Project FY24-HW-008: New Sources of Resistance to FHB and DON in Wheat

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1. What are the major goals and objectives of the research project?

Approved goals

The major goal of the project is to identify new source of FHB resistance.

Objectives

1) Screening of unique wheat-alien chromosome introgression germplasms that are available in the Wheat Genetics Resource Center at Kansas State University to identify new sources of resistance to FHB and DON in wheat.
2) Identification of genetic markers linked to FHB resistant loci in HSD2-32.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address these three items below.)*

What were the major activities?

1) Screening of 83 wheat-alien chromosome introgression germplasms for Type II FHB resistance under greenhouse condition.
2) Identification of Putative genetic loci resistant to FHB using bi parental population of CS x HSD2-32 AND Everest x HSD2-32.
3) Low-pass whole genome sequencing of HSD2-32.
4) Generation forwarding of HSD2-32 x Chinese Spring and HSD2-32 x Everest population by Single seed descent method.

What were the significant results?

Screening of wheat-alien introgression lines

A total of 83 wheat-alien chromosome introgression germplasms were screened for Type II Fusarium head blight (FHB) resistance, identifying 15 resistant lines (Fig. 1). ANOVA revealed highly significant genotypic differences ($p < 0.001$), indicating substantial variation in resistance levels among the tested lines. Replication effects were not significant ($p = 0.114$), and residual variance accounted for 15.67% of the total variance. A Tukey's Honestly Significant Difference (HSD) test ($\alpha = 0.05$) was performed to classify genotypic differences (Fig. 1). Among the tested genotypes, TA7709 exhibited the lowest FHB severity score (0.7), demonstrating the highest level of resistance. In contrast, the susceptible check recorded an FHB score of 10. The remaining resistant lines—TA7647, TA7557, TA2723, TA3425, TA7706, TA775, TA7689, TA7643, TA7583, TA7646, TA7558, TA7556, and TA7684—showed comparable resistance levels to the *Fhb6*-resistant check. In contrast, the wheat cultivars KanMark, Everest, and Bob Dole were classified as susceptible.

The selected resistant lines were crossed with the KanMark *ph1b* mutant for *ph1b*-induced chromosome engineering. The F1 hybrids derived from selected candidate Fusarium head blight (FHB)-resistant lines were evaluated under greenhouse conditions using point inoculation. This study aimed to assess the inheritance and effectiveness of resistance in these hybrids (Table 1).

Interestingly, only two introgression lines—TA7709 (CS/KanMark *ph1b-E. ciliaris* monosomic addition 7Y^{c}) and TA3425 (CS/KanMark *ph1b-Th. elongatum* partial amphiploid)—exhibited highly resistance to FHB. These two F2 populations were developed and planted in the FHB nursery at Rockey Ford in October 2024 for field evaluation. The result will be available in July 2025.

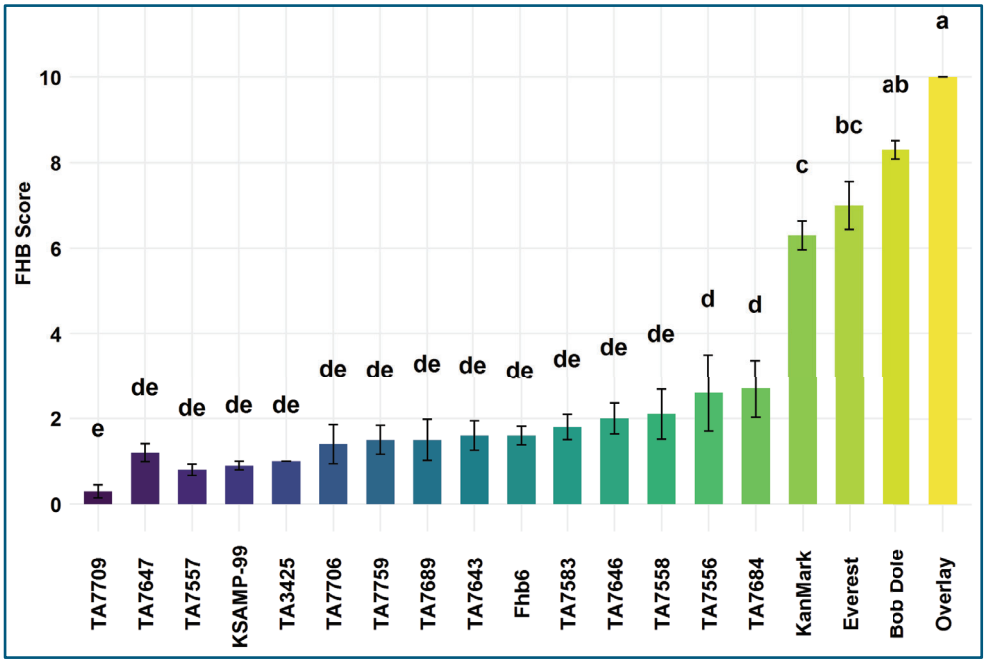

Figure 1. Candidate resistant lines identified from screening 83 wheat-alien chromosome introgression lines. Alphabets above the Standard Error (SE) bar represents the grouping of genotypes based on Tukey's HSD test (α = 0.05).
TA7709: CS-*E. ciliaris* disomic addition 7Yc; TA7647: CS-*L. racemosus* disomic addition 6Lr; TA7557: CS-*E. trachycaulus* disomic addition 5Ht; KSAMP-99: Larry-*T. monococcum* (TA2723) amphiploid; TA3425: CS-*Th. elongatum* partial amphiploid; TA7706: CS-*E. ciliaris* disomic addition 3Sc; TA7759: CS-E. trachycaulus disomic addition 7Ht; TA7689: CS-*Ae. speltoides* disomic addition 1S; TA7643: CS-*L. racemosus* disomic addition 2Lr; TA7583: CS-*E. ciliaris* disomic addition 1Sc; TA7646: CS-*L. racemosus* disomic addition 5Lr; TA7558: CS-*E. trachycaulus* disomic addition 6Ht; TA7556: CS-*E. trachycaulus* disomic addition 1St; TA7684: CS-*E. tsukushiensis* disomic addition 1Ets.

Table 1. Evaluation of F1 hybrids from selected candidate FHB-resistant lines using point inoculation under greenhouse conditions

Cross	FHB score
F1, TA3425 X KanMark ph1b	**0.7**
F1, TA7709 X KanMark ph1b	**1.7**
F1, *Fhb6* X KanMark ph1b	6.0
F1, TA7684 X KanMark ph1b	6.3
F1, TA7583 X KanMark ph1b	7.6
F1, TA7646 X KanMark ph1b	8.7
F1, TA7705 X KanMark ph1b	9.6
F1, TA7558 X KanMark ph1b	10.0
F1, TA7759 X KanMark ph1b	10.0
F1, TA7557 X KanMark ph1b	10.0
F1, TA7643 X KanMark ph1b	10.0

Identification of genetic markers linked to FHB resistant loci in HSD2-32

Identification of genetic markers associated with FHB resistant loci in HSD2-32 was done using F_2 population derived from the cross between HSD2-32 and Chinese Spring (CS) wheat. The putative candidate loci conferring FHB resistance was given in Table 2. We have identified and validated one SNP marker at 627,128,588 bp in Chr2D that was linked with a QTL region (628,574,465 – 633,228,046 bp) (Fig. 2 and Table 2) based on the SNPs derived from GBS. To design more SNP markers around the target QTL region in 2D chromosome, we sequenced the HSD2-32 genome and utilized the reference genome of Chinese Spring to identify the SNPs around the target QTL region in 2D chromosome. We designed seven SNPs around the target QTL region. Out of seven SNP markers tested, we identified one SNP marker at 623,580,344 bp in Chr2D which is present in the flanking region within the 5 Mb region of the target QTL region (628,574,465 – 633,228,046 bp). Using the F3 phenotyping data of HSD2-32 x CS we analysed the segregation pattern of the SNP marker and it was found that the SNP marker segregates in 3:1 ratio in the HSD2-32 x CS population and hence validated (Table 3). In total, we have identified and validated two SNP markers that are linked with the target QTL region (628,574,465 – 633,228,046 bp) in the HSD2-32 x CS population (Table 3). In parallel, we have identified two markers that show parental polymorphism between Everest and HSD2-32 at 623,580,344 bp and 633,199,691 bp in Chr2D that are linked with the target QTL region conferring FHB resistance in HSD2-32. These two markers segregate in 1:2:1 ratio in the HSD2-32 x Everest population (Table 4). To check the performance of this population under field condition we phenotyped this population in the Rockey ford FHB nursery. Utilizing the FHB score of HSD2-32 x Everest F2 population and the the genotyping data generated from this population from the two SNP markers, we checked the phenotypic ratio. The mean FHB score of the lines carrying this target QTL in the Evereset background does not significantly differ from the HSD2-32 or Everest or heterozygote. The mean FHB score of lines carrying HSD2-32 allele or Everest allele or heterozygote is around 6.0 for both the SNP markers tested. Out of 246 HSD2-32 x Everest F2 plants, 31 plants recorded the score of 0-2 (resistant to FHB); 70 plants recorded the score of 3-5 (moderately resistant); 57 plants recorded the score of 6-8 (susceptible) and 72 plants recorded the score of 9-10 (highly susceptible). Data is not available for 16 lines (Fig. 3)

Table 2. Putative candidate loci conferring FHB resistance identified using the F2 population derived HSD2-32 and Chinese Spring

Wheat Ch. No.	Position (cM)	LOD	PVE (%)	Add	Dom
2D	629.7	3.7969	9.0667	0.1961	3.9408
4A	45.99	3.3544	4.3105	0.9865	-1.295
4A	692.99	4.8402	12.0483	0.0389	5.3667
4B	172.14	3.6907	6.032	1.2405	-1.4174
4D	496.24	3.3928	4.4294	-0.4062	-1.9039
7A	52.23	4.2488	6.6896	1.7118	-1.2696

7A	180.23	3.2742	9.5815	1.7719	-2.048
7A	719.23	4.0034	6.8826	-1.4095	-1.639

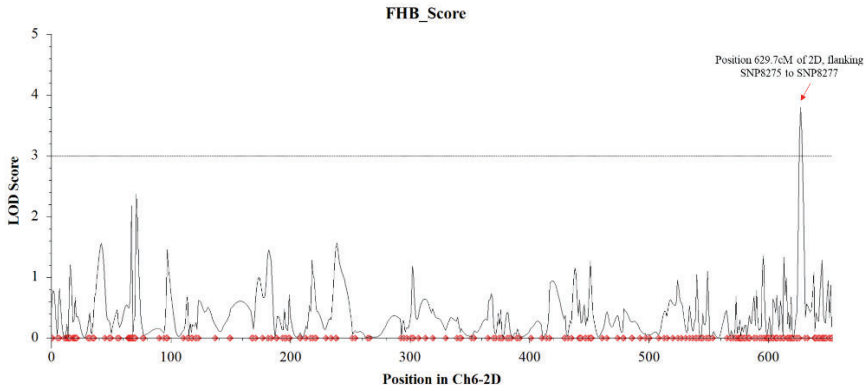

Figure 2. Position of validated QTL in the Chromosome 2D of wheat.

Table 3. Validation of SNP markers Chr2D_627128588 and Chr2D_623580344 that were used to genotype the F3 population of HSD2-32 x CS based on chi-square goodnesss of fit test

Chr2D_627,128,588	F3 FHB Score					Chi-square				
Category	Mean	Max	Min	Count		OV	EV	OV-EV	(OV-EV)^2	(OV-EV)^2/EV
Chinese Spring	6.2	9.7	2.3	24		24.0	24.5	-0.5	0.25	0.010
Heterozygote	3.8	8.5	0.0	37		74.0	73.5	0.5	0.25	0.003
HSD2-32	2.8	8.7	0.0	37						
Grand Total	4.0	9.7	0.0	98		Sample Chi-square value				0.01
Chr2D_623,580,344	F3 FHB Score					Chi-square				
Category						OV	EV	OV-EV	(OV-EV)^2	(OV-EV)^2/EV
Chinese Spring	6.0	9.7	0.0	26		26.0	24.5	1.5	2.25	0.091
Heterozygote	4.4	8.7	0.3	32		72.0	73.5	-1.5	2.25	0.030
HSD2-32	2.5	7.7	0.0	40						
Grand Total	4.0	9.7	0.0	98		Sample Chi-square value				0.12

Critical value@0.05 with d_f=1 is 3.84. If the critical value is larger than the sample's chi-square, then the null hypothesis can be rejected. Hence 3:1 segregation exist.

Table 4. Chi-square analysis of the SNP markers Chr2D_623580344 and Chr2D_633199691 that were used to genotype the F2 population of HSD2-32 x Everest

Category	**Chr2D_623,580,344**	**OV**	**EV**	**OV-EV**	**(OV-EV)^2**	**(OV-EV)^2/EV**
Everest	49	49	61.5	-12.5	156.25	2.5
Hetro	135	135	123	12	144	1.2
HSD2-32	62	62	61.5	0.5	0.25	0.0
Grand Total	246	246	246			

				Sample Chisquare value		3.7
Category	**Chr2D_633,199,691**	**OV**	**EV**	**OV-EV**	**(OV-EV)^2**	**(OV-EV)^2/EV**
Everest	53	53	61.5	-8.5	72.25	1.2
Hetro	125	125	123	2	4	0.0
HSD2-32	68	68	61.5	6.5	42.25	0.7
Grand Total	246	246	246			0
				Sample Chisquare value		1.9

Critical value@0.05 with d_f= 2 is 5.991. If the critical value is larger than the sample's chi-square, then the null hypothesis can be rejected. Hence 1:2:1 segregation exist.

We forwarded the HSD2-32 x CS polulation from F_6 to F_7 generation by single seed descent (SSD) method. We forwarded the BC_1F_5 population of HSD2-32 x CS to BC_1F_6 by SSD. Also, we phenotyped the F_2 population of HSD2-32 x Everest in the Rockey ford FHB nursery and forwarded the HSD2-32 x Everest to F_4 generation by SSD.

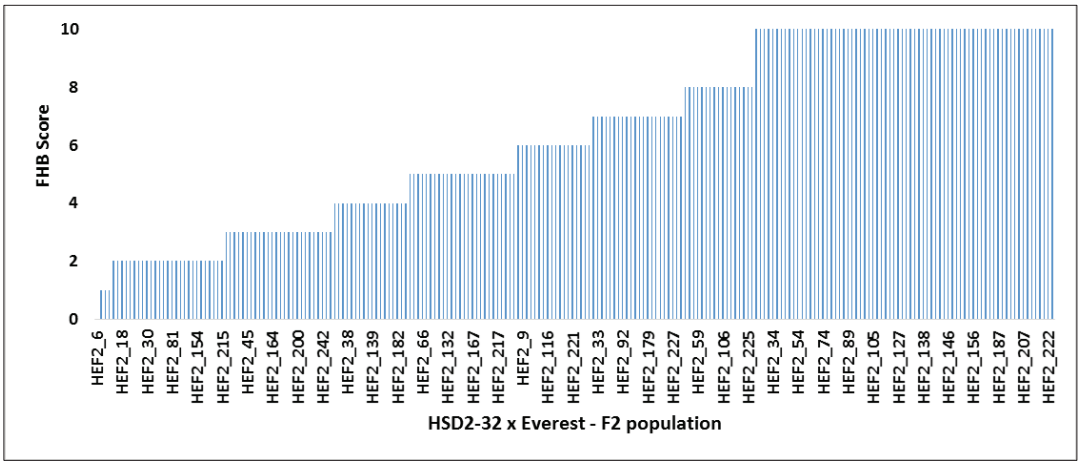

Figure 3. FHB score of HSD2-32 x Everest population screened in the FHB nursery at Rocky Ford during spring 2024.

List key outcomes or other achievements.

1) Identification of two novel FHB resistant source (TA7709 and TA3425) having stable inhetitance of FHB resistance.
2) Identification of two SNP marker at 627,128,588 bp and 623,580,344 bp in Chr2D that was linked with a QTL region (628,574,465 – 633,228,046 bp) segregates in 3:1 ratio in the HSD2-32 x CS population was identified.
3) Two SNP marker at 623,580,344 bp and 633,199,691 bp in Chr2D that was linked with a QTL region (628,574,465 – 633,228,046 bp) segregates in 1:2:1 ratio in the HSD2-32 x Everest population was identified.

3. What opportunities for training and professional development has the project provided?

NA

4. How have the results been disseminated to communities of interest?

We are currently developing recombinant inbred lines (RILs) involving HSD2-32 in two different genetic backgrounds. The identified SNP markers linked to FHB resistance will be tested in these RILs to assess their stability across different genetic backgrounds. Once validated, the SNP markers will be made available to the FHB research community for integration into FHB resistance breeding programs. HSD2-32 has already been made available to the FHB community, and the estimated timeline for SNP marker distribution is early 2027.

5. What do you plan to do during the next reporting period to accomplish the goals and objectives?

1) Chromosome engineering of FHB resistant lines (TA7709 and TA3425).

2) Genetic mapping of FHB resistance locus using the RIL population of HSD2-32 x CS (F7 and BC1F6) and HSD2-32 x Everest (F4) to reconfirm and supplement the F2 mapping data.