Washington Grain Commission

Project #: 3019-4548

Progress Report Year: 3 of 3; Final Report

Title: Pre-breeding for Root Rot Resistance Using Root Morphology Traits

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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. The aim of this project was to characterize resistance 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 as a spring wheat we could perform roughly nine generations in three years. The resistances appear to be 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 either *synthetic* lines (artificially generated by combining the genomes of AB and D wheats to reconstruct the bread wheat genome) or crosses between synthetics and other CIMMYT wheat lines. Thus, they 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 breeders would be comfortable crossing into their breeding populations. We also wanted to use the same cultivar, Louise, as the recipient of all of the sources of resistance to 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 F3 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. All of the populations will be screened in the field one more time before the end of the funding period (July 2015). Following selection in the field, lines from the Synthetic 30, 182 and 201 sources will be ready for amplification and comparative analysis. Lines from the other two sources will be advanced one more generation in the greenhouse.

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

Resistance Source	Current gen.	# lines	In 2015 Spring Nursery
Synthetic 30	BC2-F5	6	BC2-F5:6
Synthetic 182	BC2-F4	12	BC2-F5:6
Synthetic 201	BC2-F4	9	BC2-F5:6
Synthetic 172	BC2-F3	10	BC2-F4 (and BC1 derived mapping population)
CIMMYT 3104	BC2-F4	8	BC2-F5 (and BC1 derived mapping population)

In years 2 and 3of the project, we successfully screened two large populations of BC1-F5 derived lines from the Synthetic 172 and CIMMYT 3104 sources in the field. They were scored for stunting or non-stunting in fields with high levels of disease pressure. First, we evaluated stunting in Year 2 in the field by monitoring plant height at PCFS 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. In past years, we had difficulty in managing *Rhizoctonia* plots free of pests such as wire worm and in border effects between the green and clean plots. In years 2 and 3 of the project we produced field data we are more confident in. Because of the difficulty in measuring resistance in the field however, we plan to screen these populations one more time in Spring 2015. The resistance of these lines has also been evaluated in greenhouse assays. 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. In collaboration with Deven See, the CIMMYT 3104 population was genotyped using SSR markers. Marker analysis of both populations are now being performed with a high density of DNA markers using the newest technology, genotype by sequencing (GBS).

Impact:
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 likely to be due to multiple genes; given its multigenic nature, resistance is expected to be durable. Genetic improvement of wheat and barley will contribute to current management by rotation, fungicides and green bridge control, and will enhance profitability and sustainability of dryland cereal cropping.

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Project PI(s): Pat Okubara, Scot Hulbert
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Objective	Deliverable	Progress	Timeline	Communication
1. Select and advance resistant wheat lines in the field and greenhouse. Transfer six sources of resistance to a common spring wheat (cultivar Louise) background.	Five different sources of resistance crossed at least three times to Louise to place them in a popular adapted wheat background. One source was dropped because resistance appeared inferior to the other five. Advanced RIL lines for two populations for mapping the resistance genes. Protocols for producing field environments in which Rhizoctonia is present or absent; protocols for scoring stunting in the field and greenhouse.	BC_1F_7 recombinant inbred line (RIL) populations derived from synthetic wheats Synthetic 172 x Louise and CIMMYT 3104 x Louise have been advanced completed and phenotyped successfully for two years. These two sources, as well as Synthetics 30, 182 and 201 have all been crossed to Louise three times and resistant line selection is underway from the backcross two progeny. CIMMYT 3220 was dropped due to poor performance.	Multiple resistant lines with three crosses to Louise in their backgrounds from five different sources of resistance will be available for amplification by the end of the year 3 funding period.	1) Okubara PA, Mahoney A, Hulbert SH (2013) Dryland Field Day Technical Report. 2) WGC Research Reviews, annually. 3) Hulbert Cook Chair review, annually. 4) PCFS Field Day 2013. 5) Okubara PA, Mahoney A, Hulbert SH (2014) Dryland Field Day Technical Report.
2. Develop molecular markers for tracking the resistance during breeding. Two of the sources of resistance are being used to make mapping populations from the breeding pedigrees developed as they are integrated into the Louise background.	Markers that can be used by breeders for tracking resistance in cultivar development; this is particularly important with traits like Rhizoctonia resistance with are difficult to track phenotypically.	The CIMMY 3104 x Louise mapping population (175 RILs), were genotyped using 162 polymorphic SSR markers. A putative QTL with a LOD score of 4.2 was identified for shoot length reduction within a 24 cM region on the long arm of chromosome 2B. Both mapping populations have been phenotyped; for two years, marker analysis of both will be complete before the end of year three funding.	Phenotyping and high density marker analysis of both populations will be complete by July 2015. Data analysis and publication will take another year.	See above
3. Evaluate and compare the nature of the tolerance or resistance from the different sources. The six sources of resistance are all very different but once these resistances are placed in the common spring wheat background, we will be able to compare them more efficiently.	Information on whether the synthetic-derived lines carry tolerance or true resistance to *Rhizoctonia;* information on whether they carry resistance to other *Rhizoctonia species, Pythium* and *Fusarium;* protocols to compare lines for resistance under controlled environments	Greenhouse scoring of the two mapping populations with Rhizoctonia was completed; data will be compared to field resistance data to see if the same genes are controlling resistance to both traits. Comparisons of root architecture between the five sources of resistance will commence once the resistant BC2-derived lines are available.	Greenhouse and field comparisons indicate they are mostly different. Final comparisons will be made once BC2-derived lines are completed and resistances are mapped in the two mapping populations.	See above
New objective in 2014: Verify that the AUS28451 line does have some level of resistance or tolerance to *Rhizoctonia* and/or *Pythium* and examine the inheritance of this resistance by planting the population in our green-clean assays in Pullman.	Possbile germplasm that can be used for breeding for multiple resistances.	We have performed preliminary tests for *Rhizoctonia* resistance on the AUS28451 and found it to be promising. We have scored approximately 120 recombinant inbred lines from a AUS28451 x Louise in the field in 2014, but they need to be scored one more time. More field testing will be performed before the end of the funding period.	We will re-score the parent line and the whole AUS28451 mapping population at PCFS this spring in our green-clean trials.	None yet