Wheat Research Progress Report - Final

Project #: 3019-5850

Title: Mutation Breeding for Tolerance to Roundup, *Rhizoctonia* Root Rot, and Drought.

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All breeding programs capitalize on genetic variation to improve crop plants. Genetic variation can result from naturally occurring and selected mutations or changes in plant genes. Mutation breeding uses chemical or radiation treatment to increase the genetic variation in plants, allowing the isolation of new genes for tolerance to environmental stress and to pathogens. Mutation breeding is a good method for isolating needed genes that have not been seen in wild populations, and has the advantage that the germplasm developed is not considered to be genetically modified. A well known example of mutation breeding is the herbicide resistance gene in Clearfield wheat. Mutation breeding is a pre-breeding method that provides new germplasm to breeding programs. This project has successfully used mutation breeding to identify wheat plants carrying new genes providing drought tolerance, *Rhizoctonia* tolerance, and tolerance to the herbicide Roundup Ultra.

Accomplishments:

The project has isolated new drought-tolerant, *Rhizoctonia* tolerant and glyphosate tolerant germplasm, which are being introgressed into adapted spring wheat germplasm. To summarize our accomplishments to date:

1. We have used three approaches to develop wheat with resistance to Roundup Ultra at 32 oz/A (2x label) applications.
2. We identified and genetically characterized the line Scarlet-Rz1, a line of Scarlet carrying a gene for Rhizoctonia root rot tolerance in the Scarlet background.
3. New Rhizoctonia tolerant lines have been identified in cultivars Louise, Hollis, and Macon.
4. We identified mutants with increased sensitivity to the drought hormone ABA in three genetic backgrounds, Chinese spring, Scarlet, and Zak. Greenhouse and field evaluations of have shown that a subset of these lines use water more efficiently and show increased resistance to preharvest sprouting.

Results:

1. Screening for Roudup Ultra herbicide resistance.

Weed control is one of the most challenging problems facing wheat farmers in the PNW. Competition from weeds can reduce yield and product value. Moreover, some weeds can increase problems with pathogens by acting as a green bridge. Glyphosate (Roundup™) resistance would be particularly useful because in addition to be an excellent herbicide, it also has anti-fungal activity that can be used to combat stripe rust. Original screens identified a single mutant, GT-Louise, with resistance at 16 oz/A (1X) application rates. Three strategies have been undertaken to obtain resistance at 2x (32 oz/A) and 4x (64 oz/A) application rates: a) large scale screening for new Roundup tolerant mutants; b) re-mutagenesis and screening for GT-Louise with enhanced Roundup resistance; c) increase resistance by "gene pyramiding" of independently isolated mutations giving increased Roundup tolerance. These approaches have succeeded in identifying wheat lines with higher resistance to commercial application rates, but have not yet resulted in a line that can consistently resist 4X application rates.

a) Large scale screening for new Roundup tolerant mutants.

Large scale screens for Roundup resistant lines were performed in the greenhouse (Plant Growth Center) and in the field in cultivars Louise, Hollis, Tara 2002 and Macon. A summary of all screens totaling over 7 million plants and subsequent retests performed are shown in Table 1. The best candidates isolated thus far were Tara 2002 line FR1-15 with 89% survival of 1X rates, Louise FR1-33-6 showing 97% survival at 1X. A massive Field screen performed at 4X application rates in Spillman Farm in 2008 recovered two candidates. These candidates showed poor fertility suggesting that they do not have full resistance to 4X rates. Retest experiments will be performed following a seed increase.

Table1. Screening spring wheat mutants for resistance to Roundup Ultra.

	Greenhouse 2005/06	Field 2006 (1X)	Field 2007 (2X)	Field2008 (4X)
M2 screened	614,000	1.5 million	1 million	3.8 million
M2 putatives isolated	24	157	100	2
M3 Greenhouse Retest 12 oz/A or higher	5	25		Experiment in process
'07/08 Field Retests, 1X	1	10	na	na
2X	0	1	7	na

Because greenhouse conditions are gentler than field conditions, plants that showed resistance to Roundup Ultra in greenhouse experiments were further examined in a controlled field trial in 2008 in collaboration with Dr. Ian Burke. Rows of plants spaced 14 inches apart were sprayed at the 5-leaf stage with a hooded boom sprayer using TeeJet XR 80015 spray nozzles 12 in above the canopy. A total of 11 lines showed limited survival at 2X (32 oz/A) application rates. A single line, Louise FR1-62, showed 99% survival at 2X application rates but also grew as a dwarf. No plots showed more than a single survivor at the 4X (64 oz/A) application rate. This experiment suggests that it is not possible to isolate single gene mutations that provide resistance to 4X application rates in wheat. Future progress in this area will require the development of multi-gene resistance which will require more time and effort.

b) Re-mutagenesis and screening for GT-Louise with enhanced Roundup resistance.
GT Louise, was re-mutagenized using EMS and screened for two-gene enhanced resistance. Of the 48 GT M3 seeds re-mutagenized, 43 germinated and were selfed. Out of 13,706 M2 re-mutagenized GT Louise screened 448 M2 plants survived. For all candidates, the main tiller showed some damage, and secondary tillers emerge within 10 days. The best candidates resulting from this method were Re-mut GT-Louise 3.7-11 and 3.9 showing 100% survival at 1X application rates, Re-mut GTL 3.1, 3.3, and 3.5 showing 25% survival at 2X application rates. A total of three lines showed limited survival of 2X application rates in the field 2008.
c) Increase resistance by "gene pyramiding" of independently isolated mutations. Another strategy used was to cross independent tolerant lines to each other in order to enhance the tolerance expression through gene pyramiding. 147 crosses been made between some of the individual tolerant plants. When treated with RoundupUltra at 2X (32 oz/A) in the greenhouse as segregating F2 populations, the two best candidates showed 19/38 and 22/38 survival rate suggesting that this approach is the most effective method for increasing resistance to Roundup. In the 2008 field trials, 16 lines showed rare survivors at 4X application rates. A total of 7 F3 EGT and 9 F2 IGT populations showed segregation of individuals surviving 2X application rates. Dose-response experiments are required to better characterize the resistance in these lines.

2. Characterization of a *Rhizoctonia* tolerant wheat plants.

Rhizoctonia root rot is an important, yield-limiting disease in direct-seed wheat production systems in the PNW. Under severe disease pressure, plants are stunted, creating bare patches in the field that can severely limit grain yield. If direct-seed systems are to succeed, varieties adapted to the diseases associated with reduced tillage or direct seed environments must be developed. Although differences in disease response to Rhizoctonia root rot have been detected among adapted spring wheat varieties, all varieties tested were susceptible. Using mutation breeding, we have developed a line derived from Scarlet that shows resistance not only to *Rhizoctonia solani*, but to *Rhizoctonia oryzea* and to *Pythium*. Introduction of the resistant germplasm developed by this project should increase grain yields by reducing pathogen pressure, and should improve profitability since direct seeding reduces energy input requirements and conserves soil water.

The *Rhizoctonia* tolerant line *Rz1* has been characterized in detail in collaboration with plant pathologist Dr. Patricia Okubara. Further characterization has shown that it gives resistance to damping off. When Rz1 was grown in the field in 2007, it was almost identical to normal Scarlet in terms of yield and test weight. Three backcrosses to normal Scarlet have been made. Segregation analysis of BC_1 and BC_2 progeny, suggest that *Rhizoctonia* tolerance is the result of a single gene, semi-dominant (additive) mutation. A homozygous Rz1 plant has been identified, and was used to develop a doubled-haploid mapping population using a cross of Scarlet-Rz1 to Scarlet. This population has been submitted for mapping using Diversity Arrays Technology (DArT). Scarlet-Rz1 has been crossed into spring wheat breeding lines. F3 individuals segregating for Rhizoctonia tolerance have been identified from crosses to Otis.

Screening of new mutagenized lines recovered new mutants in backgrounds more likely to lead to rapid release. Retest experiments have shown reproducible resistance in 15 lines of the 'Louise' background, 4 lines of the 'Hollis' background, and 5 lines of the 'Macon' background. These mutants will need to be backcrossed to the normal parent and further characterized to determine how they are inherited and the degree of resistance to Rhizoctonia.

3. Isolation of drought tolerant wheat plants based on increased sensitivity to the plant hormone ABA (abscisic acid) during seed germination.

Low precipitation levels limit yield potential of both spring and winter wheat grown in dry areas of eastern Washington. Improvement of yield potential in dry areas will require wheat varieties that use water more efficiently. The screen used to isolate drought tolerant wheat plants was based on previous work in Arabidopsis, canola, and maize showing that plants with increased sensitivity to the plant hormone ABA during seed germination tend to use water more efficiently under drought stress.

ABA is both the seed dormancy hormone and the drought tolerance hormone. ABA application inhibits seed germination in a concentration-dependent manner. We screened for mutants that were unable to germinate on a concentration of ABA that is normally too low to inhibit seed germination. These "ABA hypersensitive" mutants are more sensitive to ABA in germination than normal plants. ABA is the signal from the roots to the shoots to conserve water as the soil dries. By making plants more sensitive to ABA, we cause them to conserve water earlier than normal. We have found that Wheat ABA-responsive mutants (*Warm*) can improve the drought tolerance of Chinese spring and Scarlet.

The first screening was performed in a lab rat wheat genotype called 'Chinese spring'. Of the 25 lines found to be ABA hypersensitive based on seed germination, 4 appear to be drought tolerant using transpiration rate measurements. Based of segregation analysis of eight BC_1F_2 plants from backcross to normal Chinese spring, 4 mutations appear to be semi-dominant, 2 dominant, and 1 recessive. Carbon isotope discrimination has identified potentially drought tolerant lines in Chinese spring and in Scarlet backgrounds. All lines were grown on re-cropped land at Lind Farm in 2008. Preliminary data show that drought tolerant line 130 shows an average yield of 98.9, where as the normal Chinese spring shows a yield of 82.3. Further repetitions are needed to determine if this is significant.

A total of 27 ABA hypersensitive mutants were isolated in Scarlet and 4 in Zak. Greenhouse transpiration experiments in the process, and suggest that 5 of Scarlet and 1 of Zak lines show drought tolerance. All Chinese spring and Zak lines were tested by Dr. M. Sorrells of Cornell for resistance to preharvest sprouting. Many lines showed increased resistance. Those Zak lines that show the strongest PHS tolerance, Zakera0 and Zakera19 were rated as moderately resistant compared to the sensitive Zak parent.

Publications

"GLYPHOSATE-TOLERANT WHEAT GENOTYPE" Patent application filed Aug. 2007 by Washington State University.

"Mutation Breeding for Disease Resistance and other Useful Traits" Patent application filed Feb 2006 by Washington State University.

Schramm, E., Abellera J., Strader, L. , Campbell K.G., and Steber, C.M. "Can ABA signaling be used to develop drought tolerant wheat? International Wheat Genetics Symposium, paper in Conference Proceedings.

Patricia A. Okubara, Camille M. Steber, Victor L. DeMacon, Nathalie L. Walter, Timothy C. Paulitz, and Kimberlee K. Kidwell. "*Scarlet-Rz1, an EMS-generated hexaploid wheat with tolerance to the soilborne necrotrophic pathogens Rhizoctonia solani AG-8 and R. oryzae.*" Theorectical and Applied Genetics, provisional acceptance.

Presentations and Reports:

Okubara PA, "*Rhizoctonia* Tolerance in Scarlet-Rz1," WSU Extension Weed and Rhizoctonia Management Workshop, Spangle, WA. Feb. 19, 2008.

Okubara PA, "*Rhizoctonia* and *Rhizoctonia* Tolerance in Scarlet-Rz1," Columbia Country Extension Variety Research Field Day, Dayton, WA. June 30, 2008.

Okubara PA, "Rhizoctonia Tolerance in Adapted Spring Wheat." Plant Pathology departmental seminar, Pullman, WA, Oct. 29, 2008.

Okubara P, Steber C, Paulitz T, Kidwell K (2008) EMS-generated *Rhizoctonia* resistance in an adapted wheat. J Plant Pathol 90(2, Supplement), p. S2.350, Abstract no. 40.14.

Okubara P, Schroeder K, Paulitz T, Steber C and Kidwell K (2008) Controlling soilborne pathogens in wheat production systems. 2008 Dryland Field Day "Bioenergy Cropping Systems Research," Dept. of Crop and Soil Sciences Technical Report 08-1, Washington State University, Pullman, WA. p. 37-38, Abstract #35.

Steber, C.M. "Can ABA signaling be used to develop drought tolerant wheat?" Oral Presentation #38, Session on Abiotic Stress. International Wheat Genetics Symposium, August 2008.

Steber, C. September 17-20 2007, "ABA and GA signaling, bridging the gap between wheat and Arabidopsis." Plant Sciences Symposiums on Translation Seed Biolgoy: From Model Systems to Crop Improvement. University of California, Davis

Steber, C. Nov 2006, "Mutation Breeding for Tolerance to Roundup, Rhizoctonia, and Drought" presentation made to the Washington Wheat Commission in Spokane WA.

Steber, C. March 2005. ABA and GA signaling, bridging the gap between *Arabidopsis* and wheat. Invited seminar for Horticulture and Seed Biology program, Oregon State University, Corvallis, OR.