Development and preliminary evaluation of a HRWW recurrent selection base population

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Abstract

A highly diverse recurrent selection base population was established in the hard red winter wheat breeding program at NDSU. Parents were assembled with emphasis on resistance to the major diseases, e.g. Fusarium head blight, leaf and stem rust, tan spot and the septoria disease complex. These sources contribute a wide range of native and exotic resistance and adaptation genes, most of which derive from either spring wheat or less cold-hardy winter wheat. A complex cross was developed, combining genes from approximately 117 diverse genotypes contained within four parental populations. The *Ms3* gene was simultaneously established within the hybrid population such that the final F_1 will segregate 1:1 for male sterility/ fertility. The parental, intermediate and final hybrid populations were evaluated in an attempt to quantify the genetic diversity that it contains by employing phenotypic and/or marker-based evaluations.

Objectives

1. Develop a genetic male sterility based crossing system suitable for random intercrossing of large numbers of selected plants.
2. Establish a recurrent mass selection (RMS) base population containing diverse disease resistance and adaptation genes.
3. Measure the genetic variability contained in the newly established base population.

Establishment of the RMS Population

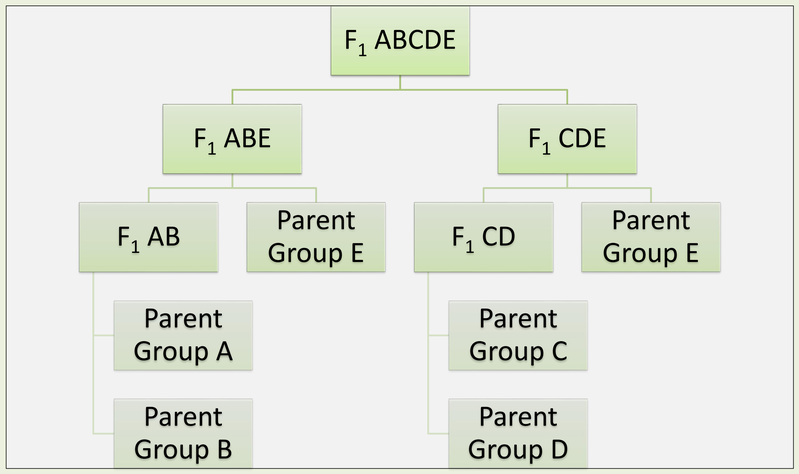

Fig. 1. Complex cross to introduce genes for agronomic diversity, quality and disease resistance into a hard red winter wheat RMS base population.

1. In the **first leg** of the complex cross (Fig. 1) 60 F_1 females from a spring wheat population (Group A), segregating for *Ms3* (Fig. 4) were hand-pollinated with one or more of 34 winter wheat genotypes (Group B) to produce F_1 : AB. The latter F_1 females were randomly pollinated with five winter-hardy varieties (Group E) to produce F_1: ABE (Fig. 2).
2. In the **second leg** of the cross the very winter-hardy variety Norstar (Parent C) was crossed with 18 North American HRS wheat lines (Group D). The resulting F_1: CD was then hand-pollinated with the five Group E parents to produce F_1: CDE.
3. The **final cross** involved random pollination of the F_1: ABE females (*Ms3*) with F_1: CDE males. The final F_1: ABCDE was **randomly interbred** to further disperse the genes contributed by the two subsets of parents.
4. In the finalized hybridization procedure (Fig. 3.) plants were grown singly in cone-tainers. At flowering, random male plants were arranged around and higher than the female plants. Female florets were cut open and mild wind agitation (fan) was used to promote pollination.

Fig. 2. First leg crossing procedure.

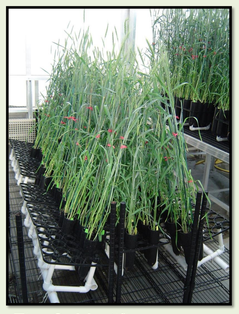

Fig. 3. Modified crossing system.

Fig. 4. Female spike.

Disease Resistance

1. Leaf rust (Table 1) and stem rust (Table 2) seedling resistance screening was done using F_2 populations AB and CD. Five *Puccinia triticina* (*Pt*) and four *P. graminis tritici* (*Pgt*) pathotypes prevalent in North Dakota were used for inoculation.
2. Tan spot insensitivity (mixed isolates; toxin) and Stagonospora nodorum insensitivity (isolate Sn4) were tested in collaboration with Dr. Tim Friesen (USDA Fargo).

Table 1. Number of seedlings that exhibited specific leaf rust infection types (ITs) following inoculation of random F_2: AB and CD seedlings.

Range of ITs	*Puccinia triticina* pathotype: MFPS		TBDJQ		THBL		TDBG		MCDL	
	F_2 AB	F_2 CD	F_2 AB	F_2 CD	F_2 AB	F_2 CD	F_2 AB	F_2 CD	F_2 AB	F_2 CD
0 to 1-[1,2]	4	7	7	1	7	0	19	7	7	0
1 to 1++[1,2]	4	8	5	3	13	0	14	10	23	2
2 to 2++[1,2]	23	20	31	23	60	23	20	28	69	59
Z[2]	3	0	0	2	0	0	0	0	0	0
X[2]	0	45	0	0	20	5	5	5	0	0
3 to 4[3]	114	66	107	115	45	119	79	95	47	87
Total	148	146	150	144	145	147	137	145	146	148
Susceptible (%)	77	45	71	80	31	81	58	66	32	59

[1] With or without associated flecking
[2] Resistant phenotypes
[3] Susceptible phenotypes

Table 2. Number of seedlings that exhibited specific stem rust infection types (ITs) observed following inoculation of random F_2: AB and CD seedlings.

Range of ITs	*Puccinia graminis* pathotype: QFCQ		QTHJ		TPMKC		TMLK	
	F_2 AB	F_2 CD	F_2 AB	F_2 CD	F_2 AB	F_2 CD	F_2 AB	F_2 CD
0 to 1-[1,2]	56	4	22	20	49	8	43	22
1 to 1++[1,2]	0	18	23	12	8	18	19	19
2 to 2++[1,2]	83	80	24	9	13	23	5	10
3 to 4[3]	4	35	4	29	1	24	7	22
Total	143	137	73	70	71	73	74	73
Susceptible (%)	3	26	1	41	0	33	1	30

[1] With or without associated flecking
[2] Resistant phenotypes
[3] Susceptible phenotypes

3. Within F_2 populations, 31-80% of seedlings were susceptible to a leaf rust race and 1-41% were susceptible to a stem rust race. However, resistance to all races occurred and there was broad variation in ITs (Tables 1 & 2; Fig. 5), indicative of the presence of varied resistance genes.
4. In both population F_2 AB and F_2 CD, the *S. nodorum* inoculation susceptibility percentages were 88 and 97, respectively. Susceptibility of the genotypes to tan spot also varied between the populations with the F_2 AB and F_2 CD showing susceptibility percentages of 85 and 63, respectively.
5. Genotype insensitivity to toxin infiltration ranged from 12-85% across both the F_2 AB and F_2 CD populations.

Fig. 5. Some of the leaf rust infection types that were encountered in the populations.

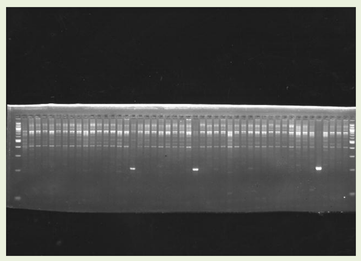

Fig. 6. PCR amplification of marker *Sr50* on the first set 48 samples from the F_2 ABCDE population.

SNP Comparison

One hundred and thirteen parental lines including 12 SDSU experimental lines; 12 MSU experimental lines; one entry from the 2010 Northern Fusarium Head Blight Nursery; 26 released winter wheat varieties; 18 North American HRSW lines as well as 24 random plants from the spring wheat recurrent selection population, plus 25 random F_1 plants from the final ABCDE hybrid population were compared. Leaf tissue from each line was used by the USDA-ARS Genotyping Center (Fargo, ND) for DNA extraction and genotyping. Single nucleotide polymorphisms (SNPs) were assayed by the Genotyping Center by employing Illumina's Infinium Assay, using the wheat 9K SNP array (Cavanaugh et al., 2013). 228 SNP markers were chosen for cluster analysis based on minor allele frequencies. A dendrogram (tree diagram) (Fig. 7) was created using the cluster procedure in the SAS statistical software program.

The dendrogram also reflected the broad variability expected among the parental groups. The two HRSW groups (of North American origin and the South African material containing *Ms3*), HRWW (Canadian) and HRWW (US) often associated in different clusters. Similarly the ABCDE hybrids tended to group together as a new cluster.

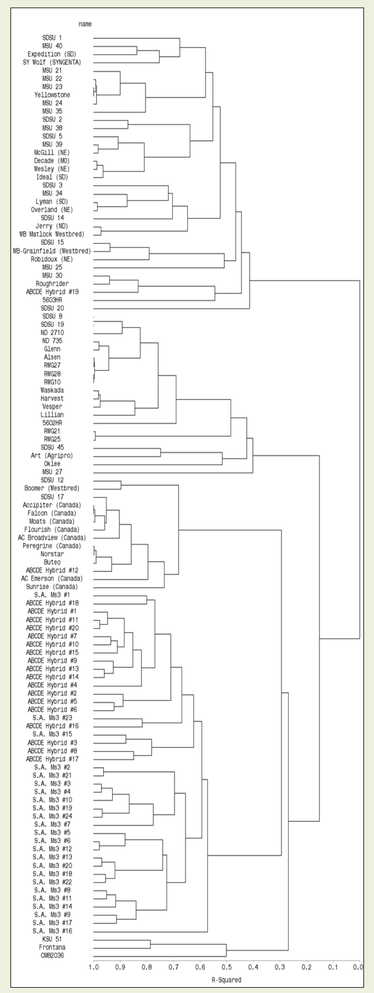

Fig. 7. Dendrogram resulting from cluster analysis (SAS) of 228 polymorphic SNPs across 113 wheat entries.

Marker Analyses

Ninety six F_2 from the F_1 ABCDE population were analyzed for molecular markers associated with specific, useful genes (*Fhb1, Lr19, Lr21, Lr24/Sr24, Lr34, Lr37/Sr38; Sr2, Sr50* (Fig. 6)). While *Lr21* did not occur in the population, the remaining genes were detected in 3 to 59% of the plants.

Summary

A highly heterogeneous base population was created that incorporates broad variation for cold-hardiness, resistance to leaf and stem rust, tan spot and *S. nodorum*. The population combines variability from distinctly different HRSW and HRWW sources and could also be very diverse in terms of traits that were not measured here such as resistance to other pests, processing quality, adaptation and yield.

Acknowledgements

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Citation

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