# Exploiting Genetic Variation for Wheat Improvement in the Northern Great Plains

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## 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-ARS Spring Wheat Core Collection?

This germplasm enhancement project is based on nested association mapping (NAM) and was initiated in 2013. It is a broad-based 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 entire project is based on the development and characterization of the Minnesota Nested Association Mapping Population (MNAMP), a large and complex germplasm resource comprised of 2,038 progeny lines derived from 25 different exotic parents that were crossed and backcrossed to RB07, a Minnesota variety with wide adaptation in the region. In phase I of this project, we developed the MNAMP and characterized it for various agronomic traits in the field and for resistance to the important diseases of stem rust (both domestic and African races), leaf rust, and also stripe rust. For phase II of the project (2018), we are: i) characterizing the genomic architecture of yield and quality traits through the genetic analysis of grain test weight, protein content and gluten strength in the MNAMP and ii) constructing comprehensive linkage maps for all 25 families of the MNAMP in order to facilitate current and future analyses of individual traits.

## Results

*Background summary for MNAMP 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 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 2,038 (Table 1)

*Phenotyping of the MNAMP in Crookston 2016 growing season.* $BC_1F_{3:6}$ seed harvested from the greenhouse in April 2016 was sown in May at the Northwest Research and Outreach Center in Crookston. Individual lines were planted as paired 4 ft. long rows spaced 24 inches apart. 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

MNAMP was hand-harvested using a sickle and then threshed with a Vogel threshing machine during the week of August 22-26. Seed of each line was cleaned and assessed for test weight and protein level. Test weights were assayed as the weight of seed (in grams) occupying a quarter pint container. These data were then converted into Kg/hL. Protein level was assayed by near-infrared reflectance (NIR).

*2017 growing season*. $BC_1F_{3:7}$ seed derived from the 2016 fall greenhouse grow-out of $BC_1F_6$ seed was planted in the field at 1Crookston in May using the same scheme as in the m as hand-harvested using a sickle and then threshed with a Vogel threshing machine during the week of August 22-24. Seed of each line was cleaned and assessed for test weight and protein level as described above.

A wide range of phenotypic diversity was observed for all of the agronomic traits scored in 2016-2017. First, with respect to the parents, days to heading of the exotic parents ranged from 49.2 (PI 189771) to 70.0 (PI 282922) compared to the recurrent parent of RB07 at 51.4 (Table 2). For plant height, the exotic parents ranged from 56.1 (PI 519465) to 99.0 cm (PI 205714) in comparison to RB07 at 75.4 cm. Spike length for the exotic parents ranged from a low of 5.2 cm (PI 199806) to a high of 10.7 cm (PI 345693) with RB07 at 6.7 cm. Test weight for the exotic parents ranged from a low of 69.3 Kg/hL (PI 181458) to a high of 85.6 Kg/hL (PI 213602) with RB07 at 85.6 Kg/hl. For many of the progeny families in the MNAMP, transgressive segregants (i.e. progeny exhibiting extreme phenotypes that exceed those of the parents) were observed for days to heading, plant height, spike length and test weight (Table 3). In most cases, the extreme phenotypes occurred in both directions: i.e. fewer and greater days to heading, shorter and taller plant heights, shorter and longer spikes, and lighter and heavier test weights than the respective parents. Transgressive segregants are useful in breeding. For example, if a breeder wanted to increase test weight in the program, he/she might consider selecting for the crossing block transgressive progeny from any family, which had a markedly higher test weight than that of RB07 (Table 1).

*2018 growing season.* For 2018, our focus was on elucidating the genetics of agronomic and quality traits in the MNAMP and identifying useful transgressive segregants that can be used in breeding. For assessment of test weight and various wheat quality parameters, we used seed harvested from 2016 and 2017 Crookston nurseries, but also conducted important validation experiments of selected families in 2018.

Test weight was assayed first on the exotic MNAMP parents and RB07 (2016 and 2017 seasons) using the protocol described above. Then, progeny from four families (with exotic parents from CItr15006, CItr14819, PI 181458, and PI 193938) exhibiting wide variation were then assayed for test weight. The same four selected families were grown in 2018 in replicated trials at Crookston and Morris for validation.

Protein content is important in the hard red spring wheat class because it commands a higher price. To identify MNAMP progeny that carry alleles for increased protein levels, we first conducted NIR assays on the exotic MNAMP parents and RB07 from the previous two seasons. Three exotic parents (CItr14819, CItr15006, and PI 181458) were found to have protein levels that were 11-17% higher than RB07 (17.2%). Progeny derived from these three exotic parents were grown in 2018 at Crookston and Morris for validation. Protein level assessments of these progeny families are in progress and should be completed by December 2018.

In bread-making, strong gluten is preferred because it produces bread loaves that rise well and hold their shape without collapsing. There are several methods for assessing the association kinetics of gluten strength. One fast and inexpensive method being used in George Annor's laboratory is the Gluten Peak Tester (GPT), a high-throughput (~5-minute test time) device that also requires relatively small seed samples (10-20 grams). In this test, a uniform gluten network is formed, resulting in a marked increase in torque. Further mixing destroys this network, resulting in a decrease in torque. Important parameters such as peak time (time to reach the maximum torque) and the maximum height of the curve are then measured with the GPT. Strong gluten types show short peak times with high peaks, while weak gluten types show late aggregation peak times with low or no peaks. Initially, GPT assays will be performed on all exotic MNAMP parents and RB07 (2016 and 2017 seasons) to select families showing broad segregation for gluten strength. Three parents (CItr14819, CItr15006, and PI 181458) were found to have high grain protein contents based on wet lab and NIR analysis and desirable gluten strength characteristics worthy of further investigation. The families of these parents were grown in the summer of 2018 at Crookston and Morris for validation and further testing of bread making quality parameters. GPT and other bread making quality assays will be completed in George Annor's laboratory by March 2019.

*Phenotyping of the MNAMP for rust resistance in the greenhouse*

The main thrust of the disease resistance research 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

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» 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 4.

*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 4). 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 4).

*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 4). 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. Family CItr14819 segregated in response to race PSTv-37 only. The best fit found for segregation was a four gene model.

*Genotyping of the MNAMP and linkage map construction.* 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 genotyping-by-sequencing (GBS) protocol by the USDA-ARS Regional Genotyping Laboratory in Raleigh, NC, under the direction of Dr. Gina Brown-Guedira. These data require great refinement in order to be useful for mapping the loci contributing to target traits. Extensive data filtering was done by removing markers with more than 20% missing data and minor allele frequency (MAF) of 0.03 or less. In the end, over 66,000 single nucleotide polymorphic (SNP) markers were generated after quality control filtering. The construction of robust and comprehensive linkage maps for all 25 families of the MNAMP are essential in order to facilitate current and future quantitative trait locus (QTL) analyses of individual traits.

We have now completed the construction of 525 linkage maps for the MNAMP: 21 wheat chromosomes per family x 25 families = 525). QTL analyses are being performed to determine the number, chromosome location and effect of loci contributing to the target agronomic and quality traits. This will be done for seed harvested from the 2016, 2017, and 2018 seasons. The same QTL analysis is also being done for the rust resistance traits based on greenhouse phenotype data. All of these analyses plus submission of manuscripts should be completed by March 2019.

## 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 greenhouse and field phenotyping tests, we have identified a number of progeny lines with agronomic, quality, and resistance traits that exceed or are comparable to RB07. These selected lines will be distributed to breeders for use as parents in their programs. The three 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.

## Materials and Methods

The key information about the materials and methods used in this investigation are described in the results section above.

## 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.

## 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 three 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 development and genetic architecture of important traits in the MNAMP, we expect to 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. Since we have already generated the population and molecular maps, it will be very easy for any future researcher to quickly and easily map their traits of interest after phenotyping.

## 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 five 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. With the publication of the wheat reference genome sequence, it will now be possible to identify gene candidates for economically important traits.

We are not submitting a grant proposal for this research project in 2019. Rather, we will use this period to fully analyze all data in order to plan the next phase of research that will be directly applicable to advancing Jim Anderson's wheat breeding program.

## Publications

Manan, F. 2017. Genetics of rust resistance in a wheat nested association mapping population. M.S. thesis, University of Minnesota, St. Paul. 140 pp.

Sallam, A.H., Steffenson B.J., and Anderson J. 2019. A Whole Genome Association Study in the Minnesota Nested Association Mapping Population. Plant and Animal Genome Meeting, San Diego, California. (in press).

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## Application and Use

**Table 1**. Individual 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 | 86 |
| **CItr15006 x RB07** | Nepal | 76 |
| **PI62364 x RB07** | Venezuela | 75 |
| **PI153785 x RB07** | Brazil | 84 |
| **PI181458 x RB07** | Finland | 95 |
| **PI189771 x RB07** | Tunisia | 94 |
| **PI193938 x RB07** | Brazil | 79 |
| **PI199806 x RB07** | Peru | 84 |
| **PI205714 x RB07** | Peru | 72 |
| **PI213602 x RB07** | Argentina | 58 |
| **PI220455 x RB07** | Egypt | 68 |
| **PI278392 x RB07** | Palestine | 70 |
| **PI282922 x RB07** | Argentina | 82 |
| **PI344018 x RB07** | Angola | 76 |
| **PI345693 x RB07** | Belarus | 96 |
| **PI374254 x RB07** | Mali | 89 |
| **PI384403 x RB07** | Nigeria | 82 |
| **PI430750 x RB07** | Yemen | 80 |
| **PI449298 x RB07** | Spain | 87 |
| **PI519465 x RB07** | Zimbabwe | 87 |
| **PI519580 x RB07** | Chile | 91 |
| **PI520033 x RB07** | Kenya | 81 |
| **PI520371 x RB07** | Syria | 78 |
| **PI565238 x RB07** | Bolivia | 93 |
| **PI623147 x RB07** | Iran | 75 |
| **Total** | | 2,038 |

**Table 2.** Data for days to heading, plant height, test weight, and spike length for the Minnesota Wheat Nested Association Mapping parents planted at Crookston MN in 2016-2017.

| LID | Accession | Origin | Days to Heading | Plant Height | Test Weight | Spike Length |
|---|---|---|---|---|---|---|
| 3 | CItr 14819 | Eritrea | 53.7 | 84.1 | 83.3 | 9.3 |
| 4 | CItr 15006 | Nepal | 54.4 | 92.4 | 81.7 | 8.7 |
| 5 | PI 62364 | Venezuela | 52.4 | 78.6 | 84.4 | 7.5 |
| 6 | PI 153785 | Brazil | 56.9 | 94.3 | 82.0 | 9.5 |
| 8 | PI 181458 | Finland | 61.7 | 83.7 | 69.3 | 8.1 |
| 9 | PI 189771 | Tunisia | 49.2 | 85.9 | 82.8 | 9.0 |
| 10 | PI 193938 | Brazil | 58.9 | 90.6 | 82.2 | 8.7 |
| 11 | PI 199806 | Peru | 54.9 | 65.4 | 81.1 | 5.2 |
| 12 | PI 205714 | Peru | 61.5 | 99.0 | 75.8 | 9.6 |
| 13 | PI 213602 | Argentina | 65.0 | 71.0 | 85.6 | 7.3 |
| 14 | PI 220455 | Egypt | 59.7 | 65.0 | 80.6 | 6.5 |
| 15 | PI 278392 | Palestine | 57.7 | 65.3 | 79.6 | 8.0 |
| 16 | PI 282922 | Argentina | 70.0 | 76.0 | 81.6 | 8.0 |
| 17 | PI 344018 | Angola | 58.9 | 69.9 | 75.2 | 7.4 |
| 18 | PI 345693 | Belarus | 57.2 | 92.3 | 78.4 | 10.7 |
| 20 | PI 374254 | Mali | 52.2 | 82.8 | 79.0 | 8.0 |
| 21 | PI 384403 | Nigeria | 49.4 | 75.6 | 78.9 | 6.9 |
| 22 | PI 430750 | Yemen | 54.2 | 70.9 | 80.3 | 9.2 |
| 23 | PI 449298 | Spain | 51.2 | 71.9 | 81.4 | 8.0 |
| 24 | PI 519465 | Zimbabwe | 50.9 | 56.1 | 76.4 | 7.7 |
| 25 | PI 519580 | Chile | 53.7 | 77.5 | 81.6 | 7.2 |
| 26 | PI 520033 | Kenya | 53.9 | 79.2 | 83.1 | 7.6 |
| 27 | PI 520371 | Syria | 49.7 | 72.8 | 76.5 | 9.3 |
| 29 | PI 565238 | Bolivia | 49.7 | 78.4 | 80.1 | 8.4 |
| 30 | PI 623147 | Iran | 53.7 | 91.3 | 78.7 | 9.7 |
| P1 | RB07 | USA | 51.4 | 75.4 | 85.6 | 6.7 |

LID=Laboratory ID number. Accessions are designated by Cereal Investigation numbers for Triticum or Plant Introduction numbers. Origin=Country of origin. Days to heading were the number of days from planting to when 50% of spikes in the row were half-emerged from boot. Plant height & spike length were measured in centimeters. Test weights were taken as the weight of seed (in grams) occupying a quarter pint container. These data were then converted into Kg/hL.

**Table 3.** Mean and range (minimum and maximum) for data on days to heading, plant height, test weight, and spike length in families of the Minnesota Wheat Nested Association Mapping Population planted at Crookston MN in 2016-2017.

| Family # | Days to Heading | | | Plant Height | | | Test weight | | | Spike Length | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max |
| 3 | 53.4 | 49 | 75 | 80.5 | 63 | 98.6 | 83.9 | 78.3 | 90.8 | 7.6 | 5.3 | 10.3 |
| 4 | 51.8 | 49.2 | 72 | 79.1 | 63.9 | 100.2 | 84.1 | 74.3 | 90.1 | 7.4 | 5.9 | 9.1 |
| 5 | 52.6 | 49 | 69 | 77.1 | 62 | 102 | 83.4 | 76.2 | 88.2 | 7.3 | 5.9 | 9.3 |
| 6 | 53.8 | 48.7 | 73 | 82 | 64 | 104.7 | 84.2 | 78.3 | 88.5 | 7.9 | 6.4 | 9.4 |
| 8 | 54.5 | 50.2 | 65 | 78.6 | 55 | 104.7 | 82.1 | 72.9 | 88.5 | 7.4 | 5.5 | 9.2 |
| 9 | 52.4 | 48.7 | 59.7 | 79.8 | 51 | 103.5 | 82.9 | 73.1 | 88.8 | 7.7 | 6.4 | 9.6 |
| 10 | 52.6 | 49.2 | 67 | 81.4 | 64.1 | 101.7 | 85.6 | 73 | 89.1 | 7.5 | 5.8 | 9.3 |
| 11 | 52.7 | 49 | 72 | 76.4 | 62.4 | 101.1 | 82.1 | 68.6 | 87.5 | 6.8 | 3.2 | 9.7 |
| 12 | 51.9 | 48.7 | 71 | 78 | 63.1 | 103.5 | 83.9 | 76.9 | 89.4 | 7.3 | 5.9 | 9.2 |
| 13 | 53.4 | 49.2 | 68 | 76.7 | 58.6 | 97.4 | 83.6 | 73.4 | 88.4 | 7.1 | 5.7 | 8.2 |
| 14 | 54 | 49.2 | 70 | 75.8 | 59.7 | 103.7 | 82 | 71.4 | 88.9 | 7.2 | 5.4 | 8.8 |
| 15 | 51.9 | 48.7 | 68 | 77.6 | 65 | 93.7 | 83.6 | 72.7 | 87.5 | 7.1 | 5.6 | 8.9 |
| 16 | 52.9 | 49.2 | 59.7 | 77.7 | 64.2 | 96.1 | 83.9 | 73.8 | 90.4 | 7.5 | 5.5 | 9.4 |
| 17 | 54.2 | 48.7 | 70 | 75.6 | 59.2 | 99 | 82.4 | 70.3 | 88.4 | 7.3 | 5.7 | 9 |
| 18 | 52.9 | 49.2 | 58.7 | 81.1 | 66 | 103.1 | 82.6 | 76.9 | 88.6 | 7.7 | 6.2 | 9.9 |
| 20 | 52.4 | 49.2 | 58.2 | 77.2 | 43 | 107 | 81.6 | 72.7 | 86.4 | 7.3 | 4.5 | 9.1 |
| 21 | 51.1 | 48 | 64.2 | 79.4 | 60 | 98.4 | 83.4 | 75.7 | 88.4 | 7.4 | 5.3 | 8.9 |
| 22 | 54.3 | 48.7 | 69 | 76.3 | 44 | 113 | 81.5 | 66.3 | 90.5 | 7.8 | 6.4 | 9.5 |
| 23 | 52.1 | 49.2 | 67 | 73.6 | 61.5 | 82 | 82.7 | 74.7 | 88.2 | 7.3 | 5.6 | 9.8 |
| 24 | 52.6 | 49 | 58.7 | 68.3 | 58 | 84.7 | 81.1 | 71.7 | 88.2 | 7 | 5.6 | 8.8 |
| 25 | 53 | 49.2 | 60.7 | 77.6 | 63.1 | 100.7 | 83.7 | 74.1 | 88.5 | 7.5 | 6.2 | 9.4 |
| 26 | 52.4 | 49.2 | 60.7 | 69.6 | 45.6 | 90.4 | 83.7 | 78.6 | 89.3 | 6.7 | 4.1 | 8.2 |
| 27 | 53.4 | 49.2 | 58.7 | 76.8 | 52 | 106.1 | 83.2 | 70.3 | 89.5 | 7.4 | 5.7 | 9.8 |
| 29 | 51.9 | 49.2 | 58.7 | 79.1 | 61.4 | 96.6 | 83.6 | 73.6 | 89.5 | 7.4 | 5.9 | 9.6 |
| 30 | 53.4 | 48.7 | 61.7 | 79.3 | 60.5 | 103.7 | 82.5 | 74.5 | 87.4 | 7.4 | 5.4 | 9.2 |

Family number refers to the exotic parent (see respective numbers in Table 1) used in the cross with RB07. For example, family #18 was derived from the cross between PI 345693 and RB07. The mean (average) and range (highest and lowest values) are provided for data on the four assessed traits within each family. Days to heading were the number of days from planting to when 50% of spikes in the row were half-emerged from boot. Plant height & spike length were measured in centimeters. Test weights were taken as the weight of seed (in grams) occupying a quarter pint container. These data were then converted into Kg/hL.

**Table 4.** 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[b] model** | **$X^2$** | **$p>X^2$** |
| (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 | 1 gene | 0.58 | 0.58 |
| P1520033 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) | TTKST | *Pgt* | 0;1-/33+ | 40 | 46 | 86 | 2 genes | 0.27 | 0.61 |
| (PI520033 x RB07) | TTKST | *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* | 2/7 | 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.

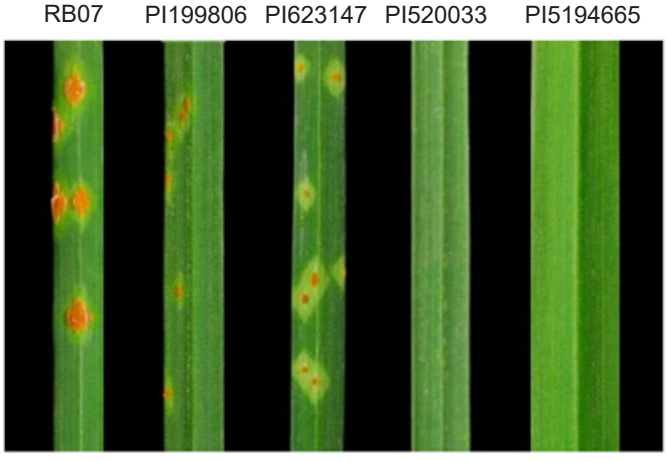


**Fig 1.** Examples of different seedling reactions of selected Minnesota nested association mapping parents to race TTKSK (i.e. Ug99 type virulence) of the stem rust pathogen (*Puccinia graminis* f. sp. *tritici*). Note the susceptible reaction of RB07.

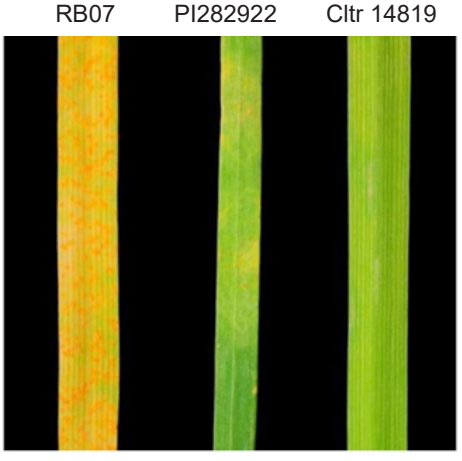


**Fig. 2.** Examples of different seedings reaction of selected Minnesota nested association mapping parents to race PSTv-37 of the stripe rust pathogen (*Puccinia striiformis* f. sp. *tritci*). Note the susceptible reaction of RB07.