Minnesota Wheat Research and Promotion Council
CROP YEAR 2013 RESEARCH REPORTING FORM
Form Due November 15, 2013

1. PROJECT TITLE

Use of recurrent mass selection to pre-breed hard red winter wheat for resistance to major biotrophic and necrotrophic diseases

2. PRINCIPAL INVESTITAGATOR (S)

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5. REPORTING PERIOD

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6. TERMINAL REPORT _____ **PROGRESS REPORT** ___X___

7. AMOUNT OF GRANT $72,000

8. PUBLICATIONS

None

9: EXECUTIVE SUMMARY

Research Question:

A recurrent mass selection pre-breeding program that is based on the dominant male-sterility gene, *Ms3*, 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, leaf and stem rust, tan spot and Stagonospora nodorum. 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. Attempting to raise the frequency of genes for insensitivity to Stagonospora nodorum and tan spot.
c. Establishing a baseline presence of the durable rust resistance genes, *Lr34/Yr18*, *Sr2* as well as the FHB resistance gene, *Fhb1*.
d. Establishing a full scale recurrent mass selection program with inbreeding and field testing of the male parents.

Materials and Methods:

A highly diverse base population that involved a range of native and exotic resistance and adaptation genes, most of which derive from either spring wheat (South Africa, Canada, Northern USA) or less cold-hardy winter wheat from the southern United States was established during 2011 and 2012. While making the crosses, the *Ms3* gene has been incorporated within the hybrid population such that the final F_1 segregates 1:1 for male sterility:fertility. This base population will now be systematically improved through selection.

Both male and female plants will be selected intensively for cold-hardiness, however, only male plants will be selected for S nodorum and tan spot insensitivity. Female plants will be derived from the F_1 produced in each season whereas male plants will be selected among the F_2 derived from the previous season. This is necessary because insensitivity is a recessive trait and a higher frequency of recessive homozygotes will occur in the F_2. Following pollination, the insensitive male plants will be subjected to continued inbreeding and field selection.

Large numbers of F_1 and F_2 seedlings will be evaluated in each season and the superior male and female plants will be randomly intercrossed. Recurrent cycles of intense selection and intercrossing of the selected plants will serve to raise the frequency of desirable genes within the base population, resulting in its gradual improvement.

In the course of the first three years, an attempt will be made to determine, and if necessary, increase the frequencies of the *Sr2*, *Lr34* and *Fhb1* genes in the base population. An attempt will also be made to introduce additional rust resistance genes (*Lr53*, *Lrbi*, *Sr26*, *Sr39* and *Sr50*).

Cold-hardiness

A protocol is being developed that will be used to subject emerging seedlings to freezing conditions in order to kill and remove spring type and less winter-hardy segregates from the population. The protocol has to provide homogeneous test conditions (with respect to cold-hardening, soil moisture, sub-zero temperature exposure) and it has to be repeatable.

Sensitivity to Pyrenophora tritici-repentis

Upon completion of the cold-hardiness test and vernalization period, selected seedlings will be transplanted in cone-tainers filled with Sunshine Mix and fertilized with Osmocote Plus 15-19-12. A single plant will be planted in each cone. At the two-leaf stage the plants will be inoculated with a mixture of *Pyrenophora tritici-repentis* races (consisting of race 1, race 3, race 5 and an Arkansas isolate). The race isolates will be multiplied and conidial suspensions prepared in sterile distilled water. The seedlings will be inoculated with a hand-held sprayer and placed into a humidity chamber at 21°C with 100% relative humidity for 24 h. Following inoculation, the plants will be moved to a growth chamber at 21°C and a 12:12 h day:night cycle. Plant symptoms will be evaluated seven days later employing a 1-to-5 scale lesion-type rating system developed by Lamari and Bernier (1989), with 1 being resistant, 2 moderately resistant, 3 moderately resistant or moderately susceptible, 4 susceptible, and 5 highly susceptible. Plants with ratings of 2 or less will be retained and used as male parents.

During the first selection cycles, the seedlings will be evaluated for insensitivity to tan spot only. As soon as the frequency of tan spot insensitivity has been raised satisfactorily, selection for S nodorum insensitivity will be implemented.

Seedling leaf rust and seedling stem rust resistance screening

Seedlings at the 2-3 leaf stage will be inoculated with spores suspended in distilled water containing a drop of wetting agent (Triton). Pathotype MFPS will be used for inoculation as it is virulent on the winter wheat backgrounds used during backcrossing; however, it is avirulent on *Lr53* and *Lrbi*. Pathotype QFCQ will be used to select for the presence of *Sr39* in the Norstar background. Following spraying of the plants with the spore suspension they will be covered and sealed inside transparent plastic bags to maintain high humidity for 24 hours (22ºC for leaf rust; 24ºC for stem rust). Following inoculation, the plants will be transferred to a growth chamber (24ºC; day:night cycle = 18:6 h). Disease symptoms (Stakman scale) will be evaluated after 7-10 days.

Marker-assisted selection

Markers will be used to detect the presence of *Sr2, Sr26* and *Sr50*. Presence of *Lr34* and *Fhb1* will be analyzed for by the USDA genotyping center in Fargo.

Results:

The base population was derived as the F_1 of a complex cross that involved ± 110 diverse varieties/advanced lines contained within five populations. It was necessary to intercross (2013) the primary F_1 population before the onset of selection in order to evenly disperse the genetic variability contained within. This intercross also served to further optimize the crossing procedure. Approximately 600 F_1 female plants (*Ms3ms3* = male-sterile) were pollinated with approximately 600 male-fertile (*ms3ms3*) plants to produce R_0F_1 seeds. Approximately 500 grams of F_1 hybrid seed were produced, with each seed potentially being a unique genotype. Following hybridization, approximately 1,750 grams of bulked F_2-seeds (R_0F_2) were harvested from the male plants. Similar to the F_1, the F_2 seeds were genetically highly diverse.

Further adaptations were made to the crossing procedure. The placement of male plant-containing cones relative to those containing female plants was changed so as to accommodate larger numbers. The positioning of plants were regularly changed (approximately every second day) so as to further randomize pollinations. Fans were placed on opposite sides of the crossing block to create a swirling air-flow over the female plants. With respect to each crossing cycle, vernalization and cold-hardiness screening is initiated from September 1st. Crosses are made during the months of January through March which has proved optimal for pollen development (the greenhouse summer temperatures get too high and result in insufficient seed set). Single seed descent inbreeding of the male parents generally requires less seed and is continued during the summer months.

In the first selection cycle, the R_0F_1 is being selected for cold-tolerance only and will serve as source of females as well as the source of R_1F_2 for selection cycle 2. Three sets of R_0F_1 seeds (600/600/300) were screened at two-week intervals. The R_0F_2 is being screened for cold-hardiness as well as insensitivity to S nodorum race 1. Selected plants from this population will be used as male parents. Preliminary assessment of the frequency of insensitivity in the base population suggested that insensitive plants may occur at a frequency of about 5-10%. Three sets of F_2 plants, two sets consisting of 2,000 individuals each and one set consisting of 1,000 individuals have been screened for cold-hardiness. It is expected that approximately 50-75% will eventually survive. These will be transplanted, inoculated and screened for S nodorum insensitivity. If necessary, further sets of plants will be cold-screened in order to retain large enough numbers of disease-insensitive plants. A growth chamber has been acquired in order to provide a controlled environment for phenotyping of the seedling populations. A preliminary assessment of the presence (frequencies) of *Lr34, Sr2* and *Fhb1* (based on markers) will be made following selection for cold-hardiness and S nodorum insensitivity.

A preliminary cold-hardiness screening test has been developed. Following cold-hardening for three weeks, batches of 2,000 seedlings are cooled to -13ºC in a programmable freezer chamber. Surviving seedlings are transplanted and vernalization is completed (56 days at 4ºC). Following vernalization a standard screening procedure to evaluate S nodorum sensitivity is conducted.

Several existing and additional resistance genes are being incorporated into the base population. These include the leaf rust resistance genes *Lr34, Lr53* and *Lrbi*; stem rust resistance genes *Sr2, Sr26, Sr39* and *Sr50* plus *Fhb1*. The genes have been backcrossed into winter-hardy genetic backgrounds (Jerry, Norstar, Decade). Crosses are continuing to now gradually integrate (backcross) it into the base population without unduly narrowing genetic diversity.

Application/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 approximately 300 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 main purpose of the project is to assemble 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 primary breeding pool. Recurrent selection is applied to gradually improve the general genetic background of the base population and to concentrate/pyramid desirable genes within. This will make it possible to annually develop new and better gene combinations that can be used in crosses with the elite breeding material. The primary diseases that are being targeted are those that are difficult to breed resistance for. Initially, the focus will be to find and accumulate genes for tan spot and S nodorum resistance. Following three to four cycles of selection, the emphasis will shift to Fusarium Head Blight and durable leaf rust resistance genes. Each year these organisms cause significant wheat yield losses and even modest changes in the average level of resistance in new cultivars will be of considerable benefit to producers.

10: RELATED RESEARCH

a. A hard red winter wheat pedigree breeding program for varietal development was initiated at NDSU during 2011. Annually, 500-700 new crosses are being made among winter wheat parents. A fourth set of crosses have been initiated. A primary problem is to acquire new genetic diversity that will provide breeding parents for continued improvement of yield, cold-hardiness, adaptation, disease resistance and processing quality.
b. A test procedure for cold-hardiness assessment is being evaluated by (i) applying the test to wheat varieties known to differ in hardiness; (ii) by selecting for cold-hardiness in two crosses of winter-hardy X less-winter-hardy wheats. In the latter case, selection will be done over 3- generations in order to assess selection progress and hence the effectiveness of selecting for this trait.
c. Several leaf rust, stem rust and FHB resistance genes are being backcrossed into cold-hardy winter wheat backgrounds. Some of these genes can be incorporated into the recurrent selection base population.

11: RECOMMENDED FUTURE RESEARCH

a. Continue to enrich the population with new resistance genes and increase the frequencies of those genes through strict recurrent selection.
b. Continue to optimize the crossing scheme and handle large numbers of individuals so as to maintain genetic diversity for those traits not being selected at any point.
c. Develop and field test inbred lines derived from the male F_1 of each crossing cycle.
d. Evaluate the possibility to implement genomic selection for yield at some later stage.

12: APPENDIX