Precise DNA Markers for Breeding for Better FHB Resistant Wheat

(Approved July 2012)

Principle Investigator: Yang Yen, Department of Biology and Microbiology, SDSU
Collaborators: Wanlong Li, Departments of Biology and Microbiology and Plant Science, SDSU
Karl Glover, Spring Wheat Breeding and Genetics, Plant Science Dept., SDSU
William Berzonsky, Winter Wheat Breeding/Genetics, Plant Science Dept., SDSU

Objectives

The goal of this proposed research is to develop more precise markers for known major quantitative trait loci (QTLs) that control resistance to Fusarium head blight (FHB-resistant) in wheat. Genes that are associated with FHB resistance/susceptibility have been identified in the first year. The identified genes were further associated with major QTLs in the second year. The specific objective for the third year is to develop polymerase chain reaction (PCR)-based markers for the major FHB resistance QTLs on wheat chromosome arms 3BS (*Qfhb1*) and 6BL.

Importance to South Dakota:

FHB is a serious problem for SD wheat production. Using precise marker in SD wheat breeding program surely will improve the breeding efficiency, which will benefit SD wheat growers helping them battling against FHB in their wheat fields.

Justification:

FHB is a devastating threat to wheat production in South Dakota. It has caused considerable income loss for spring wheat growers in South Dakota in recent years. Due to warmer climate in recent year, winter wheat acreage has expanded eastward and is in danger of suffering from FHB as well. An effective management strategy to control FHB is breeding for FHB-resistant varieties.

Breeding for better FHB-resistant varieties involves resource identification and breeding line evaluation. Since FHB resistance in wheat is quantitatively inherited and greatly influenced by environmental factors, identification and evaluation of FHB resistance are very time-consuming and labor-intensive. One way that can facilitate these processes is to apply marker-assisted selection, i.e. selecting based on existence/absence of markers of FHB resistance genes/QTLs instead of phenotype screening.

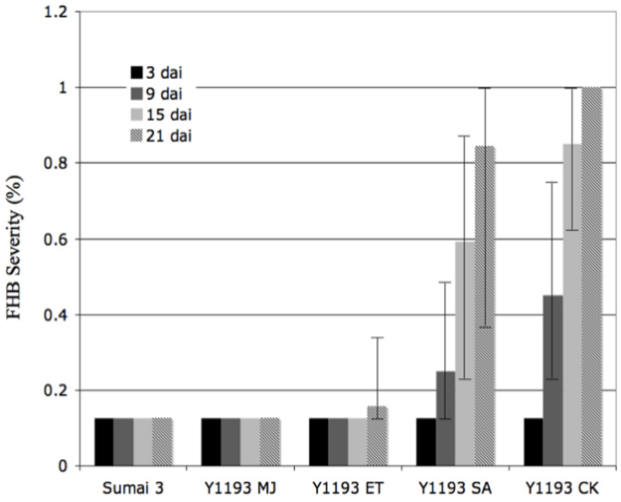

Figure 1. Mean effects of water (as mock treatment, CK), salicylic acid (SA), ethylene (ET) and methyl jasmonate (MJ) on FHB symptoms of FHB-susceptible Y1193-6 in 3, 9, 15 and 21 days after inoculation of *Fusarium graminearum* isolate Fg4. FHB-resistant Sumai 3 was used as the resistant control. Spikelets were sprayed with distilled water, 3 mM SA, 0.2 mM MJ or 1 mM Ethefon (for ET) once a day for three consecutive days before the inoculation and again injected 20 mL of the same solution into spikelets immediately before their inoculation. Data ranges, if there are, are shown.

Supported partially by SD Wheat Commission and USDA-USWBSI, several major FHB-resistant QTLs have been identified and DNA markers to these QTLs are also available. However, currently available markers are of linkage indication at the best. Linked markers are not part of the DNA sequence of the genes they represent but DNA sequences located nearby the genes. Linkage between the marker and the gene it represents, no matter how tight, can therefore be broken by chance due to genetic recombination during sexual reproduction of wheat. In fact, our study, has

suggested the occurrence of such marker-gene disassociation for *Qfhb1*within SD spring wheat breeding lines (Liu et al., 2003; 2004). In other word, marker-assisted selection using linked markers has the risk of identifying lines that do not have the gene of interest while missing lines that do have the gene. The identified and mapped genes so far are not FHB resistant genes themselves but the genomic regions (QTLs) that carry the genes. Little is known about what genes are associated with these QTLs and how they function. Obviously, we need perfect markers that are actually part of the DNA sequence of the FHB resistant genes.

In our study of the molecular mechanism of FHB pathogenesis/resistance in wheat with microarray, which was also partially supported by SD Wheat Commission, we have revealed 677 genes that changed expression during FHB pathogenesis/resistance and led to our conclusion that jasmonates (JA)/ethylene (ET) signaling pathways mediate the FHB resistance in wheat (Li and Yen, 2008) (Fig. 1). These genes likely have a role in FHB development or FHB resistance and thus are called FHB-related genes. Of them, 281 are FHB-resistance-related and 79 are FHB-susceptibility-related. Five of these genes had opposite expression patterns between the resistant and the susceptible genotypes and, therefore, are more interesting.

With the supports from USDA-ARS-USWBSI and SD Wheat Commission, we have carried out research to pair these FHB-resistance-related genes with the major FHB-resistant QTLs. A pair of near-isogenic lines (NILs) that segregating a major FHB resistance QTL *Qfhb1* (Pumphrey et al., 2007), a $F_{2:8}$ recombinant inbred line (RIL) population and a set of Chinese Spring ditelosomic/tetrasomic lines were used to do the pairing. We have paired the genes with *Qfhb1* by assaying a pair of near-isogenic lines that segregating a major FHB resistance QTL *Qfhb1* (Pumphrey et al., 2007) (Fig. 2). For other QTLs, a resistant and a susceptible pool of 10 $F_{2:8}$ recombinant inbred line population per pool and a set of Chinese Spring ditelosomic/tetrasomic lines were formed (judged by phenotype and markers) and screened. This research could shed light on the resistance mechanisms of these QTLs and the functional genes in these QTLs. The associated genes can be used for developing precise markers to the QTLs.

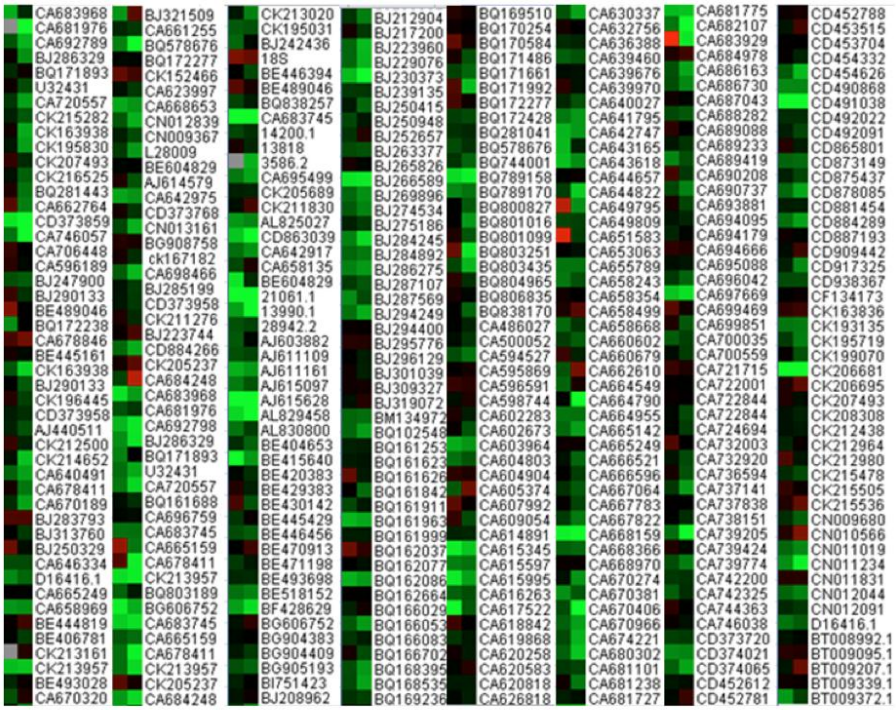

Figure 2. A heatmap showing expression difference of the FHB-associated genes between the near-isogenic lines differentiating *Qfhb1*/*qfhb1*. The value is calculated as *ΔΔCt*. Green: a positive; red: negative; black: neutral.

Therefore, through the previous supports by SD Wheat Commission, a handful of interesting materials have been created and are ready for developing precise markers to increase breeding efficiency for better FHB resistance.

Materials and Methods:
Our hypothesis is that, at least, some of these FHB-resistance-related genes are the gene components of the major FHB-resistant QTLs and therefore can be used as more precise markers for those QTLs. We plan to test our hypothesis by conducting functional genomic analysis to pair these genes with the known FHB-resistance QTLs and then develop PCR-based markers to the QTLs out of the paired genes.

Plant Materials:
A pair of near-isogenic lines that segregating a major FHB resistance QTL *Qfhb1* (Pumphrey et al., 2007), a $F_{2:8}$ recombinant inbred line population and a set of Chinese Spring ditelosomic/tetrasomic lines will be used.

Pairing the genes to QTLs:
Realtime-RT-PCR was used to do the pairing. We have paired the genes with *Qfhb1* by assaying the NILs. For other QTLs, a resistant and a susceptible pool of 10 RILs per pool were formed (judged by phenotype and markers) and screened. Total RNA samples were extracted from the FHB-inoculated spikelets of the NILs and the selected RILs and used for expression assay. The beta-actin gene that is known to be stable between FHB treatments and genotypes was used as the reference gene to estimate random errors. The comparative C_T method (Schmittgen and Livak, 2008) was use to normalized the expression data. Expression data were collected from individual plants of three biological duplicates and three technical duplicates for each gene and subjected to the Student *t*-test for significant difference between the lines at $p \leq 0.05$. A significant difference of in expression between the FHB-inoculated NILs qualified the gene as being associated with *Qfhb1*. The genes that are not significant between the NILs but significant between the two pools will be one associated with other QTLs. The paired genes should either be the genes located in the QTL regions they paired or those down stream in the resistance/susceptibility pathways that the genes of the QTLs are involved. Either way, they are the one directly make contribution to FHB resistance/susceptibility in wheat.

In FY11, a total of 458 PCR primer sets were screened. Six and 17 genes were paired with *Qfhb1* and other FHB-resistance QTLs, respectively. In FY12, pairing the 17 non-*QFhb1*genes with individual QTL by ditelosomic/tetrasomic analysis and RIL screening was conducted. Two genes (a putative invertase/pectin methylesterase inhibitor family protein gene and a glutathione-regulated potassium-efflux system protein gene) have mapped within the 3BS QTL *Qfhb1*. A gene associated with salicylic acid pathway has been paired with the major FHB QTL on chromosome arm 6BL. Piring of genes that are associated with QTLs on other chromosome arms will be continued in FY13.

Developing PCR markers for QTL-associated genes:
Genomic DNA will be isolated from sample of 14-day leaves of the resistant and susceptible genotypes. PCR primers will be designed based on the known sequences of the genes of interest to clone the genes of interest from both resistant and the susceptible genotypes. The clone genes will be sequenced and aligned for sequence polymorphisms between the resistant and the susceptible genotypes. PCR primers will then be designed to differentiate these polymorphisms and tested between the genotypes. Those primers that can differentiate the genotypes for FHB resistance/susceptibility will be the candidates for precise markers and be further tested among breeding lines. The genes of interest have been cloned. We will sequence the clones to design the primers for markers. We expect to realize this objective in FY13.

Summary of past SD Wheat Commission funding:
The following achievements have been made, at least partially, with past SD Wheat Commission funding to the PI:

1. In FY11, a total of 458 PCR primer sets were screened. Six and 17 genes were paired with 3BS QTL *Qfhb1* and other FHB-resistance QTLs, respectively. In FY12, two genes were precisely mapped in the *Qfhb1* and one gene in the 6BL QTL.

2. FHB resistant wheat variety Sumai 3 was functional genomic elucidated for the molecular mechanism of its FHB resistance with microarray assay, realtime-RT-PCR validation and physiological confirmation (Xing et al., 2004; Li and Yen, 2008). This study concluded that the FHB resistance in Sumai 3 is mediated by jasmonic acid signaling pathway. A total of 677 possible FHB-related genes were identified, of which 243 showed similar expression in both Sumai 3 and Y1193-6, 281 and 79 significantly changed expression pattern only in Sumai 3 or in Y1193-6, respectively, and five had opposite expression pattern between Sumai 3 and Y1193-6. This work paved a solid foundation for the proposed work.
3. A $F_{2:8}$ RIL population has been made between FHB-resistant Sumai 3 and FHB-susceptible Y1193-6. This population has been phenotyped for FHB-related traits (incidence, severity/disease index and DON content) both in green house and field for multiple years. This RIL population has also been genotype with 65 polymorphic SSR markers and 352 DArT markers (Basnet et al., 2007). Several FHB-resistant QTLs have been identified and mapped with this population and linked SSR markers were developed for these QTLs (Fig. 2).
4. Two other $F_{2:8}$ RIL populations have been created and analyzed for FHB resistance QTLs (Wiedel, 2008; Malla et al., 2010). These two populations involve FHB-resistant varieties Toka 66 and Abura, respectively. Both populations have Y1193-06 as the susceptible parent and thus the data can be comparable across the three RIL populations.
5. A Microsatellite Marker for tagging stem rust resistance gene Sr35 in wheat was developed (Babiker et al., 2009)
6. FHB resistance was molecularly elucidated in hard red winter wheat, especially those in SD ((Malla et al., 2010b).
7. SD spring wheat parents were screened for FHB resistance with SSR markers (Zhu et al., 2001).

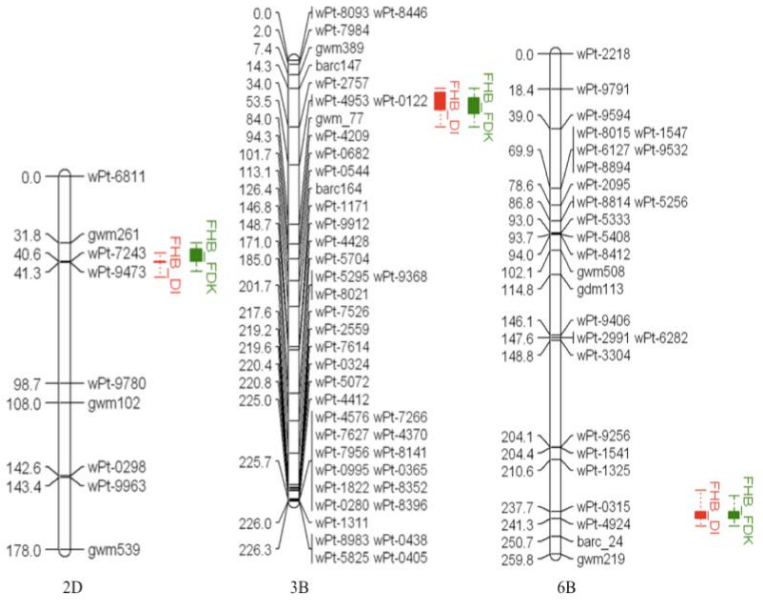

Figure 3. Wheat genetic map of chromosomes 2D, 3B and 6B for DArT and SSR markers and FHB-resistance QTLs. FHB_DI:FHB disease index; FHB_FDK:FHB damaged kernels.

Reference:

Babiker, E., A.M.H. Ibrahim, Y. Yen, and J. Stein. 2009. Development of a Microsatellite Marker for tagging stem rust resistance gene Sr35 in wheat. Aust J. Crop Sci. 3:195-200.

Basnet BR, Yen Y, Chao S, Karl D. Glover, KD. 2007. Genetic linkage mapping with dart markers to detect scab resistance QTLs in a 'Sumai-3' derived wheat population. In: Proceedings of 2007 National Fusarium Head Blight Forum, Kansas City, MO, December 2-4, 2007, pp165.

Li, G., and Y. Yen. 2008. Jasmonate and ethylene signaling pathway may mediate Fusarium head blight resistance in wheat. Crop Sci. 48:1888-1896.

Liu, D.C., Y. Yen and A.M. Ibrahim. 2003. Screening elite South Dakota winter wheat for SSR markers linked to Fusarium head blight resistance. Proc. SD Acad. Sci. 82:19-25.

Liu, D., K.D. Glover and Y. Yen. 2004. MAS Efficiency in Improving Scab Resistance in Spring Wheat: A Look From the Reverse Angle. *In*: Proceedings of the Second International Wheat Scab Symposium, Dec. 11-15, 2004, Orlando, FL. USA, pp. 94-97.

Malla, S., A.M.H. Ibrahim, Y. Yen, W. Berzonsky, K.D. Glover and J. Stein. 2010. Quantitative Trait Loci Analysis of Novel Fusarium Head Blight Resistance in Tokai 66. Am J Agri Biol Sci 5:62-69.

Malla, S., A.M.H. Ibrahim, Y. Yen, W. Berzonsky, K.D. Glover and J. Stein. 2010. QTL Analysis of a Putative Novel Source of Resistance to Fusarium Head Blight in Hard Winter Wheat. Intl J Plant Breed 4:47-54.

Pumphrey MO, Bernardo R, Anderson JA. 2007. Validating the *Qfhb1* QTL for Fusarium head blight resistance in near-isogenic wheat lines developed from breeding populations. Crop Sci 47:200-206.

Schmittgen, T.D. and K.J. Livak. 2008. Analyzing real-time PCR data by the comparative C_T method. Nat Proto 3:1011-1108.

Wiedel, N. 2008. Identification of FHB Resistance QTL in Tokai66. MS Thesis, South Dakota State University.

Xing, D.-H., Y. Yen, Y. Jin and J. Rudd. 2004. Study of genes important to scab pathogenesis and resistance in wheat. *In*: Proceedings of the Second International Wheat Scab Symposium, Dec. 11-15, 2004, Orlando, FL. USA, pp. 534-538.

Zhu, L.-C., J.C. Rudd and Y. Yen. (2001) Applying simple sequence repeat (SSR) markers in screening *Fusarium* head blight resistant parents. Proc. SD Acad. Sci. 76: 21-24.