Exploiting Genetic Variation for Wheat Improvement in the Northern Great Plains

Brian Steffenson, Dept. of Plant Pathology, U of M

Research Questions

Crop improvement is predicated on exploiting genetic variation. Without this variation, breeders cannot advance germplasm for any of the important traits of interest to growers. This project seeks to answer the question: to what degree can we enhance economically important traits in Minnesota wheat varieties using diverse germplasm from the USDA Spring Wheat Core Collection?

This germplasm enhancement project is based on nested association mapping (NAM) and was initiated in 2013. It is a broad-base program that will provide a rich source of genetic diversity for many traits that are or may become important to wheat growers in the region. This includes, but is not limited to: yield, protein content, milling and baking quality, root growth, stand establishment, nitrogen use efficiency, water use efficiency, canopy conductance (see submitted project by Walid Sadok), and disease and insect resistance. The activities completed for the calendar year of 2017 include i) the genetic characterization of stem, leaf, and stripe rust resistance in the Minnesota Nested Association Mapping Population (MNAMP), ii) a second year grow-out and agronomic trait evaluation of the entire MNAMP in Crookston, and iii) the genotyping of the MNAMP through the genotype-by-sequencing (GBS) protocol.

Results

Background summary for NAM population development. Based on single nucleotide polymorphism (SNP) marker data provided by the Triticeae Coordinated Agricultural Project (TCAP), the Spring Wheat Core Collection (SWCC) held by the USDA-ARS National Small Grains Collection was grouped into four subpopulations based on their degree of relatedness. We then selected 409 accessions that represent the greatest genetic and geographic diversity in the SWCC. These 409 accessions were designated as the “Spring Wheat Diversity Collection” (SWDC) and evaluated in the field for various traits. As expected, a wide range of phenotypic diversity was observed for many traits in the SWDC. Together with UM wheat breeder Jim Anderson, we winnowed down the 409 accessions of the SWCC to the workable number of 30 based on: a) genetic diversity as assayed by SNP markers, b) desirable phenotypes in field nurseries (i.e. normal heading date, short-stature, good straw strength, disease resistance, etc.), and c) diversity for geographic origin. These 30 Nested Association Mapping Parental Selects (NAMPS) were sown in the 2013 fall greenhouse for crossing with variety RB07, selected by Jim Anderson as the common parent because of its wide adaptation to the spring wheat growing areas of Minnesota and the Dakotas. In December 2013, the first crosses of the NAMPS were made with RB07 in the greenhouse. All but five of these crosses were successful. In the end, we developed a 25-parent NAM population based on the crosses listed in Table 1. Crossed seed from these hybridizations were planted in the 2014 winter greenhouse for backcrossing to RB07. This was done to recover more of the superior genetic constitution of RB07 since some of the NAMPS are not adapted to the Midwest production region. About 100 BC_1 crossed seeds from each cross were obtained and planted in the 2014 fall greenhouse with harvest of BC_1F_2 seed (1st selfed generation) occurring at the end of December. One arbitrarily selected seed (single seed descent) from each of ~2,500 BC_1F_2 plants was grown in the 2015 winter greenhouse and harvested as BC_1F_3 seed (2nd selfed generation) in March-April 2015. A single BC_1F_3 seed from each line was sown in the 2015 spring greenhouse in April and harvested in June as BC_1F_4 seed, representing the third selfed generation. In the 2015 fall greenhouse, two BC_1F_4 seeds were sown and the plants bulk harvested, generating the $BC_1F_{3:5}$ (4th selfing). This same procedure was done with two seeds from the $BC_1F_{3:5}$ in the 2016 winter greenhouse, which was harvested in April. The $BC_1F_{3:6}$ seed harvested from these plants represents the fifth selfed generation. The total number of lines in the MNAMP is 2,067 (Table 1).

Activities in 2017

Genetic analysis of rust resistance. The main thrust of the research in 2017 was the evaluation of the MNAMP for resistance to the stem rust (*Puccinia graminis* f. sp. *tritici, Pgt*), leaf rust (*Puccinia triticina, Pt*), and stripe rust (*Puccinia striiformis* f. sp. *tritici, Pst*) pathogens. Since the specific focus of this study was to enhance the resistance of the Minnesota wheat breeding program to widely virulent races of these rust pathogens, we set out to identify parental combinations where RB07 was susceptible and the exotic parent was resistant. With respect to *Pgt*, we found the parent combinations of PI199806 × RB07, PI519465 × RB07, PI520033 × RB07, and PI623147 × RB07 exhibited markedly different reactions to race TTKSK (i.e. a Kenyan isolate with the same virulence as the original Ug99 isolate) (Figure 1); PI519465 × RB07 and PI520033 × RB07 exhibited markedly different reactions to race TRTTF (a virulent isolate from Yemen); and PI519465 × RB07 and PI520033 × RB07 exhibited markedly different reactions to race TTKST (a Ug99 group race from Kenya). Thus, progeny from these families were evaluated to the respective races for which they were

predicted to segregate. The common parent RB07 carries the leaf rust resistance gene *Lr21*, which was overcome by virulent races of the pathogen in 2010 after only three years of cultivation. To enhance the diversity of leaf rust resistance in the Minnesota wheat breeding program, the MNAMP parents were evaluated for reaction to the *Lr21*-virulent race TFBGQ (isolate 11US 1-2). Just one parental combination (PI282922 × RB07) differed markedly in response to this race; thus, only progeny from this family were subjected to genetic analysis. The MNAMP parents were also evaluated for reaction to two races of the stripe rust pathogen (PSTv-37 and PSTv-40) since this disease is becoming an increasingly important problem in the northern Great Plains region. PSTv-37 (isolate: 16-353) was collected from Fargo, North Dakota and is the most widely virulent race found in the United States. PSTv-40 (16-323) was collected from Logan, Utah and represents the most common race found in the country. RB07 was susceptible to race PSTv-37 (Figure 2) and moderately resistant to race PSTv-40. Two (PI181458 and PI282922) of the exotic parents exhibited very resistant reactions to both *Pst* races, and one (CItr14819) exhibited a very resistant reaction to PSTv-37 only (Figure 2). Thus, progeny from families CItr14819 × RB07, PI181458 × RB07, and PI282922 × RB07 were evaluated to race PSTv-37, and those from families PI181458 × RB07 and PI282922 × RB07 were evaluated to race PSTv-40.

For the initial genetic analysis, the segregation ratio of resistant to susceptible progeny within a family was tested for fit to various Mendelian gene models using the chi-square test. Epistatic and non-epistatic models of from one to seven segregating genes were tested, and the ones giving the best fit based on the chi-square test are presented in Table 2.

Stem rust. Four families segregated for resistance to *Pgt* race TTKSK: PI199806 × RB07, PI519465 × RB07, PI520033 × RB07, and PI623147 × RB07. The best fit for the segregation ratios observed in these families was for one gene, three genes, five genes, and one gene, respectively (Table 2). Two families (PI519465 × RB07 and PI520033 × RB07) segregated for resistance to race TRTTF, and the best fit found was for a one gene and two gene model, respectively. The same two families segregating to race TRTTF were also segregating to race TTKST. For family PI519465 × RB07, the best fit found was for a two gene model and for family PI520033 × RB07 a five gene model.

Leaf rust. PI282922 × RB07 was the only family segregating for reaction to *Pt* race TFBGQ. The segregation ratio found for this family fit best to a one gene model (Table 2).

Stripe rust. Three families segregated for resistance to *Pst* race PSTv-37: CItr14819 × RB07, PI181458 × RB07, and PI282922 × RB07. The best fit for the segregation ratios observed for these families was four genes, three genes, and five genes, respectively (Table 2). Two of the three families (PI181458 × RB07 and PI282922 × RB07) segregating for reaction to *Pst* race PSTv-37 were also segregating for reaction to race PSTv-40. The segregation for these two families to PSTv-40 fit best to a three and five gene model, respectively, the same as found in response to race PSTv-37.

Population increase and field trial. In fall 2016, 2,067 $BC_1F_{3:6}$ lines of the MNAMP were planted for another seed increase in the greenhouse. The derived $BC_1F_{3:7}$ seed from these plants was harvested in December 2016-January 2017, threshed, and prepared for planting in the field. In May 2017, the entire MNAMP, plus multiple replicates of the NAMPS, common parent RB07, and local wheat variety controls were planted at the Northwest Research and Outreach Center in Crookston. During the course of the season, data were collected on the following agronomic traits: days to heading, plant height, spike length, and number of kernels per spike. The MNAMP is an extremely large population to handle in the field. With a full crew of nine people, the entire population was hand-harvested using a sickle and then threshed with a Vogel threshing machine during the week of August 22-24, 2017. Seed of each line has been cleaned and will then be assessed for test weight, 1000 kernel weight, protein level, etc. in the future.

Genotyping of the MNAMP. To determine the genetic basis of complex traits important to wheat production, it is essential to develop molecular marker maps for segregating populations. All 2,067 $BC_1F_{3:6}$ lines of the MNAMP (plus multiple replicates of the parents) were genotyped using the GBS protocol. Over 66,000 single nucleotide polymorphic (SNP) markers were generated after quality control filtering. This number of molecular markers will be more than sufficient to conduct robust quantitative trait loci (QTL) analyses of all traits phenotyped in the MNAMP.

Application and Use

The germplasm developed from this project will serve as superior, adapted parental material for regional breeding programs aiming to enhance wheat for many different traits, including but not limited to yield, protein content, milling and baking quality, root growth, stand establishment, nitrogen use efficiency, water use efficiency, canopy conductance, and disease and insect resistance. From our 2017 disease phenotyping tests, we have identified a number of lines with good rust resistance and agronomic traits that are comparable to RB07. These selected lines will be distributed to breeders for use as parents in their programs. The two years of field testing with the MNAMP demonstrated that useful genetic diversity also exists for yield components as well as various agronomic and quality traits.

Economic Benefit to a Typical 500 Acre Wheat Enterprise

Varieties bred with one or more of the enhanced traits derived from the MNAMP will increase profitability for wheat producers in the region. The level of economic benefit will depend on the trait considered. It is well documented that rust diseases can cause yield losses in susceptible varieties ranging from about 5 to 30% during epidemic years. However, both stem and stripe rust have the potential to causes yield losses exceeding 50% or more during severe epidemics. With respect to quality traits, preliminary data revealed that several exotic parents have protein levels exceeding 17%. If higher protein levels can be bred into new varieties, the premium paid to producers could be substantial. It is important to note that this germplasm enhancement /pre-breeding project has a longer-term horizon for results. In this respect, it is similar to a breeding program since it will take several years before growers will realize direct economic benefits.

Materials and Methods

Many of the general materials and methods described in this report have been summarized in the results section above.

Genetic analysis of rust resistance. After evaluation of the NAMPS to various races of *Pgt, Pt*, and *Pst*, we identified those cases where RB07 was susceptible and one of the exotic parents was resistant. Then, progeny from the respective families were evaluated to these races to elucidate the genetics of resistance. Rust evaluations were done according to our standard laboratory protocols, and we obtained robust data on sources segregating for qualitative (Mendelian) resistance. For the more quantitative resistances, we will utilize the molecular marker maps to position all of the contributing resistance loci and their individual contribution to the overall phenotype.

Population increase and field trial. The MNAMP was increased in the greenhouse and the resulting seed planted in the field at Crookston in May 2017. The following traits were assessed during the growing season: heading date, plant height, spike length, number of kernels per spike, and lodging. All pots were harvested by hand using a sickle and threshed in the field. The seed has now been cleaned and is ready for the next steps in phenotyping for milling quality and other important quality traits.

Genotyping of the MNAMP. To map the loci underlying important traits, the MNAMP and parents were genotyped. To generate tissue for DNA extraction, plants were grown for 14 days in the greenhouse and the leaves harvested and then desiccated before sending to the USDA-ARS Regional Genotyping Laboratory in Raleigh, NC, under the direction of Dr. Gina Brown-Guedira. The standard GBS protocol for genotyping was applied to the MNAMP and generated over 66,000 SNP markers after quality control filtering.

Related Research

The MNAMP is a large germplasm resource that will be useful to many different wheat researchers—now and in the future. Through funding by the Minnesota Wheat Research and Promotion Council, we have constructed this large complex population of agronomic pertinence to the Midwest region through use of the adapted common parent of RB07; conducted two field trials of the MNAMP to obtain valuable agronomic data and to generate sufficient seed stocks for quality trait assessments; and obtained robust genotyping data that can be used by all researchers aiming to characterize the genetic basis of their target traits. As a case in point, Walid Sadok evaluated the NAMPS for canopy conductance and found several parents that difference markedly for this parameter from RB07. He is now evaluating the progeny from these sets of parents to map the genes contributing to canopy conductance. Canopy conductance has great potential for enhancing productivity of wheat cropping systems because higher levels are associated with higher yields.

The NAMPS have been distributed to other researchers in the region so they can phenotype this germplasm for traits of interest. These cooperators include Ruth Dill-Macky and Madeleine Smith at the University of Minnesota; Francois Marais, Shaobin Zhong and Senay Simsek at North Dakota State University and Karl Glover and Shaukat Ali at South Dakota State University. Some of these individuals have found large differences between RB07 and the 25 exotic parents for their target traits. With the publication of our work on the genetics of rust resistance in the MNAMP, we will likely see other researchers requesting this germplasm for their research. We will make public all data generated from this project via the T3 database administered by USDA-ARS.

Recommended Future Research

Our initial studies on the genetics of rust resistance in the MNAMP were very fruitful. We were able to determine the genetic basis of resistance to stem rust, leaf rust and stripe rust. Moreover, we identified a number of lines with good rust resistance and agronomic traits that are comparable to RB07. Through four years of work, we have developed the resources for many future research projects on wheat: a large NAM population structured on a widely adapted variety RB07; sufficient pure seed stocks of each MNAMP line for distribution and testing by other researchers; and a robust genotyping dataset for mapping traits of interest. Our future objectives for the MNAMP will focus on the genetic basis of agronomic and quality traits. From our two seasons of field testing, we have observed high levels of genetic diversity for many important traits. As one example, we tested the NAMPS for protein and discovered two that had levels exceeding 17% over two seasons. We will pursue this line of research by characterizing the genetic basis of protein content in the two families derived from these parents.

Table 1. Inidividual crosses and numbers of progeny in each family of the Minnesota Nested Association mapping Population

Cross Combination	Country of origin for exotic parent	Number of progeny within each family
CItr14819 x RB07	Eritrea	91
CItr15006 x RB07	Nepal	77
PI62364 x RB07	Venezuela	76
PI53785 X RB07	Brazil	86
PI181458 x RB07	Finland	96
PI189771 x RB07	Tunisia	95
PI193938 x RB07	Brazil	80
PI199806 x RB07	Peru	86
PI205714 x RB07	Peru	72
PI213602 x RB07	Argentina	59
PI220455 x RB07	Egypt	68
PI278392 x RB07	Palestine	71
PI282922 x RB07	Argentina	82
PI344018 x RB07	Angola	76
PI345693 x RB07	Belarus	96
PI374254 x RB07	Mali	90
PI384403 x RB07	Nigeria	85
PI430750 x RB07	Yemen	81
PI449298 x RB07	Spain	87
PI519465 x RB07	Zimbabwe	89
PI519580 x RB07	Chile	92
PI520033 x RB07	Kenya	85
PI520371 x RB07	Syria	79
PI565238 x RB07	Bolivia	93
PI623147 x RB07	Iran	75
Total		2,067

Table 2. Segregation of BC_1 (backcross one) recombinant inbred line (RIL) families of the Minnesota nested association mapping population to three races of the stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) one race of the leaf rust pathogen (*Puccinia triticina*), and two races of the stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*)

				Observed			Gene model	Chi square value	Probability
Family	**Race**	**Pathogen**	**Parental IT**	**R[a]**	**S[a]**	**Total**	**Gene model[b]**	**X^2**	**$p>X^2$**
(PI199806 x RB07)	TTKSK	*Pgt*	21/4	14	71	85	1 gene	3.3	0.1
(PI519465 x RB07)	TTKSK	*Pgt*	0;/4	54	35	89	3 genes	0.58	0.58
(PI520033 x RB07)	TTKSK	*Pgt*	;1-/34	68	15	83	5 genes	0.11	0.23
(PI623147 x RB07)	TTKSK	*Pgt*	22+/4	22	45	67	1 gene	2.3	0.2
(PI519465 x RB07)	TRTTF	*Pgt*	12/33+	17	68	85	1 gene	1.2	0.3
(PI520033 x RB07)	TRTTF	*Pgt*	0;1/4	28	51	79	2 genes	2.2	0.14
(PI519465 x RB07)	TTKSK	*Pgt*	0;1/33+	40	46	86	2 genes	0.27	0.61
(PI520033 x RB07)	TTKSK	*Pgt*	0;1-/3+4	68	14	82	5 genes	2.01	0.16
(PI282922 x RB07)	TFBGQ	*Pt*	21/3	24	56	80	1 gene	1.1	0.3
(CItr14819 x RB07)	PSTv37	*Pst*	2/7	68	23	91	4 genes	1.70	0.19
(PI181458 x RB07)	PSTv37	*Pst*	2/7	59	33	92	3 genes	1.51	0.22
(PI282922 x RB07)	PSTv37	*Pst*	1/7	56	12	68	5 genes	1.39	0.24
(PI181458 x RB07)	PSTv40	*Pst*	3/6	52	36	88	3 genes	0.06	0.81
(PI282922 x RB07)	PSTv40	*Pst*	1/6	65	17	82	5 genes	0.41	0.52

[a] R is the number of resistant lines and S is the number of susceptible lines observed within a family.
[b] Seven different epistatic and non-epistatic Mendelian gene models were tested for each family x pathogen race combination; however, only the one giving the best fit based on the chi-square test is given. Models were based on 1,2,3,4,5,6 and 7 segregating genes.

RB07 PI199806 PI623147 PI520033 PI5194665

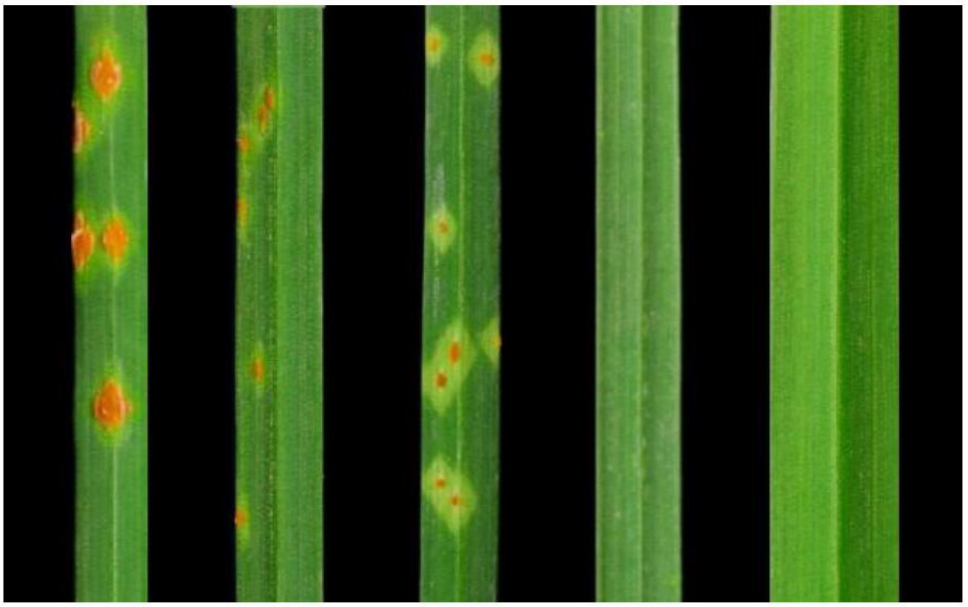

Fig. 1. Examples of different seedlings reactions of selected Minnesota nested association mapping parents to race TTKSK (i.e. Ug99 type virulence) of the stem rust pathogen (*Puccinia graminis* f. sp. *tritici*). Note the susceptible reaction of RB07.

RB07 PI282922 CItr 14819

Fig. 2. Examples of different seedling reactions of selected Minnesota nested association mapping parents to race PSTv-37 of the stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*). Note the susceptible reactions of RB07.