Washington Grain Commission
Wheat and Barley Research Annual Progress Reports and Final Reports

Project #: 3019-4548

Progress Report Year: 1 of 3

Title: Pre-breeding for Root Rot Resistance

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Cooperators: Timothy Paulitz, Deven See

Executive summary:

Rhizoctonia solani AG8 and *R. oryzae*, soilborne fungal pathogens of wheat and crops used in rotation with wheat, causes root rot, stunting and bare patch. Rhizoctonia root rot is one, but not the only disease causing green bridge problems in spring cereals in the PNW. The aim of this project is to characterize resistance or tolerance to Rhizoctonia and other greenbridge-promoted diseases identified from several synthetic wheat lines and transfer the resistance to the cultivar Louise. The cultivar Louise was selected because it has a relatively good root system already, and enhancing its resistance to Rhizoctonia would create a valuable germplasm asset for the breeding programs. It is also already a popular cultivar and we could perform roughly nine generations in three years by using a spring wheat. The resistances are controlled by the additive effects of several genes in each of the sources of resistance, so backcrossing the resistance to adapted germplasm takes many more generations than backcrossing a single gene trait, like many of the rust resistance genes used in the breeding programs. After each cross to Louise, the progeny lines are advanced 3-4 generations under selection for resistance to try to collect and maintain all of the genes from the resistant parent. In addition, the sources of resistance were *synthetic* lines; artificially generated by combining the genomes of AB and D wheats (Table 1) to reconstruct the bread wheat genome. Thus, the original sources were all poorly adapted to the PNW and still exhibited some of the wild characteristics of the AB and D genome parents, (e.g. difficult threshing). We felt that at least three crosses to an adapted cultivar would be required to develop lines that could be evaluated for performance in field trials. We also used the same cultivar, Louise, as the recipient of all of the sources of resistance so that the resistances could be compared in the same genetic background. The original sources of resistance all had very different root systems making it impossible to tell which aspects of these root systems were associated with resistance. Once the resistances are transferred into the same genetic background, analysis and comparisons of the root systems would be more informative.

The project focused on five sources of resistance that are listed in Table 1 along with the progress we have made in crossing these resistances to Louise. All five sources have now been crossed to Louise at least three times. The backcross 2 (BC2) designation indicates the original cross to Louise was followed by two more crosses with multiple generations of selection in between. The F4 to F5 designation indicates the numbers of generations of self-fertilization and selection that have been conducted after the BC2 cross. For two of the sources of resistance,

Synthetic 172 and CIMMYT 3104, we also advanced large BC1 derived populations of lines for mapping the resistance genes. In the past year all of the BC2 lines were screened one more time in field assays under severe green bridge conditions and the most resistant lines were amplified in the greenhouse for performance testing in field plots in Year 2 of the proposal.

Table 1. Progress in crossing resistance from five different sources into the cultivar Louise

Resistance Source	# lines	Stage in 2015	Pedigree*
Synthetic 30	4	BC2-F5:6	**CROC_1**/AE.SQ. (210)
Synthetic 182	12	BC2-F5:6	**CROC_1**/AE.SQ. (518)
Synthetic 201	9	BC2-F5:6	**68112/WARD**//AE.SQ. (369)
Synthetic 172	10	BC2-F5:6	**SNIPE/YAV79//DACK/TEAL**/3/AE.SQ. (904)
CIMMYT 3104	8	BC2-F5:6	(**CROC1**/ AE.SQ. (224)//OPATA/3/PASTOR)

* Durum wheat parent (A&B genomes) are in bold font, wild diploid parent (D genome) in shaded font.

In year 1 of the project, we completed field evaluation of two large populations of BC1-F5 derived lines from the Synthetic 172 and CIMMYT 3104 sources. They were scored in multiple years for stunting or non-stunting in fields with high levels of disease pressure. In the these field assays, we evaluated stunting by monitoring plant height in plots in which the green bridge was not controlled ("green"), and comparing them to plants grown at adjacent plots in which the green bridge was controlled by glyphosate ("clean"). Molecular diagnostics showed that *R. solani* AG8 was present at moderate to high levels and thus resistance to this pathogen was expected to be a component of resistance to the green bridge conditions. The resistance of these lines has also been evaluated in greenhouse assays. Results of disease assays with the CIMMYT 3104 x Louise population are summarized in Figure 1. Note the relative resistance of the CIMMYT 3104 and Louise parents compared to the 190 lines generated from the cross. While multiple pathogens are present in our field assays (*Pythium*, etc.) the greenhouse assays are conducted with *Rhizoctonia* only, so results differ to some extent. However the lines that perform best in the greenhouse assays generally performed well in the field assays. This supports our hypothesis that resistance or tolerance to Rhizoctonia is a major component of the resistance to green bridge conditions in the field.

In collaboration with Deven See, several hundred genetic markers were scored on the CIMMYT 3104 x Louise population using genotype by sequencing (GBS) in the past year. The marker data was then used to create a genetic map on which to integrate the resistance data. Genomic locations (QTL) on three chromosome arms were identified in which resistance from the CIMMYT 3104 parent mapped. A manuscript describing the five sources of resistance and the locations of the resistance QTL is in preparation. GBS mapping of the Synthetic 172 x Louise population has been initiated.

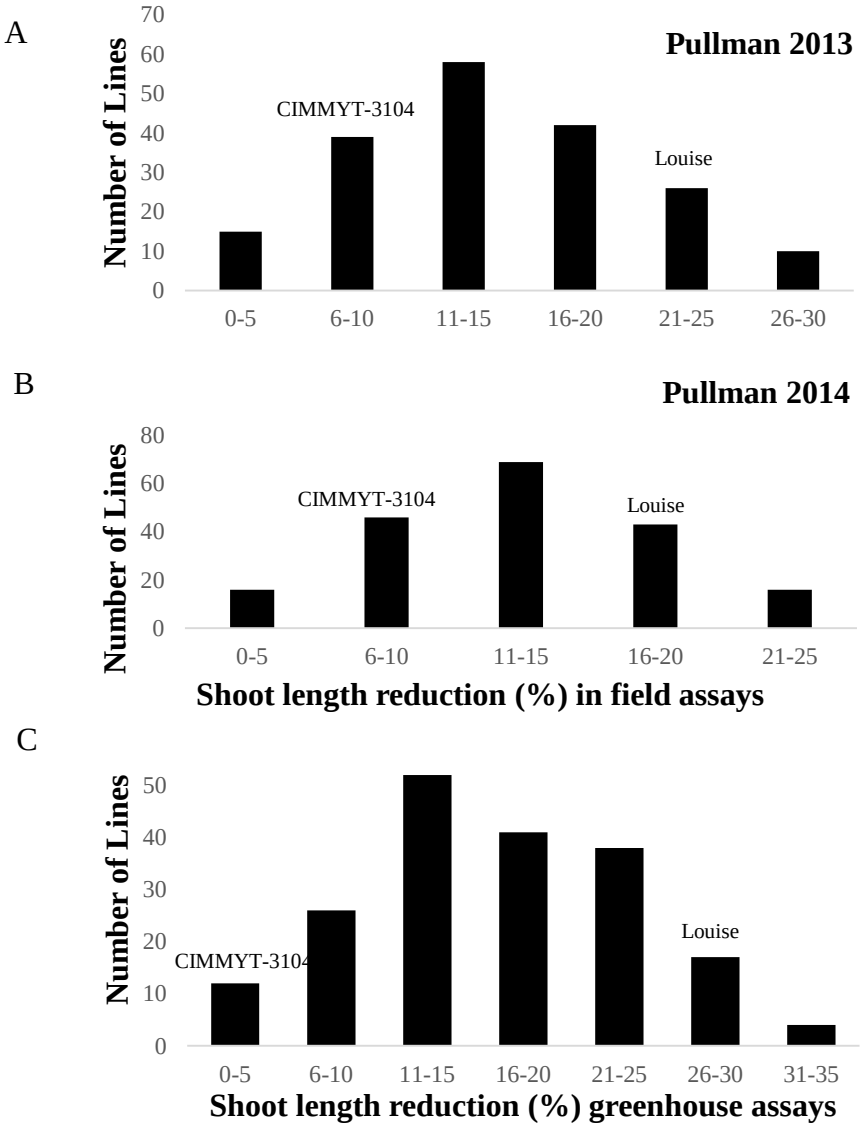

Figure 1. Root rot damage in 190 lines from a CIMMYT 3104 X Louise cross after planting in severe green-bridge field conditions (a and b) or in Rhizoctonia infested soil in the greenhouse. Root rot was estimated as % stunting compared to control rows. Results for the Synthetic 172 population were similar.

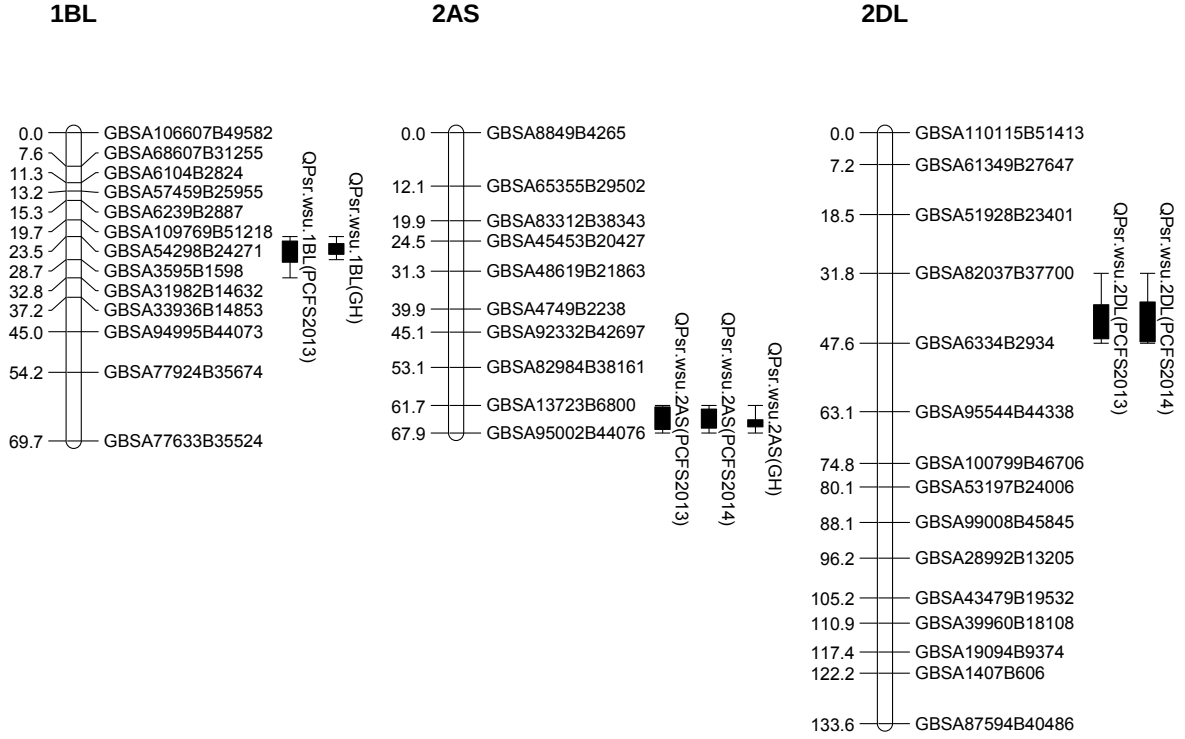

Figure 2. QTL mapping of genes contributing to resistance in the CIMMYT 3014 x Louise mapping population. The figure shows maps of three chromosome arms that carry genes that contribute to resistance or tolerance to green bridge diseases in field plots and in greenhouse assays with Rhizoctonia.

Impact:

In the past year, the main impact of project was to advance the resistance to Rhizoctonia and green bridge associated diseases to wheat lines adapted to PNW wheat production. Genetic resistance is a cost-saving resource for controlling plant pathogens, but this resource is not available to wheat breeders and growers for *Rhizoctonia* anywhere in the world. Yield loss of wheat and barley due to *Rhizoctonia* and other soilborne pathogens is estimated at 10%, but can be as high as 40% in direct seeded systems in field with high inoculum levels. Estimated yield potential to be gained from control of these pathogens would amount to over $100 million per year for the Washington wheat and barley industries. The resistance to stunting in synthetic wheats is apparently due to multiple genes with small effects, as indicated by our mapping data with the CIMMYT 3104 x Louise population. Given its multigenic nature, resistance is expected to be durable, but will not be simple to move between lines. Genetic improvement of wheat and barley resistance to root rot will contribute to current management by rotation, fungicides and green bridge control, and will enhance profitability and sustainability of dryland cereal cropping.

WGC project number: 4548
WGC project title: Pre-breeding for Root Rot Resistance
Project PI(s): Scot Hulbert & Pat Okubara
Project initiation date: July 1, 2015
Project year: Report for year 1 of 3

Deliverable	Progress	Timeline	Communication
Obj. 1. A set of novel Rhizoctonia/ green bridge associated disease resistant lines that can be used to develope PNW spring wheat cultivars. Molecular markers linked to genes controlling resistance in two synthetic lines.	Mapping of genes in the first mapping population was completed with molecular markers linked to three genes contributing to resistance. Multiple (four to 13) BC2 lines from each of the four sources of resistance were selected and amplified this year.	The BC2 lines have now been amplified and we will have sufficient seed to plant three replicate plots at two locations in year 2. We will also perform genotype-by-sequence marker generation and genetic mapping of resistance genes in the second mapping population.	Progress will be reported at the wheat research review and the Cook Chair review. An article on the synthetic lines will be submitted to *Wheat Life* if solicited. An article describing the sources of resistance and the mapping in the first mapping population will be submitted to Phytopathology in February.
Obj. 2. Multi-location yield trial data on the BC2 derived lines from each of the five sources of resistance, to identify which has/have best benefits in different types of field environments.	The BC2 lines for this objective were selected and amplified in the past year.	In year 2 and 3 of the proposal, we will identify the best performing BC2 lines that appear to be best adapted to PNW growth conditions. We will have sufficient seed for testing in two locations in year 2.	Progress will be reported at the wheat research review and the Cook Chair review.
Obj. 3. A more rapid and economical means of selecting and advancing *Rhizoctonia* resistant plants.	This objective has been completed.		A manuscript by Okubara et al. is in press in the journal Plant Disease.
Obj. 4. Information on whether the synthetic wheats carry true resistance or tolerance, and how similar or unique root morphology traits are in these lines. These will be available at the end of Year 1.	We completed the BC2 lines so the resistances are all in similar genetic backgrounds.	We will complete greenhouse resistance screens and quantification of root morphology variables for the five sources of resistance in Yeasr 2 and 3.	Progress will be reported at the wheat research review and the Cook Chair review. The BC2 lines and their root characteristics will be described in a germplasm release article.