Use of Recurrent Mass Selection to Pre-breed Hard Red Winter Wheat for Resistance to Major Biotrophic and Nectrotrophic Diseases

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Research Questions

A recurrent mass selection pre-breeding program that is based on the dominant male-sterility gene, *Ms2*, is being implemented to facilitate the continuous development of improved hard red winter wheat breeding parents with high levels of winter-hardiness coupled with effective resistance against major diseases such as Fusarium head blight (FHB), leaf and stem rust, tan spot, bacterial leaf streak (BLS) and Stagonospora nodorum blotch (SNB). This long term pre-breeding program will operate in parallel with the conventional winter wheat breeding program and will serve to systematically assemble (pyramid) new useful genes in adapted genotypes that can be used more effectively in crosses with the elite germplasm.

In the first three years (this funding application), selection will focus on:

a. Increasing the level of cold-hardiness in the population.
b. Raising the frequency of genes for insensitivity to SNB and tan spot.
c. Establishing the durable rust resistance genes, *Lr34/Yr18, Sr2* as well as the FHB resistance genes, *Fhb1* and *Fhb5A*.
d. Introducing additional major and minor gene resistance to the cereal rusts.
e. Introducing BLS resistance.
f. establishing a regular recurrent mass selection program with inbreeding and field testing of the male parents

Materials and Methods

Recurrent selection is based on consecutive cycles of intense selection followed by random intercrossing of the selected plants. The hybrid progeny from each cross cycle then constitutes the next population with which the procedure will be repeated. Successful application of this selection strategy requires an ability to readily and randomly intercross large numbers of plants. This can be done in wheat by employing a dominant gene for male sterility such as *Ms2.*

A diverse base population for recurrent mass selection is being developed. The *Ms2* gene has been incorporated within the hybrid population such that the F_1 always segregates 1:1 for male sterility: male fertility. The base population will be systematically improved through selection. Both male and female plants will be selected, however, only male plants will be evaluated in the field. Female plants will be derived from the F_1 produced in each season whereas the male plants used in a particular season will be selected among advanced generation segregates derived from crosses made in preceding years. Following their use in a crossing block, the male parents will also be evaluated for possible commercial use.

All plants will be grown in containers. In each season the spikes of selected, male-sterile females will be cut open to facilitate pollination. During pollination, selected female plants will be positioned lower and surrounded by selected male plants. Mild wind agitation (fan) will be used to enhance cross-pollination. The containers will be arranged in a manner that will promote randomness of pollination.

Characterization and selection of parent plants will be based on markers (where available), seedling screening (rust, tan spot and SNB resistance) and field screening (winter survival, disease resistance, yield and quality).

At the onset of selection (first three years), field tested male parents will not yet be available. While field testing and inbreeding of the male component is being implemented, interim male parents will be derived from backcross breeding attempts and a routine pedigree breeding program.

Results

In total three cycles of crosses will be made during the project funding period. The first cross was completed in February/March, 2013.

<u>The second cross:</u> A second crossing cycle was completed in February/March, 2014, when the following was done: (a) The *Ms2* male sterility gene was found to give more complete and regular penetrance of male sterility than *Ms3* and therefore introduced. In coming cycles it will completely replace *Ms3*. (b) Additional disease resistance genes that have been backcrossed into winter-hardy HRWW backgrounds (2011-2013) were introduced through new male parents. These included: *Lr34, Lr53* and *Lr62* (leaf rust); *Sr22, Sr26, Sr39* and *Sr50* (stem rust); and *Fhb1* (FHB). (c) A facility to do marker-assisted screening was developed and used to select for the stem rust resistance genes. In the third cross cycle (January/February, 2015) the number of markers and numbers of plants screened with markers will be increased further.

<u>Development of Norstar near-isogenic parents with pyramided resistance:</u> Since Norstar is believed to be the most winter-hardy genotype available, backcrosses aimed at pyramiding combinations of resistance genes in its

genetic background were initiated in 2013. The purpose is to include the pyramided genotypes among the male parents in future recurrent selection crossing blocks. Five B_3F_2 populations, each segregating for *Fhb1* and *RhtB1b* (semi-dwarfing gene) plus *Sr39 & Lr34; Lr53; Sr2; Sr26,* and *Sr50*, were obtained and are currently being screened for the selection of homozygotes to be used as parents.

Selection of future male parents with resistance/insensitivity to tan spot and SNB: In 2014, inbreeding coupled with seedling selection was initiated with random male-fertile F_2 plants from an earlier RMS base population that involved 110 winter and spring habit varieties and lines. The F_2 generation was screened for tan spot resistance using inoculum from a mix of races. Plants were inoculated at the two-to-three leaf stage, approximately seven days after their 56 day vernalization period and later moved to a growth chamber. Evaluations were done seven days after inoculation, where only the resistant and intermediate individuals were selected, and the 803 selected F_3 subsequently advanced through unselected single seed descent (SSD). In parallel, seeds of each lineage were planted in a greenhouse without vernalization so as to be able to identify and remove those families that had the spring growth habit. In the next phase approximately 600 F_4 will be divided into two equal populations that will be screened respectively for SNB (one isolate) and tan spot (mixed inoculum of five races). The most resistant/insensitive plants will be kept and allowed to self in order to obtain $F_{4:5}$ inbred lines. Selected $F_{4:5}$ lines from the two disease groups plus controls will be evaluated as a single group (replicated trial) with individual isolates/races and toxins to identify the best lines which will then be used as parents. The same material will also be field planted and evaluated for winter-survival.

Attempt to identify additional, potentially diverse and useful variation for resistance/susceptibility to tan spot and SNB that can be utilized for parent selection and pre-breeding: A collection of 50 genotypes are being evaluated as additional sources of resistance to tan spot and SNB. Lines developed from these will be used as male parents in future crossing blocks. The 50 lines include the following: Twenty three lines that are either $F_{4:6}$ SSD derived inbred lines or DH lines that derive from two crosses, i.e. cross 11M225 = RWG10/Jerry (21 lines) and cross 11M237 = RWG28/Norstar. Lines RWG10 and RWG28 are spring wheats with pyramided resistance to FHB, tan spot and SNB developed by Dr. Steven Xu (USDA–ARS, Northern Crop Science Laboratory, Fargo). The genes present in RWG10 (pedigree = BG282/3*Alsen) include *tsn1, snn2, QTs.fcu-1BS*, and *Fhb1*. BG290 (pedigree = BG290/3*Alsen) has the same genes as RWG10, plus an additional tan spot insensitivity QTL, i.e. *QTs.fcu-3BL*. The selections showed good winter survival in 2014 and each has *Fhb1*, yet they were not tested for the presence of tan spot or SNB insensitivity. Another ten lines were obtained from Dr Art Klatt (Plant and Soil Sciences, *Oklahoma State University*). These are derivatives from crosses between hard red spring and synthetic wheat. The synthetics have an accession of *Aegilops tauschii* in their pedigrees and were selected based on their resistance to tan spot (field). Five winter wheat lines of international origin (Chile, France, Belgium, Sweden and Bosnia-Herzegovina) that were reported to be resistant to tan spot are also included. The remaining lines constitute selections from the NDSU HRWW breeding program that have good winter survival as well as FHB and BLS resistance.

Preparation for making the third cross (January/February, 2015): Male and female populations have been planted and vernalized since October 2014 in preparation for a third set of crosses. (a) The F_1 female component derive from the 2014 RMS crossing block and will be selected following seedling inoculation and marker screening for the presence of three leaf rust resistance genes, four stem rust resistance genes plus *Fhb1*. (b) The new male parents will include winter-hardy cross and backcross derivatives with the following genes: *Lr19, Lr34, Lr46, Lr50, Lr51, Lr53, Lr56, Lr62* and *Lr68* (leaf rust); *Sr2, Sr22, Sr26, Sr35, Sr39*, and *Sr50* (stem rust); *Fhb1, Fhb5A* (FHB); unknown, but strong resistance to BLS (field-selected, 2 sources); tan spot and SNB resistance/insensitivity (3 sources). Marker-aided screening of this material will begin in December 2014 and will be extended to also include additional leaf and stem rust resistance genes.

Application and Use

The (pre-breeding) population will be continuously enriched with respect to useful disease resistance and adaptation genes. Following each (annual) selection cycle the best male-fertile F_2 plants will be harvested separately and the F_3 field planted for continued inbreeding and selection. In this manner new and diverse segregating families will be established each year. Superior inbred lines selected in the F_5 to F_7 will be utilized in the crossing block of the main breeding program and will also be evaluated further for possible commercialization.

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.

continued on page 64

continued from page 63

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 are not currently available in the HRWW primary breeding pool. Pre-breeding through recurrent selection 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 varieties with better resistance gene combinations and yield stability.

Related Research (effort)

A hard red winter wheat pedigree breeding program was initiated at NDSU during 2011. Annually, 500-700 new crosses are being made among winter wheat parents. A primary aim is to broaden the spectrum of disease resistance genes available for varietal development. 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 is meant to supplement and facilitate the pedigree breeding effort.

Recommended Future Research:

a. Continue to enrich the population with new resistance genes and increase the frequencies of those genes through recurrent selection.
b. Develop and field test inbred lines derived from the male F_1 of each crossing cycle.
c. Evaluate the possibility to implement genomic selection for the numerous disease resistance genes, in particular, the many QTL associated with resistance to the necrotrophic pathogens.