

**Project FY22-HW-001: New Sources of Resistance to FHB and DON in Wheat**

---

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

The major goal of the project is to identify new source of FHB resistance. The objectives of the project are 1) Detection of alien introgressions in HSD2-32 and 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?**

Objective 1: Genomic DNA of wild wheat relatives such as *Secale cereale* (RR), *Thinopyrum ponticum* (JJJJJJ<sup>s</sup>J<sup>s</sup>J<sup>s</sup>), *Th. elongatum* (EE), *Th. intermedium* (JJ<sup>v</sup>J<sup>v</sup>S<sup>t</sup>S<sup>t</sup>), *Dassypyrum villosum* (VV), and *Pseudoroegneria spicata* (S<sup>t</sup>S<sup>t</sup>) were used as probes to detect alien introgression in HSD2-32 by pachytene genomic *in situ* hybridization (GISH). Additionally, skim-seq was employed to identify alien introgressions in HSD2-32.

Objective 2: Earlier in this project we have identified and validated one SNP marker at 627128588 bp in Chr2D that was linked with a QTL region (628574465 – 633228046 bp). We continued screening of the additional markers in that QTL region to determine the polymorphic markers between HSD2-32/CS and HSD2-32/Everest. We forwarded the HSD2-32 x CS polulation from F4 to F5 generation by single seed descent (SSD) method. In parallel we forwarded the BC1F2 population of HSD2-32 x CS to BC1F3 by SSD. Also, we phenotyped the HSD2-32 x Everest F2 population in the Rockey ford FHB nursery.

**What were the significant results?****i) Detection of alien introgressions in HSD2-32**

HSD2-32 (2n=42) is FHB resistant and it is derived from the cross involving Ganmei8 (*Trielytrigia* 2n=56, AABBDEE) and *Thinopyrum intermedium* (2n=42, JJ<sup>s</sup>J<sup>s</sup>S<sup>t</sup>S<sup>t</sup>). However, the exact pedigree information of HSD2-32 is unavailable. GISH using total genomic DNA of *Thinopyrum elongatum* and/or *Th. intermedium* as probes and skim-seq analysis did not detected any *Thinopyrum* introgressions in HSD2-32. However, HSD2-32 had sizable regions in chromosome arms 2DL, 5BL, and 6AS which are not able to map to the wheat reference genome. This gives us a clue that HSD2-32 may have introgressions from other wheat relatives conferring FHB resistance.

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

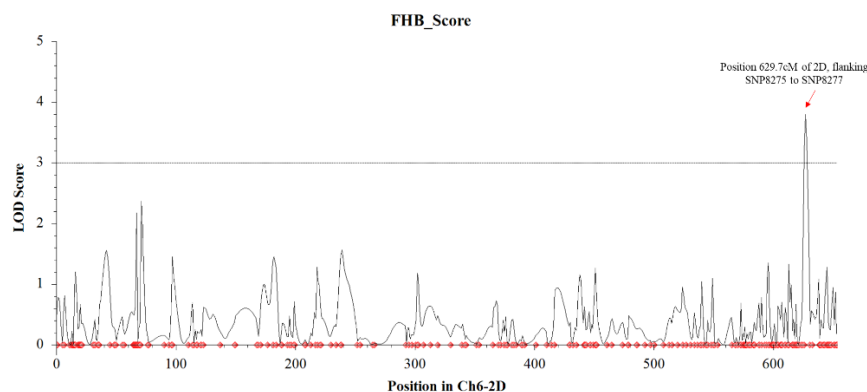
Identification of genetic markers associated with FHB resistant loci in HSD2-32 was done using F<sub>2</sub> population derived from the cross between HSD2-32 and Chinese Spring (CS) wheat. The putative candidate loci conferring FHB resistance was given in Table 1. We have identified and validated one SNP marker at 627128588 bp in Chr2D that was linked with a QTL region (628574465 – 633228046 bp) (Fig 1; 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 CS 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 623580344 bp in Chr2D which is present in the flanking region within the 5 Mb region of the target QTL region (628574465 – 633228046 bp). Using the F<sub>3</sub> 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 2). In total, we have identified and validated two SNP markers that are linked with the target QTL region (628574465 – 633228046 bp) in the HSD2-32 x CS population (Table 2). In parallel, we have identified two markers that show parental polymorphism between Everest and HSD2-32 at 623580344 bp and 633199691 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 HSD-32 x Everest population (Table 3). 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 marker, we checked the phenotypic ratio. The mean FHB score of the lines carrying this target QTL in the Everest background does not significantly differ from the HSD2-32 or Everest or heterozygote. The mean FHB score of lines carrying HSD allele or Everest allele or Heterozygote is around 6.0 for botht 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. 2).

We forwarded the HSD2-32 x CS polulation from F4 to F5 generation by single seed descent (SSD) method. We forwarded the BC1F2 population of HSD2-32 x CS to BC1F3 by SSD. Also, we phenotyped the F2 population of HSD2-32 x Everest in the Rockey ford FHB nursery and forwarded the HSD2-32 x Everest to F3 generation by SSD.

**Table 1.** 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 1. Position of validated QTL in the Chromosome 2D of wheat.**

**Table 2.** 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 goodness of fit test

Chr2D_627128588	F3 FHB Score				Chi-square				
Category	Mean	Max	Min	Count	OV	EV	OV-EV	(OV-EV) <sup>2</sup>	(OV-EV) <sup>2</sup> /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_623580344	F3 FHB Score				Chi-square				
Category					OV	EV	OV-EV	(OV-EV) <sup>2</sup>	(OV-EV) <sup>2</sup> /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

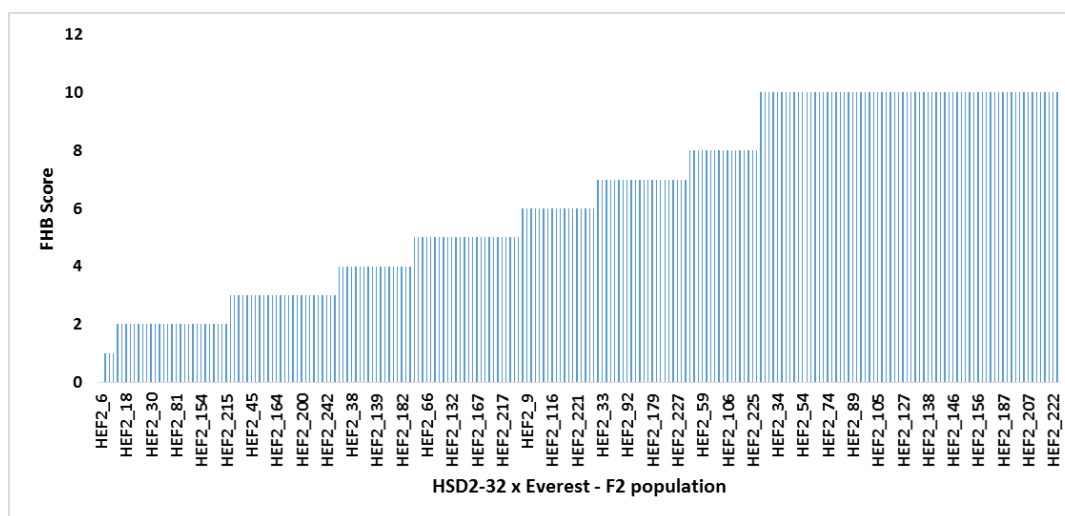
Critical value@0.05 with  $df=1$  is 3.84. If the critical value is larger than the sample's chi-square, then the null hypothesis can be rejected. Hence 3:1 segregation exist.

**Table 3.** 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_623580344	OV	EV	OV-EV	(OV-EV) <sup>2</sup>	(OV-EV) <sup>2</sup> /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_633199691	OV	EV	OV-EV	(OV-EV) <sup>2</sup>	(OV-EV) <sup>2</sup> /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		<b>1.9</b>

Critical [value@0.05](#) with  $df=2$  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.



**Figure 2. 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 and characterization of novel sources of FHB resistance in HSD2-32.
- 2) Two SNP marker at 627128588 bp and 623580344 bp in Chr2D that was linked with a QTL region (628574465 – 633228046 bp) segregates in 3:1 ratio in the HSD2-32 x CS population was identified.
- 3) Two SNP marker at 623580344 bp and 633199691 bp in Chr2D that was linked with a QTL region (628574465 – 633228046 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?

The PI of this project trained Metin Tuna, Faculty at Namik Kemal University, Turkey and Izamar Olivas Orduna, PhD student at KAUST (King Abdullah University of Science and Technology), Thuwal, Makkah, Saudi Arabia in cytology and provide hands on experience to them in various cytogenetic techniques.

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

The results of this project were presented in 2023 National FHB Forum