

Pre-breeding of HRWW to Achieve Multiple Disease Resistance

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Research Questions

NDSU initiated a HRWW pedigree breeding program in 2011. Parallel pre-breeding projects to introgress genetic variation that was lacking in the founder breeding germ-plasm have been conducted since; in particular to obtain resistance to major wheat pests such as Fusarium head blight, the wheat rusts and leaf spot diseases. Useful resistance has been transferred from diverse sources of spring wheat and less winter-hardy winter wheat. The acquired genes occurred mostly singly in introgression lines that had poor adaptation to local conditions. During the report period, three approaches have been followed to systematically integrate the new variability into the pedigree breeding program for the development of inbred lines with broader resistance spectra and superior winter survival.

First: Very specific gene combinations (pyramids) to attain complex FHB resistance in winter-hardy genetic backgrounds were pursued. Crosses were mostly based on the winter-hardy varieties Jerry and Norstar and pyramiding was strongly reliant on marker-assisted selection.

Second: Development of broad-based gene pyramids that also involve resistance to other diseases were initiated using the newest pure lines that were (annually) selected in the pedigree program. Molecular markers, bio-testing and yield assessment (field) identified promising and more complex gene combinations for follow-up crosses to produce still better combinations. Single seed descent (SSD) inbreeding and doubled haploid (DH) development were used to speed up the process.

Third: A recurrent selection (RS) pre-breeding population was coupled with the pedigree breeding program to produce additional, random F1 hybrids in each year. These additional hybrid progenies have also been tested to select potentially useful, pyramided genotypes. Annually, the best pyramided genotypes from all three pre-breeding attempts are included in pedigree program crosses to produce about 500-600 new segregating families.

Results

(i) Pre-breeding to obtain and evaluate pyramids of specific FHB resistance genes

Pyramids involving FHB resistance QTL were developed and are listed below. Those with *Fhb6* were completed in 2017 and are now being evaluated (greenhouse) for the gene's ability to complement *Fhb1* and *Qfhs.ifa-5A*. Homozygotes have not yet been derived with respect to all of the pyramids based on the PI277012 QTL; however, the available lines will be used in breeding program crosses as from 2019. (*chart see below*)

Cross # and QTL combination	Pedigree
Pyramids involving Sumai 3-derived QTL in CM82036	
11M221-24-1 (<i>Fhb1</i> , <i>Qfhs.ifa-5A</i>)	CM82036/Jerry
14K456-K-1 (<i>Fhb1</i> , <i>Qfhs.ifa-5A</i>)	CM82036/Jerry/3/ <i>Lr56-157</i> /Superb//4*Jerry
14M7 (<i>Fhb1</i> , <i>Qfhs.ifa-5A</i>)	<i>Fhb6</i> /Jerry//Radiant/3/14K456-K-1
15K353 (<i>Fhb1</i> , <i>Qfhs.ifa-5A</i>)	RWG21/Jerry//14K456-K-1
Pyramids involving <i>Fhb6</i> ex <i>Elymus tsukushiensis</i>	
14M7 (<i>Fhb1</i> , <i>Fhb6</i>)	<i>Fhb6</i> /Jerry//Radiant/3/14K456-K-1
14M7 (<i>Fhb1</i> , <i>Fhb6</i> , <i>Qfhs.ifa-5A</i>)	<i>Fhb6</i> /Jerry//Radiant/3/14K456-K-1
Pyramids involving PI277012-derived QTL	
15K353 (<i>Fhb1</i> , <i>Qfhb.rwg-5A.1</i>)	RWG21/Jerry//14K456-K-1
(<i>Qfhb.rwg-5A.1</i> , <i>Qfhb.rwg-5A.2</i>)	PI277012/Grandin// 2*Novus-4 (= RWG21/Jerry)
(<i>Fhb1</i> , <i>Qfhb.rwg-5A.1</i> , <i>Qfhb.rwg-5A.2</i>)	PI277012/Grandin//14K456-K-35F-9/3/14K456-K-1

(ii) Pre-breeding to develop pyramids with broader based resistance

Annually, the most promising pedigree breeding program crosses have been identified for generation acceleration and scrutiny to identify plants with good phenotype and broad resistance. The first comprehensive evaluation of the progenies occurs when they attain pure line status and shows agronomic merit for inclusion in preliminary (Junior), un-replicated field plot trials. The progenies derived for this purpose and their status are summarized below.

Cross cycle	Lines developed	Field evaluation		
		Final (F ₄) single spike selection	DH/ F ₅ pure lines	Un-replicated Junior plots
2015	332 SSD lines from 8 crosses	2017	2018	2019
	344 DH lines from 8 crosses	-	2018	2019
2016	394 SSD F ₄ lines from 22 crosses	2018	2019	2020
	259 SSD F ₄ lines from one cross	2019	2020	2021
2017	634 DH lines from 8 crosses	-	2019	2020
	332 SSD F ₄ lines from 23 crosses	2019	2020	2021
2018	450 DHs from 9 crosses	-	2021	2022
	± 350 SSD lines initiated (25 crosses)	2020	2021	2022
	± 400 DH lines initiated (8 crosses)	-	2022	2023

As shown, despite the use of generation acceleration methods, the process of identifying pyramided genotypes that are both winter-hardy and high yielding is unavoidably time-consuming. The first selections (from 2015 crosses) of pre-breeding effort (ii) will be comprehensively evaluated in 2019 for resistance to FHB, individual leaf and stem rust races, tan spot, Septoria nodorum blotch, and appropriate molecular markers. The best selections (pyramids) will be involved as first-time parents in the RS and pedigree program crosses of 2020. Similarly, the benefit of having used parents with pyramided resistance in the past (2018) season's crosses will not be realized until 2023/24. Thus, the process of convergent breeding to attain multi-pest resistance is complex and requires ongoing effort.

(iii) Pre-breeding based on recurrent selection

Recurrent selection cycles have been integrated in the NDSU pedigree breeding program. Selected pedigree-bred lines are annually crossed as male parents with selected male-sterile F₁ plants from the RS population. These short RS breeding cycles contribute additional hybrid combinations, require minimal additional input, increase target gene frequencies, maximize recombination, accelerate random gene pyramiding and maintain broad genetic variability. Annually, diverse RS generated F₂ families are fed back into the pedigree breeding program to be evaluated with the conventional crosses.

Since 2017 a proportion of RS male parents are chosen to specifically address new pre-breeding needs, which are:

- (a) Recently, the incidence and severity of natural stripe rust infections appeared to be on the increase, necessitating the acquisition and use of resistant breeding material.
- (b) There is a need to also breed white hard winter wheat without significantly increasing the current breeding effort.

(c) The newly developed FHB resistance gene pyramids occur in highly related genetic backgrounds, which limits their utility. It is necessary to continue to transfer these genes into more diverse germplasm.

In February 2017 RS male-sterile F₁ were pollinated with male parents that were both white seeded and stripe rust resistant to generate F₁. Male-sterile F₁ from the latter crosses will again be pollinated with new sources of pyramided FHB resistance QTL and selected with the purpose to develop more diverse white and red seeded inbred lines with multiple disease resistance. Similar hybrid populations will be generated in subsequent years.

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 numerous cycles of un-interrupted, meticulous crosses; strict phenotypic and statistical evaluation and selection. This purpose is better served through targeted pre-breeding utilizing accelerated pure line development and marker-facilitated selection. The genetic material and gene pyramids developed in the course of this project will however, not only ensure that the breeding program reach maximum productivity sooner; it also has commercial potential and we will continue to evaluate it in yield trials.

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» **Materials and Methods**

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 have not been available in the winter wheat 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.

Standard plant breeding methodologies (convergent- and backcrosses; doubled haploid production and modified (with selection) single seed descent inbreeding) have been followed in the pyramiding attempts. 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. 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 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.

Related Research

The project was 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 was meant to directly supplement and facilitate the main pedigree breeding effort.

Recommended Future Research

- a. Continue to acquire and evaluate new resistance and adaptation genes and increase the frequencies of those genes within the pedigree program base population.
- b. Build ever more complex and versatile gene combinations in genetically diverse and high yielding backgrounds (new breeding parents) that would facilitate the selection of improved cultivars.