

**Washington Grain Commission  
Wheat and Barley Research Annual Progress Report**

**Project #:3144**

**Progress Report Year:**       3   of   3  

**Title:**                     ***Breeding Wheat Varieties With Efficient Control of ROS Production***

**Cooperators:**             *Dr. Michael Pumphrey, Department of Crop and Soil Sciences.*

**Executive summary:**     *This project aims to advance toolbox for breeding drought and heat tolerant wheat varieties. Our approach is based on the fact that harsh environmental conditions, including heat and drought, increase production of free radicals also known as Reactive Oxygen Species (ROS). ROS diminish the yield by damaging cells inside plant body. Plants alleviate the ROS damages using so-called "scavenging" mechanisms. Varieties with higher scavenging activity would yield better in hot and dry climates. We want to identify genetic markers with more efficient ROS scavenging and introduce these markers into breeding programs. Previously, our laboratory developed a technique for measuring ROS scavenging under the greenhouse condition.*

***In Year 1** (growth season 2019) the suitability of our technique for analysis of material in the field was tested using 14 spring wheat varieties in Lind, Moses Lake, and Spillman farms. We found that our technique detected variability of the ROS scavenging in the field-grown material. One of the tested varieties, Kelse, was amongst varieties with more efficient ROS scavenging.*

***In Year 2** we collaborated with Dr Pumphrey to phenotype ROS scavenging in 180 RILs of the biparental population Kelse x Scarlet in Lind. This RIL population has been genotyped. It means we can use this material to identify genetic markers of efficient ROS scavenging. The material was collected on May 29 when the maximum day temperature reached 88°F. Despite delays caused by COVID-19 pandemics, we completed the measurements. The results demonstrate significant variability of ROS scavenging activity in the population.*

***In Year 3** in collaboration with Dr. Pumphrey we repeated phenotyping of 180 RILs of the biparental population Kelse x Scarlet in Lind and in Othello. Two replicates were planted at each location. The leaf material in Lind was collected on July 1, 2021 when the air temperature was 93°F. Due to the lack of precipitation in April and May of 2021 the soil was very dry and all plants were very stressed: leaves were curling and plants were*

yellowing. The Othello site was affected to the dust storm that bowed away soil with the fertilized. As a consequence, the amount of fertilizer was not even across the field and plants were of different size and developmental stage. This variability in material was not suitable for measuring peroxisomes. In this way we have lost two replicates out of four. Two replicates measured in Lind were not sufficient to get statistically significant data. Another activity in Year 3 was measured activity of ROS scavenging enzymes catalase, ascorbate peroxidase and guaiacol peroxidase in Kelse and Scarlet in response to heat and drought stress in the greenhouse conditions. This experiment demonstrated that of three enzymes, only activity of guaiacol peroxidase increased in Kelse under stress conditions, whereas activity of other enzymes was not affected. No differences in the enzyme activity were detected in Scarlet. This outcome is consistent with the hypothesis that ROS scavenging system is more activity in Kelse, but the fact that activity of only one enzyme was increased suggests these three peroxidases is not the key ROS scavenging mechanisms in these varieties.

#### **Impact:**

1. We optimized phenotyping ROS scavenging in field trials by developing optimal procedures for sample collection and transportation.
2. We used this technique to produce phenotyping data on ROS scavenging in the Kelse x Scarlet population under heat and drought stress. This information will be used to identify genetic marker of efficient ROS scavenging. In the long-term this will contribute to breeding drought and heat-tolerant wheat varieties.
3. The project provided training to undergraduate student Jessica Fisher, graduate student Kathleen Hickey, and post-doctoral scientist Taras Nazarov.
4. The outcomes of this project were used as preliminary data for two successful grant proposals. First, USDA Research and Extension Experience for Undergraduates aims at training students in mechanisms of stress resiliency. Second, Foundation for Food and Agriculture Research (FFAR) proposal that aims at identification of genetic markers of more active ROS scavenging system. Two labs in WSU, Pumphrey and Smertenko, one lab in CIMMYT (Matthew Reynolds) and one lab in Flinders University, Australia (Kathleen Soole) are collaborating on this project. This grant will allow significantly expand the scope and accelerate our research by giving us access to more germplasm and resources. In particular we have funding for one post-doctoral scientist to work on this project as well as funding for reagents, consumables and cost of the field work.

#### **Outputs and Outcomes:**

WGC project number: 3144

WGC project title: **Breeding Wheat Varieties With Efficient Control of ROS Production**

Project PI(s): Smertenko

Project initiation date: June 10, 2019

Project year (X of 3-yr): 3 of 3

Objective	Deliverable	Progress	Timeline	Communication
Objective#1. Determine correlation between peroxisome abundance and ROS homeostasis under drought and heat stress.	Measure peroxisome abundance in spring wheat varieties grown under different climate conditions.	Measured peroxisome abundance in 7 soft white and 7 hard spring wheat varieties Diva, Louise, Melba, Ryan, Whit, Seahawk, Tekoa, LCS Luna, SY Selway, Alum, Chet, Dayn, Glee and Kelse grown at Spillman Farm, Moses Lake, and Lind.	Completed	Was communicated at the annual 2020 Review Meeting
Objective#2. Identify spring wheat genotypes with low ROS production.	Screen genetically diverse population for genotypes with more active ROS scavenging	Peroxisome abundance was used as the proxy of ROS scavenging. Measured peroxisome abundance in 180 RIL lines of mapping population Kelse x Scarlet growth in Lind. We collected leaf material on May 29 2020 when the temperature was between 80 and 88°F. It means plants experienced both heat and drought stress. In 2021 we collected material in Lind on June 1 when the temperature was between 92°F and 94°F. We could not collect material in the second location, Othello, because fertilized was blown away with the top layer of soil by strong wind. Two replicated in Lind were not sufficient to generate statistically reliable data.	Completed	Will be communicated at the annual 2022 Review Meeting
	Expression of peroxisome biogenesis genes	We measured transcription level of two peroxisome biogenesis proteins: catalase and PEROXIN11C. We found that transcription of both genes was higher in Scarlet than in Kelse.	Completed	Will be communicated at the annual 2022 Review Meeting
	Measure ROS homeostasis	We measured activity of three ROS scavenging enzymes catalase, ascorbate peroxidase, and guaiacol peroxidase. We found that only activity of guaiacol peroxidase was increased.	Completed	Will be communicated at the annual 2022 Review Meeting
Measurable impact in 1 year	Identify genotypes with low ROS production under drought and heat and start screening breeding populations for low ROS production trait.	Kelse was found to have lower ROS production in field and greenhouse studies.		
Measurable impact in 2 years	Identification of breeding lines with low ROS production under heat and drought.	Kelse x Scarlet mapping population was phenotyped in Lind and generated suitable data. Published one article in Wheat Life. Recorded two Wheat Life podcasts.		

Measurable impact in 3 years	Field trials of lines with low ROS production under heat and drought and laboratory eperiments	Activity of one ROS scavenging enzymes, guaiacol peroxidase was higher in Kelse than in scarlet. Transcription of peroxisome biogenesis genes under head and drought stress is higher in Scarlet than in Kelse. Two grants were funded: USDA and FFAR. Published one article in Wheat Life.		

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