Washington Grain Commission
Wheat and Barley Research Annual Progress Report

Project #: 13C-3019-3687 (55); AWD003580

Progress Report Year: *__1__ of __3__ (maximum of 3 year funding cycle)*

Title: *A Genetic Arsenal for Drought Tolerance, Getting to the Root of the Problem*

Cooperators: *Karen A. Sanguinet, Kimberly Garland-Campbell, Timothy Paulitz*

Executive summary: Dryland farms in eastern Washington experience yield losses due to drought stress and disease pressure from soil-borne pathogens and 2021 was no exception. Improving wheat root structure can help to resist such stresses by increasing access to water as well as selecting for traits that confer tolerance to belowground stresses. Changes in tillage practices have changed the soil structure and pathogen load, altering root-microbiome interactions. Thus, there is an urgent need for understanding and improving both the root system and structure to improve stress tolerance. We found cooler canopy temperatures were associated with better yield under drought and healthier roots suggesting that breeding for more efficient root systems can improve the performance of Washington wheat under drought. Lignin content and accumulation has been linked with different stress tolerances and responses in crop plants because it lends rigidity to plant cell walls and increases in response to drought, heavy metals, salinity, and pathogen attack. Therefore, managing overall lignin content is a key step for generating wheat lines with improved stress tolerance. We have been working to understand how lignin content and deposition in winter wheat roots contributes to stress tolerance. To this end, we study a landrace from Iran called AUS28451 and the winter wheat variety Louise, which have different lignin contents in their root systems. We found that both roots and shoots of AUS contain less S lignin than G lignin, whereas Louise has higher amounts of S lignin. S-lignin is correlated with the ability to breakdown biomass (i.e. more S lignin means plant biomass breaks down more easily). We also performed in silico characterization of the *CAD* and *COMT* gene families that help biosynthesize lignin. We have identified root-specific *CAD* and *COMT* genes and are working to understand how they are regulated in response to stress. In addition, we are running qRT-PCR experiments to determine how lignin genes are expressed in roots in response to different environmental cues and in different varieties. We have also identified *cad* and *comt* mutants in the Kronos background to examine loss of function phenotypes and contributions of the subgenome copies to root architecture and stress responses.

Impact: According to USDA data, farmers in Washington planted 70,000 acres of wheat in 2021, down from 90,000 in 2020 with decreased yields of 2.6 million bushels at an average 38 bushels per acre compared to 6.4 million bushels at 90 bushels per acre in 2020. In years where water is sparse like in 2021, wheat yields can decrease by more than half. Our research goal is to improve the reliance of wheat root systems and understand the contribution of lignin to improved drought and stress tolerance. Certainly, there is a long way to go, but our goal is to help breeders and farmers by understanding the physiological constraints to water movement in wheat and hope to improve resource allocation from roots to the shoots as well as optimize lignin composition and ratios.

WGC project number: 13C-3019-3687
WGC project title: A Genetic Arsenal for Drought Tolerance, Getting to the Root of the Problem
Project PI(s): Karen A. Sanguinet, Kim Garland-Campbell, Timothy Paulitz
Project initiation date: 7/1/2021
Project year (X of 3-yr cycle): 1 of 3

Objective	Deliverable	Progress	Timeline	Communication
1.Examine accumulation of lignin and other aromatic polymers in wheat roots in Louise, AUS28451 and correlate that with stress responses	Determination of the lignin content of shoots versus roots of Louise and AUS28451 as well as other aromatic polymers that may contribute to stress tolerance. We will be able to determine if lignin or potentially another compound contributes to stress tolerance in AUS roots	In year 1, we were able to determine the lignin content in roots and shoots of AUS28451 via a collaboration with Dr. John Ralph at UW-Madison.	In year 1, lignin and aromatic analysis was performed in shoot and roots of the parental lines. We will continue with selections in subsequent years 2 and 3.	We are currently working on two manuscripts, one describing the total lignin analysis pipeline and the other will be a report of the disease resistancs and stress tolerance of the lines. A paper was submitted in collaboration with Dr. Smertenko's group and an article was published in wheat life in October 2021.
2. Identify the CAD and COMT lignin biosynthetic genes in wheat	Since we determined that there is more lignin of AUS root systems, this will enable us to determine which of the CAD and COMT enzymes contribute to lignin content in both wheat roots and shoots. This will then serve as a marker for breeders to assess lignin content.	In year 1, the *in silico* analysis was performed. Years 2 and 3 will examine activity of the genes via molecular techniques in both shoots and roots and RNA expression analysis.	The in silico analysis of the *CAD* gene familiy was completed in year 1, whereas, year 2 will focus on the *COMT* gene family. Molecular follow-up experiments will be run in year 3.	We have drafted a paper for the CAD gene family in wheat and we are working on qRT-PCR data as a final step prior to submission. Characterization of the COMT gene family will occur in year 2. All data will be shared with growers in field day abstracts, articles, and podcats.
3.Determine the influence of CAD and COMT on root architecture and stress resistance in the Kronos tilling population of wheat	Our data as well as a recent publication in sorghum indicate that there is a link between lignin content, disease responses within the plant and root architecture. Therefore, we will use the Kronos TILLING population of wheat to functionally test the role of mutations in CAD and COMT on stress tolerance and root architecture.	In year 1, mutations in CAD and COMT genes of interest were identified in Kronos. The lines will be grown out and we are working on backcrosses to Kronos. There was some sterility in some of the lines either from other background mutations or from CAD/COMT. The mutant lines once confirmed, will be analyzed for stress tolerance and root growth/architectural traits.	In year 1, mutations in CAD and COMT genes were identified and planted in the greenhouse. The mutants will be assayed for stress responses in years 2 and 3.	Since are working on growing Kronos and crossing the mutant lines, most of the reporting for this aim will not occur until year 3.