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: what degree can we enhance economically important traits in wheat 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 long-term and 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, and disease and insect resistance.

Results

Summary of population development activities. Based on single nucleotide polymorphism (SNP) marker data provided by the Triticeae Coordinated Agricultural Project (TCAP), the Spring Wheat Core Collection 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 Spring Wheat Core Collection. 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 cultivar RB07, selected by Jim Anderson as the recurrent parent. In December 2013, the first crosses of the NAMPS were made with RB07 in the greenhouse. All but five of these crosses were successful; thus, we developed a 25-parent NAM population from the accessions 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 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 in April 2015. Another generation advance of this population (harvesting BC_1F_4 seed) was made during the 2015 spring greenhouse season. To further increase homozygosity in the NAM population and at the same time increase seed for the larger field plots sown at Crookston in 2016, we planted BC_1F_4 seed in the 2015 fall greenhouse. The BC_1F_5 seed from these BC_1F_4 plants were harvested in December 2015 and planted in the 2016 spring greenhouse. See the timetable and status summary for the project under the Recommended Future Research section.

Activities in 2016. The BC_1F_5 seeds harvested from BC_1F_4 plants in December 2015 were sown in the 2016 spring greenhouse. The BC_1F_6 seeds from these BC_1F_5 plants were harvested in March 2016 and prepared for spring planting at Crookston. The final number of lines in the Minnesota Nested Association Mapping Population (MNAMP) was 2,240. MNAMP was planted on May 16, 2016 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. Natural infections of several important wheat diseases occurred in the nursery. Therefore, we also assessed the reaction of each MNAMP line and respective parents to the bacterial leaf streak (BLS) (*Xanthomonas translucens*), leaf rust (*Puccinia triticina*), and stripe rust (*Puccinia striiformis f.* sp. *tritici*) pathogens.

A wide range of phenotypic diversity was observed for all of the agronomic traits scored in 2016. First, with respect to the parents, days to heading of the exotic parents ranged from 51 (PI 519465) to 70 (PI 282922) compared to the recurrent parent of RB07 at 50 (Table 1). For plant height, the exotic parents ranged from 58 (PI 519465) to 98 cm (PI 205714) in comparison to RB07 at 79 cm. Spike length for the exotic parents ranged from a low of 4.2 cm (PI 199806) to a high of 10.5 cm (PI 345693) with RB07 at 7.0 cm. For nearly every one 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 and spike length (Table 2). Moreover, in most every case,

the extreme phenotypes occurred in both directions: i.e. fewer and greater days to heading, shorter and taller plant heights, and shorter and longer spikes than the respective parents. The few exceptions where transgressive segregants in both directions were not found include MNAMP family #13 for plant height and families #6, #18, #27, and #30 for spike length (Table 2). Transgressive segregants are useful in breeding. For example, if a breeder wanted to increase the length of the spike and hence seed number in the program, he/she might consider selecting for the crossing block transgressive progeny from family #18 with a spike length of 10.5 cm (Table 2), a significant increase over the 7.0 cm spike length of RB07 (Table 1).

In addition to the agronomic traits, we also observed phenotypic diversity in the parents for reaction to BLS. RB07 is moderately susceptible to BLS giving a reaction of 5 on a 0 to 9 scale (Table 1). PI 278392 is highly resistant (reaction=1) to this disease (Table 1) and can therefore serve as a valuable donor for BLS resistance in its derived MNAMP family #15 (Table 2). Other families showed strong transgressive segregation for resistance to BLS and can also serve as sources of resistance.

The NAMPS were grown out in 2015 at St. Paul, and the seed harvested from these plots was sent to the Wheat Quality Laboratory at North Dakota State University under the direction of Dr. Senay Simsek. One striking result from this analysis was the high protein levels observed in some of the parents, several of which had levels exceeding 17.5%. This test will be repeated on the NAMPS grown in 2016.

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 has been cleaned and will then be assessed for test weight, 1000 kernel weight, protein level, etc. In the fall greenhouse 2016, BC_1F_6 seed was planted for increase. The derived BC_1F_7 generation seed will be planted and evaluated for various agronomic and disease reaction traits in the field in 2017. To generate molecular markers for mapping traits of interest, we will subject the MNAMP to genotype by sequencing. This service will be performed by the USDA-ARS Regional Genotyping Laboratory in Raleigh, NC, under the direction of Dr. Gina Brown-Guedira. We have already collected tissue from BC_1F_6 plants and sent them to North Carolina.

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, and disease and insect resistance. Our first year of field data demonstrated that useful genetic diversity exists in the MNAMP for yield components, quality traits, and disease resistance.

Materials and Methods

The MNAMP will be planted again in the field in spring 2017 and assessed for the following traits: heading date, plant height, spike length, number of kernels per spike, and lodging. In addition, we will assess the field reaction of lines to any diseases that develop in the nursery. For the 2017 field trial, the plot size will be larger to generate sufficient seed quantities for milling and baking tests at NDSU. In 2015, the mapping parents were evaluated to the widely virulent African stem rust (*Puccinia graminis f.* sp*. tritici*) races of TTKSK (isolate synonym Ug99) and TRTTF inside the Biosafety Level-3 (BSL-3) greenhouse. Significantly, several NAMPS were resistant to either race TTKSK or TRTTF, and one was resistant to both (PI 519465). These results hold great promise for enhancing the resistance of Minnesota wheat varieties to widely virulent African stem rust races. Additionally, several NAMPS possess resistance to the stripe rust and leaf rust pathogens. We will evaluate each of the MNAMP families whose parents exhibit differences in reaction to these rust pathogens. The data from these phenotyping tests will be merged with the genotyping data to provide valuable information on the number and chromosomal location of genes controlling resistance. These data will be valuable for introgressing the resistance traits into the Minnesota breeding program.

The NAMPS have been distributed to other researchers in the region so they can phenotype the germplasm for traits of interest. These cooperators include Ruth Dill-Macky and Madeleine Smith at the University of Minnesota; Francois Marais, Shaobin Zhong & Senay Simsek at North Dakota State University and Karl Glover, Shaukat Ali, and Bill Berzonsky at South Dakota State University. We expect to receive phenotype data from their trials in the near future and will provide seed of any MNAMP families whose parents exhibit differences in the target traits under study.

Economic Benefit to a Typical 500 Acre Wheat Enterprise

Cultivars 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. Several exotic parents have protein levels exceeding 17%. If higher protein levels can be bred into new cultivars, the premium paid to producers could be substantial. Incorporating new resistance to a disease like BLS also can provide significant benefits during epidemic years. It is important to note that this pre-breeding project has a longer-term horizon for results. In this respect, it is similar to breeding programs since it will take several years before growers will realize direct economic benefits.

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Related Research

To effectively map and transfer genes controlling traits in the MNAMP, genotyping must be done. Currently, genotyping by sequencing is the best method for generating a sufficiently large number of molecular markers at a reasonable cost. The Minnesota Wheat Research and Promotion Council kindly provided funds for service so we can complete mapping work on select traits in 2017. Other colleagues in the region also have expressed a strong interest in evaluating the MNAMP for specific traits of importance to their programs. They have been sent seed of the NAMPS so they can choose which segregating families to pursue for genetic analyses. We will make public all of the data we have generated in this project via the T3 database administered by USDA-ARS.

Recommended Future Research

Now that the MNAMP is developed, we will evaluate it for many traits of importance to regional wheat producers, including agronomic traits (yield, lodging, etc.), milling and baking quality (flour yield, protein, absorption, mixing time, loaf volume, etc.), and disease resistance (rusts, root rots, bacterial leaf streak, etc.). Other colleagues in the region also have expressed a strong interest in evaluating the MNAMP for specific traits of importance to their programs. We will assist them by providing seed and genotype by sequencing data for their research. To follow the steps and progress of this research, I have provided the following timeline and status update.

Timetable and Status of the Minnesota Nested Association Mapping Population Project

2013 Fall GH:

--Plant NAMPS in August-September, make crosses to RB07 in November and harvest crossed (F_1) seed in December. *(Status: Completed)*

2014 Winter GH:

--Plant crossed seed in late December-early January, make backcrosses to RB07 in March, and harvest BC_1 crossed seed in April. *(Status: Completed)*

--Establish a genetically pure seed increase of the original 25 NAMPS. *(Status: Completed)*

2014 Summer field:

--Plant BC_1 crossed seed from each cross combination in the field at St. Paul in April. *(Status: Postponed until fall greenhouse season to ensure no populations are lost due to weather-related calamities).*

--Disease and agronomic trait assessments of original 25 NAMPS and RB07. *(Status: Completed)*

2014 Fall GH:

--BC_1 crossed seed (generating BC_1F_1 plants) was planted from each cross combination in the greenhouse and harvested in December (represents 1st selfed generation). *(Status: Completed)*

--Collate and analyze data taken on the NAMPS from the field. *(Status: Completed)*

2015 Spring GH:

--BC_1F_2 seed was planted (for single seed descent) and harvested in April (represents 2nd selfed generation). *(Status: Completed)*

--NAMPS were screened against African stem rust races TTKSK and TRTTF at the seedling stage. *(Status: Completed)*

--Seed of NAMPS and RB07 were distributed to cooperators around the region so they can test them for traits of interest. Parents that differ from RB07 for a particular trait can be mapped in the derived MNAMP families. *(Status: Pending)*

2015 Late spring-Summer GH:

--BC_1F_3 seed planted in April and harvested in July and August (3rd selfed generation). *(Status: Completed)*

--Collate all data collected on the NAMPS and RB07 by our cooperators and by us. *(Status: Pending)*

2015 Summer Field

--Disease and agronomic trait assessments of original 25 NAMPS and RB07. *(Status: Completed)*

2015 Fall GH:

--Plant BC_1F_4 seed in greenhouse in August-September and harvest in December. (4th selfed generation) *(Status: Completed)*

--Test NAMPS and RB07 for leaf rust reaction at the seedling stage. *(Status: Completed)*

2016 Winter GH:

--Plant BC_1F_5 seed in greenhouse in January and harvest in March (5th selfed generation). *(Status: Completed)*

2016 Summer field:

--Plant BC_1F_6 lines of the MNAMP (and parents) at Crookston and obtain year 1 phenotype data from the field. *(Status: Completed)*

--Collate all data collected on the MNAMP and parents by our cooperators and by us. *(Status: Pending)*

2016 Fall GH:

--Plant BC_1F_6 seed, extract DNA from seedlings, and send to USDA-ARS for genotype by sequencing *(Status: Completed)*

--Plant NAM population (and parents) segregating for various traits and obtain first experiment phenotype data from the greenhouse. *(Status: In progress)*

2016 Winter GH:

--Analyze genotype by sequencing data for the MNAMP.

--Plant MNAMP (and parents) segregating for various traits and obtain second experiment phenotype data from the greenhouse.

2017 Summer field:

--Plant MNAMP (and parents) at Crookston and obtain year 2 phenotype data from the field.

--Collate all data collected on the NAM population (and parents) by our cooperators and by us.

2017 Fall:

--Analyze data.

--Identify and distribute advanced lines with enhanced traits to regional breeders for crossing in their programs.

--Write up manuscripts for publication.

--Continue evaluations of derived materials until variety candidates are identified.

2017-2019:

--Continue evaluation of the MNAMP for other traits of interest to regional producers.

--Distribute complete MNAMP to cooperators who are interested in exploiting specific traits important to their programs.

--Conduct mapping studies of additional traits in the MNAMP.

--Conduct validation studies of identified genes in advanced populations of the Minnesota wheat improvement program.

--Continue evaluations of derived materials until variety candidates are identified.

Appendix

Table 1. Data for days to heading, plant height, spike length, protein level and bacterial leaf streak (BLS) reaction for the Minnesota Wheat Nested Association Mapping parents.

LID	Accession	Origin	Days to Heading	Plant Height	Spike Length	Protein %	BSL
3	CItr 14819	Eritrea	55	80	8.5	15.2	4
4	CItr 15006	Nepal	56	89	9.0	19.9	6
5	PI 62364	Venezuela	55	79	8.2	16.1	5
6	PI 153785	Brazil	55	92	9.2	13.8	6
8	PI 181458	Finland	70	70	7.0	15.3	4
9	PI 189771	Tunisia	55	86	7.7	15.6	4
10	PI 193938	Brazil	57	87	8.0	16.3	4
11	PI 199806	Peru	56	64	4.2	17.6	5
12	PI 205714	Peru	63	98	8.7	18.7	5
13	PI 213602	Argentina	55	66	6.0	18	3
14	PI 220455	Egypt	61	69	6.0	17.3	3
15	PI 278392	Palestine	59	75	7.2	16.7	1
16	PI 282922	Argentina	70	76	8.0	16.9	7
17	PI 344018	Angola	57	71	6.5	17.6	3
18	PI 345693	Belarus	57	97	10.5	15.9	5
20	PI 374254	Mali	60	75	8.0	14.4	3
21	PI 384403	Nigeria	55	73	7.0	15.4	4
22	PI 430750	Yemen	53	69	9.5	14.7	2
23	PI 449298	Spain	52	70	9.0	14.6	3
24	PI 519465	Zimbabwe	51	58	8.0	18	4
25	PI 519580	Chile	53	74	7.0	15.2	4
26	PI 520033	Kenya	54	81	7.0	17.6	4
27	PI 520371	Syria	51	71	10.0	15.4	6
29	PI 565238	Bolivia	51	74	8.5	15.3	5
30	PI 623147	Iran	55	86	5.5	16.6	5
P1	RB07	USA	50	79	7.0	15.8	5

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. Protein content data were provided by Senay Simsek at North Dakota State University A 0-9 scale was used for assessing the reaction of lines to bacterial leaf streak where 0=most resistant and 9=most susceptible.

Table 2. Mean, range and standard deviation for data on days to heading, plant height, spike length and bacterial leaf streak (BLS) reaction in families of the Minnesota Wheat Nested Association Mapping Population.

Family #	Days to Heading			Plant Height			Spike Length			Bacterial Leaf Streak		
	Mean	Range	Std	Mean	Range	Std	Mean	Range	Std	Mean	Range	Std
3	53.5	49-75	5.5	80.8	43-57	8.03	7.6	5.5-10.0	0.9	2.7	1-6	1.2
4	51.8	43-72	4.6	78.6	59-98	8.2	7.5	5.2-9.2	0.9	3.2	1-7	1.4
5	52.4	49-69	4.6	77.3	62-102	8.04	7.3	5.5-9.0	0.7	3.1	1-6	1.1
6	53.3	48-72	4.5	82.7	65-107	8.1	7.8	6.7-9.2	0.6	2.6	0-7	1.2
8	54.4	48-65	3.3	80.1	55-106	10.1	7.2	5.5-9.5	0.7	2.6	1-5	0.9
9	51.9	48-59	2.9	79.2	51-102	9.3	7.6	6.3-9.5	0.7	2.6	1-5	0.9
10	52.3	49-70	4.1	81.4	66-104	8.1	7.3	5.5-9.2	0.7	2.5	1-5	0.9
11	52.3	49-68	4.3	75.5	54-99	8.1	6.7	3.0-9.7	1.7	3.1	1-8	1.3
12	52.7	48-71	3.8	76.9	63-99	8.1	7.3	5.5-9.2	0.7	3.2	1-6	1.1
13	53.4	49-68	3.7	77.3	66-103	7.1	7.1	5.2-8.2	0.6	2.9	1-7	1.4
14	54.1	49-70	4.1	77.5	57-104	10.7	7.2	5.7-9.0	0.7	3.1	1-7	1.2
15	51.7	48-75	4.3	76.4	60-92	6.5	7.1	5.5-9.0	0.7	3.2	1-7	1.3
16	52.6	47-70	3.2	77.4	59-102	8.4	7.4	5.5-9.0	0.7	2.4	1-7	1.1
17	54.4	48-70	6.2	75.8	54-97	8.1	7.2	5.5-9.2	0.7	2.8	1-6	1.1
18	52.9	49-65	2.7	81.3	65-112	8.7	7.7	6.0-10.5	0.8	2.8	1-6	1.2
20	51.8	49-60	2.8	76.1	43-108	8.7	7.2	4.5-9.0	0.8	3.8	0-9	1.6
21	50.5	48-66	2.8	77.5	58-100	8.5	7.3	5.3-9.0	0.7	4.1	2-9	1.5
22	54.2	48-69	2.8	77.1	44-113	14.1	7.8	6.2-9.2	0.6	3.3	1-7	1.2
23	51.8	48-67	2.8	73.3	58-85	5.1	7.4	5.5-10.0	0.7	2.7	1-5	1.1
24	52.4	48-64	2.8	67.6	55-83	7.1	7.1	5.5-9.0	0.8	2.9	1-6	0.9
25	52.9	49-69	2.8	78.3	60-105	8.1	7.5	5.7-9.0	0.7	2.9	1-6	1.0
26	52.1	49-65	2.8	69.7	53-86	8.6	6.7	4.7-9.0	0.7	2.8	1-7	1.1
27	53.1	48-57	2.8	77.1	51-106	11.7	7.3	5.7-10.0	0.8	2.7	1-6	1.1
29	51.5	48-62	2.8	79.5	63-101	7.4	7.4	6.0-9.5	0.7	3.1	1-7	1.2
30	53.2	48-65	2.8	81.1	62-103	9.4	7.3	5.5-9.0	0.8	3.1	1-6	1.1

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), range (highest and lowest values) and standard deviation (amount of variation in dataset) 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. A 0-9 scale was used for assessing the reaction of lines to bacterial leaf streak where 0=most resistant and 9=most susceptible.