Pre-Breeding of HRWW to Achieve Multiple Disease Resistance

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Research Questions

The NDSU HRWW breeding program was started (2011) with germplasm that was 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. Since then, useful resistance has been transferred from many diverse spring and winter wheat sources. The acquired genes are now being used 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 then 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 yielding commercially useful genotypes.

The best pyramided genotypes from both attempts are annually employed in: (a) conventional (pedigree) crosses to produce 500-600 new segregating families, and (b), recurrent selection (RS) inter-crosses to produce 1,500+ diverse F_1 hybrid seeds that additionally can be tested for the recovery of pyramided genotypes.

Results

Attempts to derive and evaluate pyramids of specific resistance genes

Resistance to FHB is a primary breeding objective and in the past year we aimed to: (i) substantially increase the proportion of routine breeding program crosses that involved at least one parent with some level of FHB resistance (mostly *Fhb1*), and to evaluate additional resistance QTL for their ability to interact additively with *Fhb1*. (ii) Although only a limited number of near-isogenic and inbred lines with *Fhb1* have been obtained for use as cross parents, these were used extensively in the development of new segregating populations. The production and confirmation of pyramids of known resistance genes have been difficult in the absence of robust markers (only available for *Fhb1* and *Fhb6*), however, satisfactory progress has been made.

Pyramiding of *Fhb1, Qfhs.ifa-5A, QTL5AS* and *QTL5AL*: Analysis of a cross between Novus-4 (*QTL5A-1* and/or *QTL5A-2)* and 14K456-K-1 (*Fhb1* and *Qfhs.ifa-5A*) was continued. 400 F_2 were screened with *Fhb1* and *Qfhs.ifa-5A* markers and two sets of plants were identified, i.e. (i) 17 F_2 homozygous for *Fhb1* only, and (ii) 19 F_2 homozygous for both *Fhb1* and *Qfhs.ifa-5A*. Within each set of $F_{2:3}$ families, four F_3 plants per family were grown and leaves were cut on each for doing 9K SNP analyses. This was done to find additional, mapped chromosome 5A marker loci that could aid in the interpretation of the data. The 144 F_4 sub-families were then evaluated for FHB type II resistance in a replicated greenhouse trial. The data from this experiment are presently being analyzed. Preliminary indications are that *QTL5A-1* from PI277012 occurs within the same chromosome region and produces a similar effect to *Qfhs.ifa-5A*. Like *Qfhs-ifa-5A* it appears to add to the *Fhb1* effect and *Fhb1* plus *Qfhs-ifa-5A* pyramids appear to be like Fhb1 plus QTL5A-1 pyramids. However, due to the suspected heterogeneity of the donor source, RWG21, the second PI277012 locus, *QTL5A-2*, has not been transferred to our winter material. A new attempt therefore needs to be made to also transfer and evaluate *QTL5A-2*. In addition to the above information, the experiment yielded numerous inbred lines each homozygous for two FHB QTL. We planted these lines in the field in the fall of 2017 and will to continue to evaluate them as part of the routine breeding program. Pyramids of *Fhb1* with *QTL3A*: A near-isogenic line, Norstar-*Fhb1*, was crossed with the F_1: Frontana (*QTL3A*)/Norstar and the F_1 marker screened to identify dihybrid plants. Following self-pollination, 200 F_2 progeny were marker-screened to identify the *Fhb1* homozygotes. The selected homozygotes were then tested with a *QTL3A* marker and 34 F_3 families that are homozygous for both QTL were identified. Since Frontana is a HRSW, the lines also segregate for winter habit and winter types need to be identified before the two groups of homozygotes will be compared in a greenhouse trial during 2018. Pyramids of *Fhb1* with *Qfhs.ifa-5A*, and *Fhb6*: F_1 heterozygotes from the cross: *Fhb6*/Jerry//Accipiter were crossed with 12DH172 (*Fhb1* and *Qfhs.ifa-5A*). The F_1 was marker-screened to identify trihybrid (*Fhb1, Qfhs.ifa-5A, Fhb6*) plants. F_2 have been derived and will be screened in 2018 to identify ±100 *Fhb1* homozygotes. The latter homozygotes will then be marker screened to derive selections homozygous for *Fhb1* only, *Fhb1* & *Qfhs.ifa-5A; Fhb1* & *Fhb6*; and *Fhb1* & *Qfhs.ifa-5A* & *Fhb6*. Such plants will be used for comparison of the QTL effects in a greenhouse FHB trial. Pyramids of *Fhb1* with *Lr35/Sr39* and *Lr53*. Near-isogenic (Norstar background) F_3 material that is homozygous for *Fhb1* but segregates or the rust resistance ➨➨

is currently being screened to identify pyramids having all the genes. *Fhb1* pyramids with durable leaf rust resistance genes (*Lr34, Lr46* and *Lr68*). Doubled haploids that appear to contain pyramids of the four genes were selected using molecular markers.

Development of broad genetic base pyramids

Single seed descent (SSD). A large set of modified (greenhouse selection for Type II FHB resistance applied in the F_2) SSD inbred lines has been developed from the F_2 of 21 diverse crosses (made in 2016) and the inbred lines (approximately 400) were field planted as $F_{3:4}$ lines in September 2017. The crosses include 13 that segregate for Fhb1 plus a 5A FHB QTL (*Qfhs.ifa-5A* or *QTL5A-1*); six that segregate for *Fhb1* only; and two that segregate for *QTL5A*). All the crosses involved parents with significant resistance to leaf and stem rust. The material will be selected phenotypically and with markers to recover more complex resistance combinations.

Doubled haploids (DHs). 394 DHs were derived from eight crosses. The cross parents were selected such that each cross provides for an opportunity to derive lines having *Fhb1* plus a chromosome 5A FHB resistance QTL. In addition the parents contributed one or more of the resistances: *Lr53, Lr56, Sr24, Sr2*, TS, and SNB. The material has been marker-screened and planted for field evaluation in 2018. Useful pyramided lines with acceptable cold-hardiness will be identified for continued use in convergent crosses.

Seedling phenotyping and marker screening. Junior, Senior and Elite trial entries (totaling 490 entries) were inoculated as seedlings and scored with six prevalent leaf rust pathotypes/collections; four stem rust races; tan spot (mixed inoculum) and Stagonospora nodorum (Sn4). All the lines have also been evaluated with markers for the presence of *Fhb1, Lr34, tsn1, Sr2, Lr24, Yr17*, 1BL.1RS and 1AL.1RS. Lines with significant single gene resistance (such as *Fhb1, Yr17, Sr24, Lr50, Lr53, Lr56, Lr59, Lr62, Sr39/Lr35*, and *Sr50*) or combinations thereof were identified. One hundred and forty and 150 of the Senior and Junior lines have been sent to Washington State University and Kansas State University, respectively, for stripe rust (field) resistance screening. Additional resistance sources were identified and introduced as parents in the 2017 crossing block: Five leaf and stem rust resistant entries from the 2016 Regional Germplasm Observation Nursery; seven NDSU breeding lines with resistance to leaf, stem and stripe rust and five NDSU lines with good resistance to stripe rust; two SDSU lines (2015 Northern Regional Performance Nursery) with the stripe rust resistance gene, *Yr17*; three 2016 RGON lines with stripe rust resistance as well as strong resistance to all US and East African stem rust races; and six NDSU inbred lines with promising tan spot and S nodorum resistance plus excellent stem rust resistance. All these sources were included in crosses with the aim to derive more complex combinations.

Accelerated gene pyramiding through recurrent selection. Breeding of high yielding, winter-hardy wheat cultivars with good processing quality and stable, multiple disease resistance is complicated by the polygenic nature and low to medium heritability of the traits. Conventional gene pyramiding often relies on some form of backcrossing that limits genetic diversity and sets ceilings for traits such as yield. To improve gene pyramiding in the NDSU pedigree breeding program, parallel recurrent selection cycles are being incorporated since this past season. A “co-evolving” pre-breeding population is being established in which selected pedigree-bred F_6 lines will annually be crossed with selected male-sterile RS F_1 plants. Initially, male parents for RS will derive from conventional pedigree program crosses only; however, when fully implemented, 80% of the male parents will derive from RS and 20% from pedigree program crosses. Evaluation of inbred lines for cold-hardiness and resistance to FHB, leaf rust, stem rust, stripe rust, SNB and TS will utilize and combine markers, field and greenhouse phenotyping as appropriate. Shortened RS cycles will increase target gene frequencies, maximize recombination, accelerate random gene pyramiding and maintain broad genetic variability. Annually, highly diverse RS generated F_2 families will be fed into the pedigree breeding program and evaluated with the conventional crosses.

A set of 38 diverse inbred lines has been identified from the 2017 field trials. These lines will contribute a broad range of resistance genes and will be randomly inter-crossed as male parents with selected female F_1 in February–March 2018.

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 seventh 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. 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. In addition, a pyramiding scheme often needs to be modified as new results and cross progenies become available. Molecular marker characterization (DNA extraction and use of marker systems such as microsatellite, SCAR, EST, 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 appropriate markers are not available or it is used to confirm marker results. In the past three seasons, stripe rust infections occurred regularly and it is apparent that breeding for resistance to this devastating disease has now become a necessity. Only low levels of resistance have been found in the breeding population and we are in the process of acquiring and implementing more resistance sources. 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

The project is conducted in support of the NDSU hard red winter wheat pedigree breeding program. 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 main pedigree breeding effort. In addition it also feeds into a recurrent selection scheme that is designed to increase the rate at which new resistance genes can be recombined into new, useful combinations.

Recommended Future Research

It is difficult to simultaneously incorporate multiple advantageous genes affecting many different traits, in a cultivar. 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 start 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.

A recurrent selection (RS) base population has also been established. RS is a breeding strategy that can greatly facilitate gene pyramiding. We started to integrate RS steps with the pedigree program in such a way that it will yield numerous additional cross combinations that will be evaluated for gene pyramids. In each season the 30-40 best new inbred lines from the pedigree program will be employed as male parents in RS crosses. At the same time, approximately 200-300 diverse (pre-selected) RS F_2 families will be exported to, and evaluated with, the pedigree program-derived F_2 families.

Publications

Marais, G.F., McCallum, B., Kolmer, J.A., Pirseyedi, S-M., Bisek, B.R. and Somo, M. (2017). Registration of spring wheat sources of leaf rust resistance genes *Lr53, Lr56, Lr59* and *Lr62*. J. of Plant Registrations (In print).

Mohamed Somo, Seyed Mostafa Pirseyedi, Xiwen Cai, and Francois Marais (2017). Engineered versions of the wheat *Lr62* translocation. In print: Crop Science. https://dl.sciencesocieties.org/publications/cs/first-look