

**Washington Grain Commission
2023 Wheat and Barley Research Final Report**

Project #: 3019-3687_GR00012279

Progress Report Year: __3__ of __3__

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

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Executive summary

Dryland farms in eastern Washington experience yield losses due to drought stress and disease pressure from soil-borne pathogens, which are becoming more frequent owing to changing weather patterns. 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 and also used the Chinese Spring variety of wheat as a reference for molecular and phylogenetic analyses.

Introduction

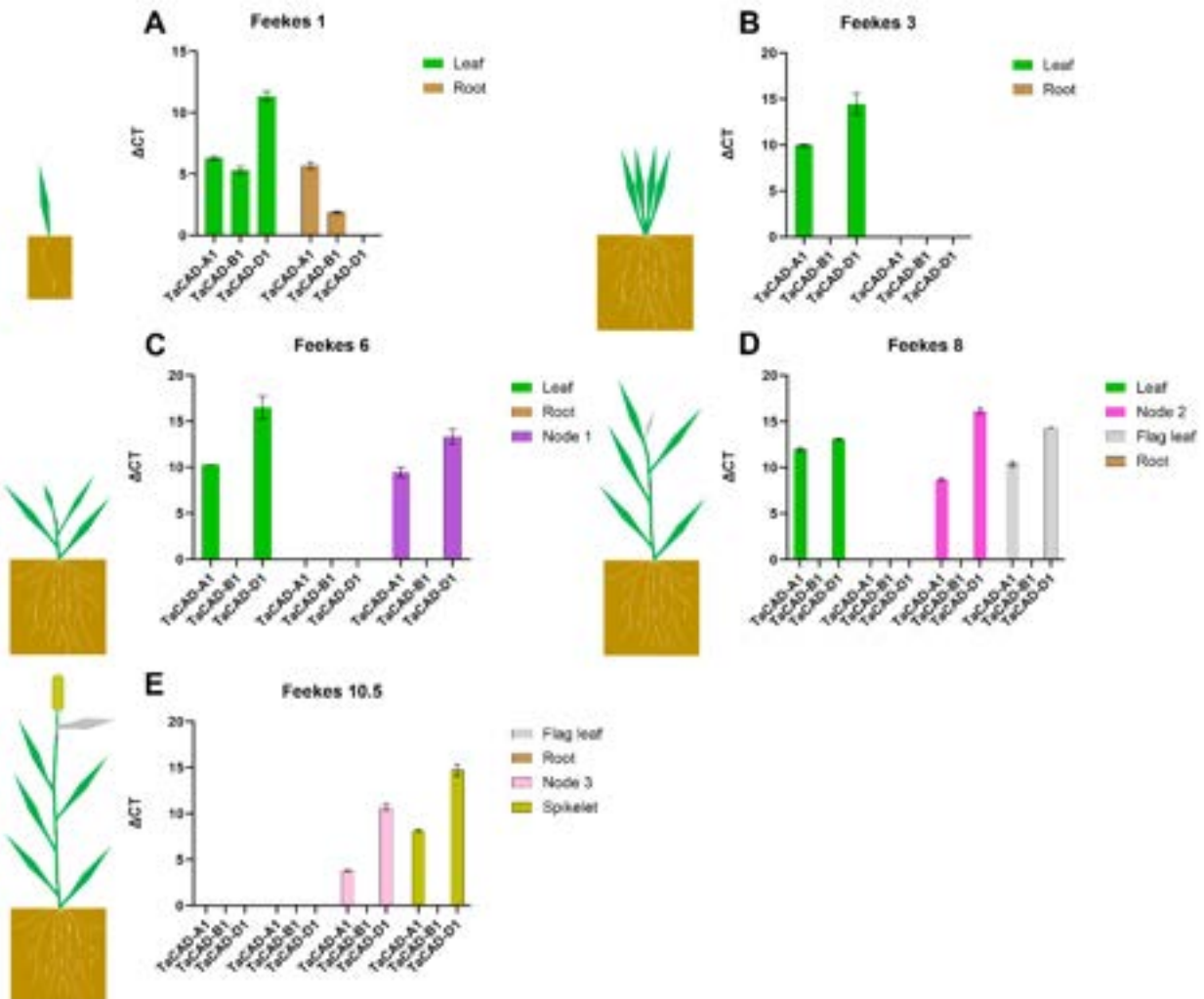
The overall goal of the project was to determine the role of lignin in wheat roots for drought tolerance and disease resistance and will provide insight into the genes that can be developed as markers for lignification during root development and in response to stress to select for more stress-tolerant root systems.

Approach

Our approach was to carefully characterize the differences in lignin content and drought responses between the Iranian landrace AUS28451, Louise, and the reference line Chinese Spring. First, we attempted several methods for lignin quantification and initiated a collaboration with Dr. John Ralph at UW-Madison. We also performed *in silico* characterization of the *CAD* gene family, developed quantitative markers for *CAD1* gene expression, performed RNAseq analysis in Louise and AUS28451 in response to drought.

Results

Figure 2. Expression of Class I CAD genes at Feekes 1, 3, 6, 8, and 10.5 in roots, leaves, nodes 2 and the flag leaf using subgenome-specific primers in qRT-PCR



We have also identified *cad* and *comt* mutants in the Kronos background and in a hexaploid wheat line to examine loss of function phenotypes and contributions of the subgenome copies to root architecture and stress responses. However, due to problems with sterility in the Kronos tillering lines that work ended up not being very fruitful. Therefore, we performed RNAseq analysis to look at the genes that are differentially expressed in AUS28451 vs Louise in both shoots and roots in response to drought. We are now working on analysis of these data with a focus on CAD and COMT genes as well as root-specific genes that are differentially expressed in response to drought. These data will lay the groundwork for the next funding cycle.

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. The short-term impact of this project is that we were able to characterize the *CAD* gene family in hexaploid wheat that and found all subgenome copies were expressed albeit at different developmental stages. We have laid the ground work for studying *CAD* gene expression and provided a phylogenetic framework. We also found quantification of root lignin to be very challenging and perhaps not the best breeding target because of the dynamic nature of lignin and differential responses to stress and depending on the developmental stage. This has allowed us to redirect efforts back into root development and nutrient use efficiency in future work.

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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
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Project year (X of 3-yr cycle): 3 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 2, we processed data and determined a repeat analysis was needed to clear up some inconsistencies between repeats. Mainly, aromatic analysis of root tissues is technically challenging. Therefore, we will regrow samples to repeat the analysis, and are still working on refining the data. In the meantime we have used other qualitative methods.	In year 1, lignin and aromatic analysis was performed in shoot and roots of the parental lines. We performed data analysis on the aromatic profiles and had technical challenges, which we are complementing with staining for root lignin in Louise and AUS	We are currently working on two manuscripts, one describing the total lignin analysis pipeline and the other will be a report of the disease resistances and stress tolerance of the lines. All data will be shared with growers in field day abstracts, articles, and podcasts.
2. Identify the CAD and COMT lignin biosynthetic genes in wheat	Since we determined that there is more lignin in AUS root systems, this will enable us to determine if 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 <i>in silico</i> analysis was performed. In year 2 we designed subgenome specific primers to examine activity of the CAD gene expression. We also grew and harvested roots for RNAseq analysis in roots to assay expression of all CAD genes in normal vs. drought conditions. We also worked on characterization and phylogenetic analysis of CAD and COMT families.	The <i>in silico</i> analysis of the CAD gene family was completed in year 1. Years 2 and 3 focused on the CAD gene family and experimental validation of markers for CAD and COMT gene expression in response to drought.	We are finalizing a paper for the CAD gene family in wheat to be submitted in early 2024. We are also working with a review article regarding stress lignin with Dr. Laura Bartley's group in the IBC at WSU. All data will be shared with growers in field day abstracts, articles, and podcasts.
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. In year 2, the lines were grown out, but there were issues with sterility in some of the lines either from other background mutations or from CAD/COMT. Therefore, we attempted to analyze individual mutant lines for stress tolerance and root growth/architectural traits in year 3. As an alternative approach, we used pangenome comparisons to identify a CAD deletion in hexaploid wheat in the Landmark variety.	In year 1, mutations in CAD and COMT genes were identified and planted in the greenhouse. In year 2, we found much sterility and failed crossing to generate double mutants. In year 3, we will focus on individual CAD mutant in the Landmark hexaploid wheat line.	Growing Kronos and crossing the mutant lines was attempted in years 1-3, but we had poor germination and sterility in these lines, which make confirmation of the mutants challenging. We took an alternative approach and identified a CAD mutant in Landmark which is being analyzed. Our data will be reported in a WheatLife article and prepared for a peer-reviewed publication. In addition, all data will be shared with growers in field day abstracts, articles, and podcasts.