Pre-breeding of HRWW to Achieve Multiple Disease Resistance

G. Francois Marais, Dept. of Plant Services, NDSU

Research Questions

A new NDSU HRWW breeding program was started in 2011. Current hard red winter wheat germplasm is generally lacking in resistance to major diseases such as Fusarium head blight, leaf rust, stem rust, bacterial leaf streak, tan spot and Septoria nodorum blotch. In the past five years, useful resistance has been transferred from many diverse sources of spring wheat and less winter-hardy winter wheat. The acquired genes occur mostly singly in diverse introgression lines that will now be utilized for the systematic development of inbred lines with broader resistance spectra and superior winter survival.

Gene pyramiding is being attempted at two levels: First, very specific pyramids are being constructed. The primary aim is to attain complex FHB or rust resistance in winter-hardy backgrounds (primarily Jerry and Norstar). Such plants are being used as cross parents to rapidly disseminate multiple resistance genes coupled with cold-hardiness into the breeding population; however, they are unlikely to directly produce commercially useful genotypes. Second, more broad-based pyramids are also being pursued. These are based on annual characterization of the previous seasons' cross progeny by using molecular markers, bio-testing and yield assessment (field). Promising and more complex gene combinations involving a broader range of pests are being identified and follow-up crosses are made to produce still better combinations. These crosses aim to establish a broader base of background variability and have stronger potential for direct production of commercially useful genotypes.

The best pyramided genotypes from both attempts are annually employed as parents to produce 600-700 cross combinations in the general pedigree breeding program.

Results

Specific (narrow genetic base) gene pyramids

Confirmation of earlier derived 2-gene pyramids: SSD11M221-24-1 (= RWG10/Jerry), 14K456-K-1 and 14K-456-L-5 (= CM82036/2* Jerry) were found to contain both *Fhb1* and *Qfhs.ifa-5A*, while DH172 (=CM82036/Jerry) contains only *Fhb1*. Phenotypic results showed that the four lines have significantly reduced severity of infection, vomit toxin accumulation and FDK as compared to susceptible controls. Lines with both *Fhb1* and *Qfhs.ifa-5A* performed better than lines with *Fhb1* only. 14K456-K-1 was the most resistant and comparable to the spring donor parent, CM82036. Lines SSD11M228-19-1 and SSD11M228-57-2 carry resistance from PI277012 (*QTL5AS* and *QTL5AL*) with SSD11M228-57-2 being the more resistant of these two winter habit lines. However, it is not clear whether one or both genes had been transferred.

Pyramiding of *Fhb1*, *Qfhs.ifa-5A*, *QTL5AS* and *QTL5AL*: Cross 15K353 was made and 406 F_2 plants tested for the presence of *Fhb1* and *Qfhs.ifa-5A*. *Fhb1* could be detected with marker Umn10, while each of markers Gwm304, Gwm293 and Barc186 could be used to detect *Qfhs.ifa-5A*. Neither the *QTL5AS* markers nor the *QTL5AL* markers appeared to be suited for marker selection. As a result, progeny phenotyping will be done in an attempt to select genotypes with pyramided resistance QTL from PI277012. Also, it is not clear from the published data whether one of the genes may be the same as *Qfhs.ifa-5A*. In total, 69 *Fhb1* homozygous lines were selected, including 19 *Fhb1*, *Qfhs.ifa-5A* homozygotes; 33 *Fhb1* homozygotes segregating for *Qfhs.ifa-5A*; and 17 *Fhb1* homozygotes. Four $F_{2:3}$ progeny of each of the plants in the two homozygous classes were raised and leaf samples of each were cut for single nucleotide polymorphism (SNP) analysis and detection of the 1BL.1RS translocation. Upon ripening the plants were harvested separately. The 136 F_4 families thus derived will now be subjected to FHB evaluation utilizing 15-20 plants per family. The overall means of the *Qfhs.ifa-5A* (+) and *Qfhs.ifa* (-) groups will be compared to evaluate the effect of this QTL. Similarly, the 1BL.1RS (+) means will be compared with 1BL.1RS (-) means to determine whether the results were affected by its segregation. Finally, the within family variation and mean values will be compared to search for evidence of the segregation of additional FHB resistance QTL and an attempt will be made to find evidence of co-segregation with mapped SNP loci.

Pyramiding *Fhb1* with a 3A QTL from the spring wheat Frontana: The F_1:15M16 (= Norstar-*Fhb1*//Frontana/Norstar) heterozygous for *Fhb1* and *QTL3A* was obtained and has been planted for derivation of F_2. Approximately 100-200 F_2 plants will be typed for *Fhb1* and ±25-50 homozygotes will be identified; these will then be characterized with respect to absence/presence of *QTL3A*.

Pyramiding *Fhb1* & *Qfhs.ifa-5A* with *Fhb6*: The F_1:14M7 (= *Fhb6*/Jerry//Accipiter) was marker tested to derive F_1 suitable for continued marker-aided selection of *Qfhs.ifa-5A*. The selected plants were crossed with 14K456-K-1 (*Fhb1* and *Qfhs.ifa-5A*). The hybrid progeny will be used to establish populations for the recovery of the desired pyramid.

continued on pages 30 - 31

Pyramids involving durable (slow-rusting) leaf rust resistance genes: 414 new doubled haploids derived from eight crosses were increased and field planted (fall 2016) in un-replicated plots or single rows. Marker data suggested that 18 lines have *Fhb1* in combination with the durable leaf rust resistance genes *Lr34*, *Lr46* and *Lr68*; some lines have *Fhb1* plus two (38 lines) or one (21 lines) durable resistance gene(s). 53 lines have the three durable resistance genes but not *Fhb1*. The lines will be evaluated for cold hardiness and phenotype and the best will be used for continued field evaluation, new crosses and pyramiding.

Broad genetic base pyramids:

New (2016) crosses for continued gene pyramiding: Ten new F_1 combinations were produced and supplied to Heartland Plant Innovations for the development of 400 doubled haploids. Twenty six new F_2 populations were produced for the eventual development of 350-400 new single seed descent (SSD) inbred lines. These crosses were intended to produce new combinations of resistance genes in winter-hardy, well adapted genetic backgrounds. Each cross involved an FHB resistant parent with 1-2 of the resistance QTL *Fhb1*, *Qfhs.ifa-5A*, and the PI277012-derived 5AS and 5AL QTL. The second parent was chosen to have resistance to one or more pests (leaf rust, stem rust, stripe rust, wheat stem sawfly, bacterial leaf streak).

2015 Crosses: F_2 and F_3 from an additional 26 crosses were used to initiate single seed descent (SSD) inbreeding. The material was first subjected to screening with mixed leaf and stem rust inoculum. The more resistant seedlings were transferred to a greenhouse and evaluated for FHB resistance. Random F_3 and F_4 plants were increased and 396 rows and plots were planted in the field for evaluation of phenotype and cold-hardiness. 31 of the lines are also being analyzed with markers to detect known, major resistance loci.

The inbred lines that will be derived from the above crosses will be selected to obtain subsets of new breeding parents having more complex resistance combinations. The best combinations from each annual cycle will be used in further crosses to derive still more complex pyramids.

Use of gene combinations in the breeding program crossing blocks:

The overall purpose of these crosses is to initially establish *Fhb1* as the baseline FHB resistance in the crossing block and to systematically add to it additional FHB, rust, leaf spot, bacterial leaf streak and wheat stem sawfly resistance. 22 parental lines with moderate to very good winter-hardiness and one or more of the FHB resistance QTL *Fhb1*, *Qfhs.ifa-5A* and the PI277012-derived 5AS and 5AL QTL were crossed with 46 F_1, inbred lines and cultivars that varied widely in terms of FHB resistance, cold tolerance, known and unknown leaf and stem rust resistance genes. 662 crosses were made. The F_1 was increased and the F_2 field planted for selection in 2017.

Five newly identified lines with broad resistance to tan spot and Septoria nodorum blotch; 14 lines with stripe rust resistance and 5 lines with broad stem rust resistance were incorporated for the first time in crosses involving cold-hardy parents with some level of FHB resistance.

Application and Use

The accumulation of multiple favorable genes for disease resistance, yield, adaptation and processing quality in a breeding population is a formidable task that is only achieved through decades of un-interrupted, meticulous planning; strict phenotypic and statistical evaluation and selection. A well established, successful breeding program is built on an extensive base of phenotypic and genetic data and highly productive elite germplasm. Not surprisingly, such breeding populations are extremely valuable and well-guarded. The new NDSU HRWW program is only in its sixth year of development and clearly will require many more years of genetic improvement before it can be fully commercially competitive. To speed up introgression and genetic improvement, optimal use should be made of modern breeding tools such as accelerated inbreeding and in particular, marker-facilitated selection. The genetic material and gene pyramids that are being pursued here are absolutely necessary to ensure that the breeding effort will reach commercial competitiveness within a reasonable period of time.

Materials and Methods

No pre-determined procedure is followed for gene pyramiding as each set of target genes and donor parents presents a unique situation in terms of crossing strategy and marker/bio-test application. In addition, a pyramiding scheme often needs to be modified as new results become available. Standard plant breeding methodologies (convergent- and backcrosses; doubled haploid production and modified (with selection) single seed descent inbreeding) are primarily being followed in the pyramiding attempts. Molecular marker characterization (DNA extraction and use of marker systems such as microsatellite, SCAR, EST, SNP, etc.) is an integral part of the pyramiding attempts. Phenotypic evaluations are being done including seedling leaf and stem rust resistance screening, greenhouse FHB type II resistance screening; seedling resistance to tan spot and Septoria nodorum blotch. Phenotyping is necessary in cases where no appropriate markers are available or it is used to confirm marker results. Annual field yield trials and processing quality assessments are being done as appropriate. Naturally occurring diseases and response to stresses such as winter damage are recorded in field trials and provide valuable information on genetic background differences among pyramids.

Economic Benefit to a Typical 500 Acre Wheat Enterprise

The disease-causing pathogens targeted in the project annually cause significant wheat yield losses in the Northern Great Plains and even modest changes in the average level of resistance in new cultivars will be of considerable benefit to producers. The targeted diseases include some that are notoriously difficult to breed resistance for (for example tan spot, bacterial leaf streak, SNB and FHB) since resistance/insensitivity is based on numerous quantitative trait loci each making only a small contribution to the total resistance phenotype.

The project aims to assemble a wide spectrum of useful known and new resistance and adaptation genes through pre-breeding in winter-hardy genetic backgrounds. The majority of the target genes are not currently available in the HRWW primary breeding pool. Pre-breeding is being applied to gradually improve the general genetic background in which the newly introduced genes occur and to concentrate/assemble them into more complex combinations that will be more useful in pedigree breeding. This will make it possible to also develop new cultivars with better resistance gene combinations and yield stability.

Related Research

A hard red winter wheat pedigree breeding program was initiated at NDSU during 2011. Annually, 500-700 new crosses are being made among winter wheat parents. Many of the known genes for resistance to the rusts, FHB, tan spot, SNB and BLS are not available in winter-hardy genetic backgrounds that are adapted to North Dakota. Furthermore the resistance genes often occur singly in very diverse and poorly adapted backgrounds making it even more difficult to combine multiple genes in a single line. This pre-breeding program is meant to directly supplement and facilitate the pedigree breeding effort.

Recommended Future Research

Breeding of a new cultivar that incorporates multiple advantageous genes affecting many different traits, is difficult. The odds of being able to select such genotypes in a pedigree breeding program can be improved vastly if the breeding population gets continuously enriched with those target genes. In a well-established breeding program this stage of development is normally achieved following decades of selection and intercrossing of superior selections. However, the new NDSU program is being developed from scratch and therefore totally lacks such structure. Since a breeding cycle takes about 9 years, the NDSU program cannot even generate field-tested inbred lines that can serve as new parents (akin to an established program) until 2019. The current pre-breeding effort is therefore crucial to fill the void until 2019 and beyond. It aims to hasten the process of gene acquisition and base population enrichment and therefore should:

a. Continue to speedily acquire and evaluate new resistance and adaptation genes and increase the frequencies of those genes within the pedigree base population.

b. Build ever more complex and versatile gene combinations in genetically diverse and high yielding backgrounds (new breeding parents) that would hasten its dissemination in the breeding base population.