2003 STEEP PROGRESS REPORT

RESEARCH PROJECT TITLE: Optimizing Plant Genetics and Soil Fertility to Achieve High Grain Protein Content in Hard Red Spring Wheat

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INTERIM REPORT: (First year’s funding was allocated in October, 2003.)

PROJECT OBJECTIVES:
1. Evaluate current varieties and improved near isogenic lines (isolines) of hard red spring wheat (HRSW) for grain yield and protein response to low and high nitrogen fertilizer application rates.
2. Evaluate the most promising isolines identified in Objective 1 for agronomic potential and protein response to reduced nitrogen fertilization regimes.

KEY WORDS: hard red spring wheat, fertility, grain protein content, marker-assisted selection

STATEMENT OF PROBLEM: Developing HRSW varieties that effectively use nitrogen is important component of sustainable cropping systems geared towards maximizing profitability while minimizing environmental contamination. Our goal is to assess nitrogen use efficiency differences between the recurrent parents, Scarlet and Tara 2002, and BC₁F₂ isolines derived from these varieties, which carry a chromosomal region associated with high grain protein concentration (HGPC) from the donor parent Glupro. If protein content stability is increased in isolines with the HGPC region, or nitrogen fertilizer requirements are reduced, varieties released from this material will be of tremendous value to HRSW producers in the region.

ZONE OF INTEREST: Initially, this research will be conducted in the high rainfall zone near Pullman, WA to maximize the opportunity to detect changes in grain protein content based on fertility management strategy. Once promising isolines have been identified, field trials also will be conducted in the semi-arid and intermediate rainfall zone to customize fertility management plans for each production region.

ABSTRACT OF RESEARCH FINDINGS: Our primary objectives for 2003 were to: 1) confirm the genotypes of isolines derived from Scarlet and Tara 2002 in the chromosomal region associated with HGPC; and 2) assess these isolines, along with Scarlet, Tara 2002 and the HGPC region donor Glupro, for grain yield and protein response to low and high nitrogen fertilizer application rates in conventional and direct seeded field trials. Using primers that amply DNA sequences closely associated with the HGPC region on chromosomes 6BS, the presence or absence of the HGPC regions from Glupro was successfully determined for each isolate. Fifty-six isolines (43 Scarlet derivatives; 13 Tara derivatives) with confirmed genotypes in the HGPC region were evaluated in replicated yield trials with conventional or direct seed management at high (3.6 lb N/expected bu) or low (2.5 lb N/expected bu) fertility levels. Results from the direct seeded trial were highly variable, and were not useful for assessing differences among isolines. In the conventional trial, several isolines with promising potential were identified that had equal or improved abilities to produce protein, based on grain yield, test weight and grain protein concentration, compared to the recurrent parents. Sixteen Scarlet isolines (13 with and 3 without the HGPC region) and 12 Tara 2002 isolines (6 with and 6 without the HGPC region) are currently being evaluated for milling and baking performance at the Western Wheat Quality
Laboratory. All of these isolines will be evaluated for a second year in replicated fertility trials, and a few of the most promising lines, based on agronomic performance and end-use quality, will be evaluated in nitrogen response trials, along with Scarlet and Tara 2002, in 2004. Several isolines evaluated in this study, including a stripe rust resistant Scarlet types, appear to have potential for variety release.

RESULTS AND INTERPRETATION: To assess the impact of incorporating the HGPC region into Scarlet and Tara 2002, replicated fertility trials were conducted at two locations in the high rainfall zone in 2003. Based on field data generated in 2002, a subset of isolines were selected from among 100 candidates as potential HGPC replacements for their recurrent parents (data not shown). We noted at that time that several of the promising isolines did not appear to carry the reputed HGPC region, based on results from initial evaluations with DNA markers flanking the target region on chromosome 6BS of wheat.

To further address this concern, isolines were re-evaluated with the Nor-B2 RFLP marker, which is highly associated with the target region (Figure 1). Nor-B2 clearly distinguished the presence or absence of the targeted chromosomal region among isolines. Due to complexities associated with conducting RFLP analyses, PCR-based markers tightly linked to this region were highly desirable. Amplification product patterns derived from DNA primers provided by Dr. Jorge Dubcovsky (UC-Davis) aligned perfectly with Nor-B2 results (Figure 2). These primers were used to confirm the composition of each isolate in the HGPC region, and genotype designations were adjusted to reflect either the presence of the segment from GluPro (G) or the presence of recurrent parent DNA in this region (Scarlet (S) or Tara 2002 (T)).

We evaluated isolines (43 Scarlet BC$_2$F$_5$ derivatives; 13 Tara 2002 BC$_2$F$_5$ derivatives) along with the donor and recurrent parents for response to high (3.6 lb N/expected bu) and low (2.5 lb N/expected bu) fertilizer application rates in conventional and direct seeded production systems (Table 1). Several isolines without the HGPC region from GluPro also were included as controls. Our goal was to determine whether isolines with the HGPC region have differential responsiveness to N availability based on grain yield, grain protein content, and grain quality. Field trials consisted of a split-plot design, where nitrogen application rate and genotype were the main plot and subplot factors, respectively. Genotype by N regime plots were 5’ x 20’ with three replications. This trial was conducted with conventional tillage at Spillman Farm, whereas direct seed management was used to establish the trial at the Palouse Conservation Farm. Soil samples were taken in the early spring prior to planting to determine available soil N (KCl extractable nitrate and ammonium in the root zone), and fertilizer application rates were adjusted accordingly (Table 1). Plant biomass samples were collected for component analysis of N use efficiency factors. Total N uptake and N partitioning between straw and grain will be determined for a subset of isolines, with and without the HGPC region, to evaluate N uptake (plant N per unit of total N supply), N utilization (grain production per unit of plant N), and grain N accumulation efficiency (grain N per unit of plant N). Grain yield, test weight and grain protein concentration (Technicon NIR) were measured at harvest. Amount of protein (lbs/A) produced per genotype was calculated using the following equation: grain yield X test weight X (grain protein concentration/100).

Trial averages for test weight, grain protein concentration, grain yield, and amount of protein produced are reported in Table 2 for both sets of isolines. Variation levels were dramatically higher in the direct seeded trial compared to the conventional tillage trial, based on standard deviations (Table 2) and analysis of variance results (data not shown). Poor stand establishment and field non-uniformity had a negative impact on results from the direct seeded trial. No significant (p < 0.05) differences were detected among genotypes evaluated at this
location for most traits tested due to the high level of variation in the trial (data not shown); therefore, only data from the conventionally tilled trial are reported here (Tables 3 and 4).

Since genotype by fertility interactions were non-significant, data from high and low fertility treatments were combined for final data analyses. Significant ($p < 0.05$) differences in the amount of protein produced per acre were detected among isolines derived from both Scarlet (Table 3) and Tara 2002 (Table 4). Although Scarlet produced the largest amount of protein based on rank (Table 3), protein production for 21 isolines did not differ significantly from Scarlet, and all but one of these isolines contain the HGPC region from GluPro. In addition, all of these lines inherited stripe rust resistance from GluPro that was not present in Scarlet (data not shown). In 2001, the stripe rust resistance in Scarlet was overcome by races that were new to the region, which increases the risk of commercially producing this variety. Scarlet isolines with the HGPC region and resistance to new races of stripe rust are excellent candidates for variety release.

Results for isolines derived from Tara 2002 were even more encouraging. Seven isolines, five of which carry the HGPC region from GluPro, produced significantly ($p < 0.05$) more protein than Tara 2002 in the conventional field trial (Table 4). All seven of these lines are excellent candidates for further evaluation to access the direct impact of the presence or absence of the HGPC region on N utilization in the same genetic background (i.e. Tara 2002). It appears as if several Tara 2002 isolines have increased grain protein concentration compared to the original variety.

Grain samples from a subset of the genotypes evaluated in 2003 field trials were submitted to the Western Wheat Quality Laboratory in October 2003 for milling and baking evaluations. Based on consistency of field performance over fertility treatments and locations, sixteen Scarlet isolines (13 with and 3 without the HGPC region) and 12 Tara 2002 isolines (6 with and 6 without the HGPC region), along with parental checks, were selected for quality assessment (data not shown). Results will be used to determine the impact of the presence of the HGPC region on milling and baking performance, and to identify promising candidates to evaluate in Objective 2.

In year 2, the sixteen Scarlet isolines and 12 Tara 2002 isolines described above will be evaluated for a second year in replicated fertility trials in conventional and direct seeded production scenarios. In addition, superior HRSW isolines selected based on end-use quality performance, along with Scarlet and Tara 2002, will be grown under various N regimes established in low N soil to access agronomic potential and protein response to reduced nitrogen fertilization as proposed for Objective 2.

**IMPACT**: Nitrogen fertilizer use is an important economic and environmental concern for regional wheat producers. Reducing the risk of producing high quality HRSW varieties while minimizing nitrogen fertilizer requirements would be a tremendous asset to the region. Results from this research will generate fertility management guidelines for recent HRSW varieties released by WSU, and a new generation of HRSW varieties with enhanced nitrogen use efficiency and reduced nitrogen fertilizer requirement may be identified through this work.

**INTERACTION (COOPERATION) WITH OTHER SCIENTISTS CONDUCTING RELATED ACTIVITIES**: Dr. Jorge Dubcovsky, Dep. of Agronomy & Range Science, UC-Davis; Mr. Brady Carter, Wheat Quality Specialist, WSU

**PUBLICATIONS AND PRESENTATIONS**: none
Table 1. Management information, including spring soil test results, for conventional tillage and direct seeded fertility trials conducted at Spillman Farm and the Palouse Conservation Farm, respectively, in 2003. A high and low fertility treatment was included at each site to assess the impact of incorporating a chromosomal region conferring high grain protein concentration into the adapted hard red spring wheat varieties. Scarlet and Tara 2002

<table>
<thead>
<tr>
<th>Spillman Farm Cultural Management Practices</th>
<th>Spring Soil Test Results</th>
<th>Fertility Treatment</th>
<th>Yield Goal (bu/A)</th>
<th>Nitrogen (lb/A) Goal</th>
<th>Present Needed</th>
<th>Fertilizer Applied (lb/A)</th>
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<tbody>
<tr>
<td>Previous crop</td>
<td>Peas</td>
<td>177 lb N</td>
<td>Low</td>
<td>80</td>
<td>208</td>
<td>80</td>
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<tr>
<td>Tillage</td>
<td>chiseled, cultivate 2x</td>
<td>7 ppm S</td>
<td>High</td>
<td>80</td>
<td>288</td>
<td>117</td>
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<tr>
<td>Planting date</td>
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<td>30 ppm P</td>
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<td>Planter used</td>
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<tr>
<td>Seeding rate</td>
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<td>pH 5.6</td>
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<tr>
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<td>Herbicide applications</td>
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<table>
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<th>Spring Soil Test Results</th>
<th>Fertility Treatment</th>
<th>Yield Goal (bu/A)</th>
<th>Nitrogen (lb/A) Goal</th>
<th>Present Needed</th>
<th>Fertilizer Applied (lb/A)</th>
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<td>92</td>
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<tr>
<td>Tillage</td>
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<td>70</td>
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<td>92</td>
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<td>Planter used</td>
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<td>pH 5.6</td>
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</table>

1. Low fertility treatment based on 2.6 lb N per expected bu as recommended for soft white spring wheat.
2. High fertility treatment based on 3.6 lb N per expected bu as recommended for hard red spring wheat.

Table 2. Means and standard deviations (SD) for test weight, grain protein concentration, grain yield, and lb protein produced per acre for near isogenic lines of wheat isolines, derived from Scarlet or Tara 2002, that were evaluated in replicated fertility trials at two locations in Washington State in 2003

<table>
<thead>
<tr>
<th>Location*</th>
<th>Recurrent Parent Fertility**</th>
<th>n***</th>
<th>Test Weight (lb/bu)</th>
<th>Test Weight SD</th>
<th>Grain Protein Concentration (%)</th>
<th>Grain Protein Concentration SD</th>
<th>Grain Yield (bu/A)</th>
<th>Grain Yield SD</th>
<th>lb Protein per Acre</th>
<th>lb Protein per Acre SD</th>
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<tbody>
<tr>
<td>Spillman</td>
<td>Tara 2002 High</td>
<td>45</td>
<td>59.1</td>
<td>0.9</td>
<td>14.7</td>
<td>0.9</td>
<td>49.2</td>
<td>5.5</td>
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<td>39</td>
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<tr>
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<td>Low</td>
<td>45</td>
<td>59.7</td>
<td>0.9</td>
<td>14.6</td>
<td>0.8</td>
<td>48.5</td>
<td>5.9</td>
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<td>43</td>
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<td>Spillman</td>
<td>Scarlet High</td>
<td>135</td>
<td>57.4</td>
<td>0.8</td>
<td>15.2</td>
<td>0.5</td>
<td>49</td>
<td>4.2</td>
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<td>37</td>
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<tr>
<td></td>
<td>Low</td>
<td>135</td>
<td>58</td>
<td>0.8</td>
<td>14.9</td>
<td>0.6</td>
<td>47.5</td>
<td>4.3</td>
<td>411</td>
<td>35</td>
</tr>
<tr>
<td>PCF</td>
<td>Tara 2002 High</td>
<td>45</td>
<td>60.8</td>
<td>1.2</td>
<td>14.2</td>
<td>0.8</td>
<td>55.4</td>
<td>7.7</td>
<td>478</td>
<td>63</td>
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<td>Low</td>
<td>45</td>
<td>59.5</td>
<td>1.4</td>
<td>14.5</td>
<td>1</td>
<td>45.2</td>
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<tr>
<td>PCF</td>
<td>Scarlet High</td>
<td>135</td>
<td>56.7</td>
<td>2</td>
<td>14.9</td>
<td>0.7</td>
<td>49.3</td>
<td>9.5</td>
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<td>82</td>
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<tr>
<td></td>
<td>Low</td>
<td>135</td>
<td>58</td>
<td>1.8</td>
<td>14.3</td>
<td>0.8</td>
<td>37.3</td>
<td>9.8</td>
<td>308</td>
<td>80</td>
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</table>

*Spillman = WSU's Spillman Farm in Pullman, WA. PCF = USDA-ARS Palouse Conservation Farm, Albion, WA
**High fertility = 3.6 lb N/expected bu; Low fertility = 2.5 lb N/expected bu
***n = the number of replicated genotypes evaluated per treatment
### Table 3: Pounds (lb) of protein produced per acre* by BC$_3$ isolines of Scarlet grown in a conventional field trial at Spillman Farm in 2003. Genotypes with a "G" contain the high grain protein concentration (HGPC) region from Glupro, whereas lines with a "S" do not contain the HGPC region.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>lb protein/A</th>
<th>LSD*</th>
</tr>
</thead>
<tbody>
<tr>
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<td>460</td>
<td>A</td>
</tr>
<tr>
<td>1584-12G</td>
<td>457</td>
<td>AB</td>
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<td>ABCD</td>
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### Table 4: Pounds (lb) of protein produced per acre* by BC$_3$ isolines of Tara 2002 grown in a conventional field trial at Spillman Farm in 2003. Genotypes with a "G" contain the high grain protein concentration (HGPC) region from Glupro, whereas lines with a "T" do not contain the HGPC region.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>lb protein/A</th>
<th>LSD*</th>
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<td>3514-5G</td>
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</tbody>
</table>

*Values from high and low fertility treatments where combined due to a non-significant (p<0.05) genotype by fertility interaction.

**Values followed by the same letter do not differ significantly (LSD=32) at the 5% probability level.
Figure 1. Autoradiogram illustrating hybridization of Taq1 digested DNA from near isogenic lines (isolines) with Nor-B2, which is closely associated with the High Grain Protein Content (HGPC) QTL on 6BS. Lane 1 is a DNA standard, lanes 2 through 5 are parental lines, lanes 6 through 24 are isolines; lane 25 is a control. A "+" indicates presence, whereas "-" indicates absence of the HGPC marker.

Figure 2. Agarose gel confirming results from Figure 1 through amplification of isolines with two DNA primers supplied by J. Dubcovsky that also are linked to the HGPC QTL. Lane 1 is 100 bp DNA standard, lanes 2 through 5 are parental lines, lanes 6 through 24 are isolines and the last lane is a control. A "+" indicates presence, whereas "-" indicates absence of HGPC marker.