Project FY22-HW-001: 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?

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.)*

a) What were the major activities?

Objective 1: Genomic DNA of wild wheat relatives such as *Secale cereale* (RR), *Thinopyrum ponticum* (JJJJJJJ^{s}J^{s}J^{s}J^s), *Dassypyrum villosum* (VV), and *Pseudoroegneria spicata* (S^{t}S^t) were used as probes to detect alien introgression in HSD2-32 by pachytene GISH.

Objective 2: Earlier in this project 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). 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.

b) 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, AABBDDEE) and *Thinopyrum intermedium* (2n=42, JJJ^{s}J^{s}SS). 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_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 1. 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 1 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 2). 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 2). 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 3). To check the performance of this population under filed 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. 2)

Table1. 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

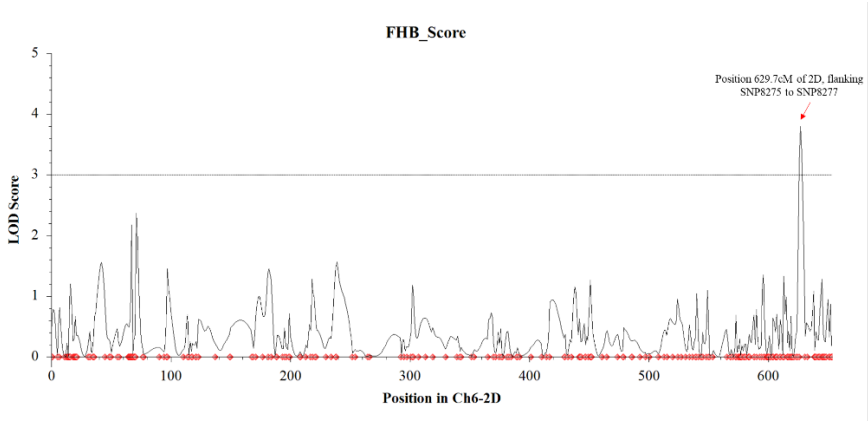

Figure 1. Position of validated QTL in the Chromosome 2D of wheat

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 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 3. Chi-square anlaysi 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 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.

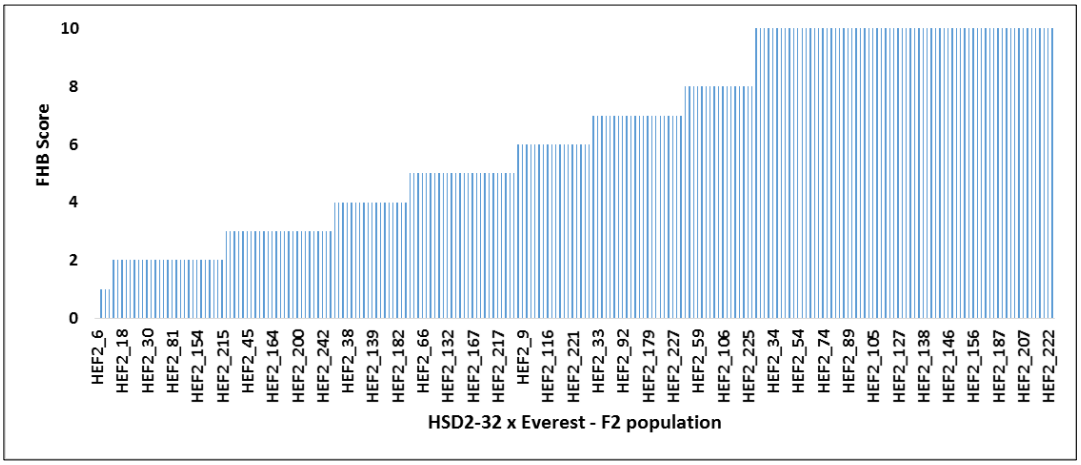

Figure 2. FHB score of HSD2-32 x Everest population screened in the FHB nursery at Rocky Ford during spring 2024

c) List key outcomes or other achievements.

1) Identification and characterization of novel sources of FHB resistance in HSD2-32.
2) 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?

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.

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

We continue forwarding the F5 population of HSD2-32 x CS and BC1F3 population of HSD2-32 x CS by SSD to develop Recombinant Inbred Line (RILs) for mapping. We planned to screen large number of chromosome engineered lines/wild germplasms to identify the novel FHB resistant lines.