated in greenhouse and in field at Aberdeen, ID as well as at Saint Paul and Crookston, Minnesota in 2015 and 2016. Based on two years' data in greenhouse and field, 17 lines showing consistent resistance were selected as the starting resistance resources. These lines have no Sumai 3 or related backgrounds and can be used to develop FHB resistant cultivars for the PNW area. The 170 lines were genotyped using high-density Illumina 90K single nucleotide polymorphisms (SNPs) assay and ten other markers. A genome-wide association analysis was conducted with mixed model (Q+K). Consistent significant SNP associations with multiple traits (incidence, severity, FHB score, and deoxynivalenol concentration) were found on chromosome 2B, 4B, and 5B. The SNPs on chromosome 3B and the SSR marker *umn10* were not detected in any of the data sets, indicating the main FHB resistance loci in this panel does not include *Fhb1* locus. In summary, the resistance resources and associated SNP markers detected in this study can be used in the development of new FHB resistant cultivars in the PNW area.

## ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-064. 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 authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

# GROWERS' NEEDS AND INDUSTRY WANTS: A RETROSPECTIVE OF TWO DECADES IN THE TRENCHES IN THE BATTLE WITH FHB

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**ABSTRACT**

My position at the University of Minnesota was created in response to the historic FHB outbreaks of 1993 and 1994 in Minnesota and North Dakota. In the two decades since I took this appointment as extension specialist, I have been part of, and witnessed large changes in HRSW production practices. At first glance the public and private research communities have made gains in combating this opportunistic and ruthless pathogen. Albeit slower than producers and industry may have wanted or needed. Yet under this veneer of success lie some facts and statistics that suggest that a repeat of 1993 and 1994 epidemics is not out of the realm of possibilities, and that complacency has no place when it comes to scab management.

# MOLECULAR MAPPING OF QTL FOR FHB RESISTANCE INTROGRESSED INTO DURUM WHEAT

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**ABSTRACT**

In the past years, great efforts have been devoted to introgress FHB resistance from tetraploid and hexaploid wheat accessions into adapted durum wheat cultivars. However, most of the quantitative trait loci (QTL) for FHB resistance existing in the introgression lines are not well characterized or validated. In this study, we aimed to identify and map QTLs for FHB resistance in durum line 10Ae564 and cultivar Joppa. 10Ae564 is a $BC_1F_8$ durum wheat line, which has FHB resistance derived from cross and backcross of the durum wheat cultivar Lebsock to PI 277012, a hexaploid wheat line carrying major FHB resistance QTL on chromosome 5A. Joppa is a newly released durum wheat cultivar with less FHB susceptibility than other durum wheat cultivars currently grown in North Dakota (ND), but no information is available on existence of QTL for FHB resistance in this cultivar. We developed a mapping population consisting of 205 recombinant inbred lines ($F_{2:7}$) from a cross between Joppa and 10Ae564. Genotyping was done with the wheat 90K-SNP chips and 6,323 polymorphic SNP markers were identified in the population. Excluding those co-segregated markers, 1,272 SNP makers were used to construct a genetic map, which consisted of 36 linkage groups with the total length of 472.14 cM. Phenotyping of the population for FHB reactions was also conducted in greenhouse for two seasons (2015GH and 2016GH), as well as in field FHB nurseries for three experiments (2015Fargo, 2015China and 2016Fargo). Meanwhile, grains of inoculated spikes collected from the 2015 greenhouse experiment (2015GH) and the 2015 Fargo field experiment (2015Fargo) were tested for DON content, referred to DON_2015GH and DON_2015Fargo, respectively. QTL analysis indicated that one QTL on chromosome 2A from Joppa and two QTL each on 5A and 7A from 10Ae564 were associated with FHB resistance. The 2A QTL was detected in the two greenhouse experiments (2015GH and 2016GH) and in two field experiments (2015Fargo and 2015China), explaining 15.4%, 17.3%, 8.8%, and 8.0 % of the phenotypic variation, respectively. The 7A QTL was detected only in the two greenhouse experiments (2015GH and 2016GH), explaining 10.4 and 12.6 % of the phenotypic variation, respectively. The QTL on 5A was detected in one greenhouse season (2015GH), one field experiment (2015Fargo), and the two DON tests (DON_2015GH, and DON_2015Fargo), which explained 20.0%, 17.9 %, 17.6, and 6.2% of phenotypic variation, respectively. The 2A QTL from Joppa was mapped to the QFhb.rwg-2A region identified in the ND durum cultivar Ben in a previous study. The 5A QTL was mapped to the same region where the major QTL Qfhb.rwg-5A.2 is located in PI 277102. However, the 7A QTL is located in a region where no FHB QTL have been reported and may represent a new QTL. The origin of the 7A QTL is not known, but it is probably from Lebsock, a parent in the pedigree of 10Ae564. This study further confirms that minor QTL exist in ND durum cultivars and combining major QTL from hexaploid wheat and native durum germplasm will be useful for improving durum FHB resistance.

## ACKOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0200-3-004. 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.