Project FY22-DU-010: Developing FHB Resistant Durum Wheat Germplasm

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1. **What are the major goals and objectives of the research project?**
   The ultimate objective of this project is to enhance the resistance in durum cultivars by removal of persistent suppression mechanism. The only objective of proposed project approved for continuation by the EC was to develop backcross derived nested association mapping (NAM) panel for the top five resistant mutants

2. **What was accomplished under these goals or objectives?**
   What were the major activities?
   The top 50 resistant backcross (BC) derived lines along the parental lines were tested at two field locations, Saint Paul & Rosemount, MN for agronomic traits such as heading time, plant heights, total heads, 30 head seed count and seed weight. The measurements were performed in 8 replicates, 4 replicates at Saint Paul & 4 at Rosemount, MN. The BC lines were consistent in performance along with the parental lines and other durum lines.

   We also performed transcriptome analysis by comparing between the M4 line (41708-72, E.25.10 and E.25.23) and the parental lines (D0-41708 and E.25) at two time points, 12h and 48h. Comparison of M4 with the parental lines provided significant details on the acquired resistance. Several genes related to grain development such as grain softness protein, alpha-gliadin, lipid transfer protein, dimeric alpha-amylase inhibitor, low molecular weight glutenin subunit, and gamma gliadin protein significantly upregulated (more than 5-fold) in response to fusarium infection in M4 lines. In addition, transcription factors such as NAC, BZIP, Ethylene- responsive transcription factor, MYB, and other genes such as NBS-LRR disease resistance protein, Pathogenesis-related thaumatin protein, defensin-like protein 4, PR1, alpha-amylase inhibitor protein, invertase/pectin methylesterase inhibitor family protein, dirigent protein, trypsin inhibitor, and detoxification superfamily proteins were found differentially expressed in M4 lines. For example, the alpha-amylase inhibitor is known to express in high concentration in response to Fusarium inoculation and would be interesting to see the susceptibility level of mutant lines. In addition to the commonly expressed genes above, the M4 lines also had a different set of genes expressed in response to Fusarium inoculations.

   We also discovered novel genes (Tables 1 and 2) which are differentially expressed in 41708-72 (M4 line) as compared to D0-41708 (parental line) and in E25.23 (M4 line) as compared to E25 (parental line). These novel genes along with the previously known genes (related to the biosynthesis of secondary metabolites, MAPK signaling, photosynthesis, starch and sucrose metabolism, plant hormone signal transduction, and plant-pathogen interaction pathways) are being tested for their potential role in acquired FHB resistance in the M4 lines. We are validating the expression of the candidate genes using PCR analysis and utilizing the durum mutant lines and by VIGS silencing.

   We also crossed five M4 lines (41708-72, E.25.11, E.25.32, E.25.23, and E.25.10) with two NDSU advance varieties lines (ND Grano, and ND Stanley). Seed setting was low in some of the crossing sets and were repeated to generate adequate seed for development of NAM lines. Currently, many are advanced to BC1F2 toward generating the nested association mapping panel as well as material for further selection by the breeding programs.

Table 1. List of novel genes discovered in 41708-72.

Gene Name	D0-41708_C vs 41708-72_C	D0-41708_48h vs 41708-72_48h
TRITD7Bv1G028510.1	9.982593187	-24.35159238
TRITD5Bv1G000590.1	10.50674524	-23.7201033
TRITD6Av1G009830.1	24.47082416	-22.55041863
TRITD2Av1G280980.1	8.698189792	-22.07918647
TRITD3Bv1G032400.1	9.878017024	-9.072717642
TRITD4Av1G020340.1	8.931652601	-8.20945587
TRITD1Av1G002790.1	11.33622174	-8.15397015
TRITD1Av1G193070.1	8.746071953	-8.130279848
TRITD1Bv1G008290.1	23.29187794	-7.224990696

Table 2. List of novel genes discovered in E25.23.

Gene Name	E25_C VS E25.23_C	E25_48h VS E25.23_48h
TRITD1Av1G040240.1	-4.99412606	-22.8636824
TRITD5Av1G206770.1	-4.99412606	-22.8636824
TRITD1Bv1G189750.1	-0.69610511	5.610171542
TRITD1Av1G087460.1	1.89261257	6.698701489
TRITD5Bv1G217150.1	0.09101065	6.825857006
TRITD1Av1G084110.1	1.80594753	6.868997512
TRITD1Av1G093090.1	1.56752937	7.7426015
TRITD1Av1G094190.1	-0.1778959	8.657498778
TRITD1Av1G104890.1	1.98687075	9.393966387
TRITD1Av1G070600.1	0.72635357	9.957724297

What were the significant results?

Identification of candidate FHB resistant genes

Development of a large NAM population for genetic characterization and introgression of identified FHB resistance loci.

List key outcomes or other achievements.

3. **What opportunities for training and professional development has the project provided?**
There was a part-time postdoctoral scientist on this project who performed all of the RNAseq and gene identification work. He accepted a position with Bayer Crop Sci. and has moved to a much larger crop improvement effort. Since then all of the work is being conducted by a part-time student technician.

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

Through meeting presentations and publications

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

Advance the NAM population and increase seeds for field evaluation in the summer of 2025.