

**Washington Grain Commission
Wheat and Barley Research Annual Progress Reports and Final Reports**

PROJECT #: 30109-3157

Progress report year: 3 of 3 Final

Title: Evaluation and Selection for Cold Tolerance in Wheat

Cooperators: K. Garland Campbell, K. Sanguinet, A.H. Carter

Executive summary:

In 2021, we rated over 600 wheat breeding lines and cultivars from public breeding and variety testing programs for survival. In 2020, and despite the slowdown due to the pandemic, we rated 924 breeding lines and cultivars from public winter wheat breeding and variety testing programs for survival. This compared well with 2019 when we tested 936 lines. Breeders used this information for selection of new experimental lines. We have evaluated the Washington Extension Winter Wheat Trials every year since 2001. The survival results for the top varieties grown according to the Washington Grain Commission Variety Survey are below (page 2). We are still analyzing data for the 2021 crop year. We observed association between our testing results and the winter injury that occurred in WA in April of 2020 and in survival notes from the Cereal Variety trials that have been collected between 2018-2020. Therefore, we are confident that our evaluation of freezing tolerance using programmable freeze chambers at the WSU Plant Growth Facility represents real world winter survival due to cold injury.

We conducted a genome-wide association study of wheat from the WSU winter wheat and ARS winter wheat programs and a doubled haploid population of Cara/Xerpha. We also did a QTL analysis in a population involving the very cold tolerant Canadian winter wheat Norstar, in collaboration with Dr. Debbie Laudencia of USDA-ARS in Albany CA, Dr. Brian Fowler (retired) of Univ. of Saskatchewan. We previously identified allelic variation at the genes for vernalization response (VRN) and for the genes controlling general cold tolerance response (CBF) genes on the group 5 chromosomes. These genes impact vernalization requirement and cold tolerance. We have now identified QTL for cold tolerance on the multiple chromosomes including 1A, 1B, 1D, 2A, 2B, 2D, 3B, 3D, 4A, 4B, 5A, 5B, 6B, 7A, and 7B. Some of these genes are major and we can use marker assisted selection to fix them in our breeding lines. Genomic selection models will be most useful to improve freezing tolerance in PNW wheat. We also know that resistance to soil borne diseases like eyespot, fusarium crown rot and snow mold is critical for winter survival in wheat.

Even though we have learned a lot about control of cold tolerance from the QTL associated with differences in the DNA code of specific genes controlling growth and flowering, we know that flowering and cold tolerance are influenced by epigenetic responses. Epigenetics refers to changes in gene expression that are caused by exposure to different environments rather than changes due to differences in the DNA code. Previously, Dr. Dan Skinner started with a single seed of the winter wheat ‘Norstar’, grew it using exposure to cold to induce flowering and harvested the seed from that plant. He then exposed several of the seeds to cold as usual for winter wheat and harvested those seeds. He called these “cold-induced flowering (CIF-Norstar)”. He also maintained some of the seeds at room temperature (no cold exposure) but he did expose

then to longer daylengths (or longer photoperiod). Wheat is daylength sensitive and flowers in response to lengthening days. Initially, few tillers flowered on the plants that were not exposed to cold. After three generations, he was able to harvest a normal amount of grain from each plant. This version of Norstar was named photoperiod induced flowering (PIF-Norstar). We hypothesized that these differences in the control of flowering were due to different gene expression, rather than to changes in the DNA code itself. We want to know which genes are affected by these differences in epigenetic expression. Our rationale is that, even if we incorporate all the DNA allelic variation (differences in DNA code) that is associated with cold tolerance in wheat; we may still alter the cold tolerance of a cultivar based on how we treat the seed during the breeding process.

We used a technique called RNA-seq to analyze gene expression data for epigenetic effects of exposure to cold in the CIF and PIF Norstar lines. We exposed 6-week-old plants of the two different Norstar lines to freezing temperature from 0-24 hours. Then we extracted RNA from each type of Norstar at each time period and analyzed it for differences in gene expression between the CIF-Norstar and PIF-Norstar lines. A large percentage of genes were expressed differently between the two Norstar lines at all time points (Fig. 1). In addition, these genes were both up-regulated and down-regulated in the CIF-Norstar vs. the PIF Norstar (Fig. 2).

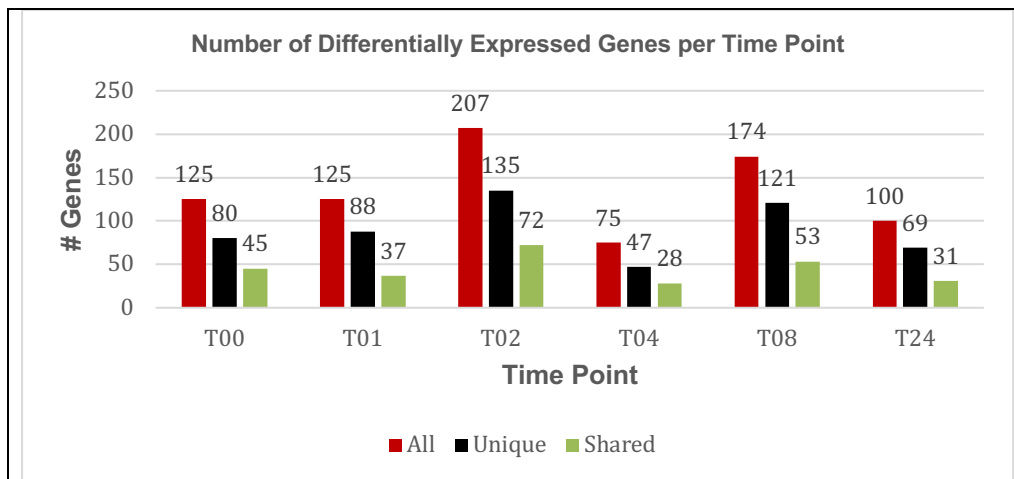
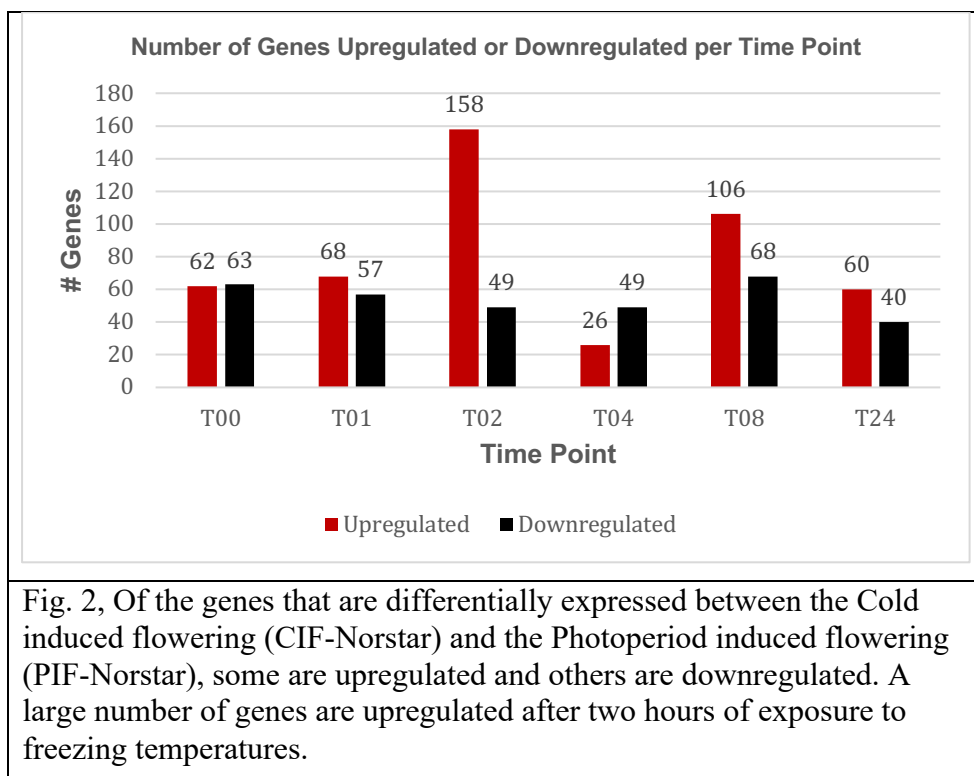


Fig. 1. The number of differentially expressed genes at each time (0-24 hours) for Cold induced flowing (CIF-Norstar) vs. Photoperiod induced flowering (PIF-Norstar). The green bars are genes that were expressed in both CIF and PIF Norstar. The black bars (unique) are the genes that were different between the two Norstar lines.



Thirty of these genes, located on different chromosomes are of particular interest. They are (Table 1). Several genes interact with genes known to be involved in cold tolerance, copper metabolism in plants, and membrane structure. We are investigating these results to better understand the genetic and epigenetic mechanisms that control cold tolerance in winter wheat.

Impact

- The data from these cold tolerance trials was published in the seed buyers guide (Table 2) so that farmers can select winter wheat varieties that are less sensitive to winter kill.
- Our results from screening the regional nurseries, and screening breeding lines has been used by winter wheat breeders to select for resistance to winter injury.
- Varieties released from the WSU winter wheat breeding program have consistently excellent cold tolerance and this tolerance has been maintained because of testing using the procedures developed by this project.
- Because of the high correlation between our artificial screening trial and winter survival in the field, we can incorporate better cold tolerance into our early generation breeding lines.
- We have identified molecular markers that are being used by breeders to select for winter survival.

Table 1. Location and proposed function of important differentially expressed genes between cold induced and photoperiod induced flowering Norstar lines during a 0-24 hour freeze treatment.	
Location (EnsemblPlants)	Proposed_Function (InterPro)
Chromosome 1A: 16,930,826-16,935,085	Ankyrin repeat; Zinc finger
Chromosome 1A: 57,680,271-57,682,134	Protein BIG GRAIN 1-like
Chromosome 1A: 252,499,278-252,521,296	WD40-repeat-domain
Chromosome 1A: 252,499,278-252,521,296	WD40-repeat-domain
Chromosome 1A: 567,316,488-567,329,062	GBF-interacting protein 1, N-terminal; UBA-like superfamily
Chromosome 2A: 171,835,609-171,840,193	Autonomous transposable element EN-1 mosaic protein (EN-1/SPM)
Chromosome 2B: 255,050,138-255,056,090	EF-domain; Ser/Thr kinase
Chromosome 2B: 386,305,098-386,312,126	Calcium permeable stress-gated cation channel 1, N-terminal transmembrane domain; 10TM putative phosphate transporter, cytosolic domain
Chromosome 2D: 107,399,802-107,407,399	Glycosyltransferase 2-like; Sugar transferase
Chromosome 2D: 507,747,730-507,757,643	Heavy metal-associated domain (HMA); Superoxide dismutases (SODs) copper chaperone
Chromosome 2D: 553,385,507-553,388,909	Major Facilitator Superfamily (MFS); Proton-dependent oligopeptide transporter (POT) family
Chromosome 3D: 398,401,967-398,407,146	Glycosyl transferase; HAD(-like) superfamily
Chromosome 4A: 143,433,126-143,436,996	CALMODULIN-BINDING PROTEIN60
Chromosome 4B: 38,783,518-38,792,831	CHASE-domain; HATPase; His-Kinase domain
Chromosome 4B: 481,492,246-481,493,603	FAS1 domain superfamily
Chromosome 4D: 93,841,069-93,841,966	Late embryogenesis abundant protein, LEA_2 subgroup
Chromosome 5A: 356,474,101-356,507,274	Armadillo-type fold; E3 ubiquitin ligase; WD40-repeat-containing domain; Zinc finger
Chromosome 5A: 361,446,165-361,448,993	CHCH; possible Cu interacting
Chromosome 5B: 402,733,811-402,734,204	ZF-HD homeobox protein, Cys/His-rich dimerization domain

Chromosome 5B: 703,291,249-703,292,516	Blue (type 1) copper protein; Cupredoxin; Phytocyanin
Chromosome 5D: 549,854,716-549,859,435	RPM1-interacting protein 4/NOI4
Chromosome 6A: 80,695,452-80,708,832	Squalene cyclase; Terpene synthase
Chromosome 6D: 152,255,321-152,260,592	Sel1-like repeat; Tetratricopeptide-like helical domain superfamily
Chromosome 6D: 332,807,360-332,814,746	RNA-modification via methylation, pseudouridylation, and thiouridylation
Chromosome 6D: 338,399,729-338,413,289	DWNN domain (apoptosis); E3 ubiquitin-protein ligase RBBP6 family; Zinc finger, RING/FYVE/PHD-type
Chromosome 7A: 122,175,829-122,187,343	GBF-interacting protein 1, N-terminal; UBA-like superfamily
Chromosome 7B: 169,462,792-169,467,243	CBF1-interacting co-repressor CIR, N-terminal domain; Pre-mRNA splicing factor
Chromosome 7B: 504,808,145-504,813,117	GDP-fucose protein O-fucosyltransferase
Chromosome 7B: 632,793,180-632,796,098	Pentatricopeptide repeat (PPR), post-transcriptional processes; Tetratricopeptide-like helical domain superfamily
Chromosome 6A: 573,490,463-573,495,427	Homeobox domain; Myb domain; SANT/Myb domain

**Table 2. Freeze Survival Of Top Acreage Winter Varieties in
WSU Cereal Variety Testing program, 2016-2020.**

Ranking Based On 2020 Variety Acreage Survey

	Percent Survival	95% LCL	95% UCL			Percent Survival	95% LCL	95% UCL
Soft White Wheat					Club Wheat			
UI Magic CL+	27%	9%	44%		ARS Crescent	65%	47%	82%
Curiosity CL+	80%	63%	98%		Bruehl	54%	37%	72%
Norwest Duet	54%	37%	72%		Pritchett	56%	38%	73%
Otto	58%	43%	73%					
Norwest Tandem	52%	32%	72%		Hard Winter Wheat			
M-press	33%	13%	53%		LCS jet	40%	23%	58%
Mela CL+	67%	49%	84%		Keldin	64%	47%	82%
Eltan	73%	62%	84%		SY Clearstone CL2	70%	52%	87%
Resilience CL+	44%	26%	62%		LCS Rocket	14%	0%	31%
Puma	50%	32%	67%		WB 4303	75%	55%	95%
SY Dayton	26%	9%	44%					
PNW Hailey	65%	41%	90%		Checks			
LCS Art deco	26%	8%	43%		Norstar	86%	75%	97%
Jasper	32%	14%	49%		Stephens	39%	28%	50%
LCS Hulk	68%	51%	86%					
Sy Assure	28%	8%	48%					
LCS Drive	23%	3%	43%					
WB 1604	42%	24%	60%					
LCS Sonic	51%	33%	68%					
Bobtail	28%	8%	49%					
SY Raptor	4%	0%	29%					
SY Ovation	17%	0%	37%					
Rosalyn	23%	5%	41%					
UI Castle CL+	30%	12%	48%					
Madsen	29%	9%	49%					

Freeze Tests were conducted under controlled conditions in The WSU Plant Growth Facility between 2016 And 2020.

These were severe survival trials. Field survival is likely to be greater, but these results have correlated with field survival over multiple years.

The precision of Confidence Intervals varies because some lines have been tested more than others.

WGC project number: 3019-3157
WGC project title: Evaluation And Selection for Cold Tolerance in Wheat
Project PI(s): Kimberly Garland-Campbell, Karen Sanguinet and Arron Carter.
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Objective	Deliverable	Progress	Timeline	Communication
1. Evaluate Washington winter wheat variety trials and the hard spring wheat trials.	Ratings for freezing tolerance for commonly grown and new winter wheat cultivars and hard spring cultivars and breeding lines.	The Washington Cereal Variety Trial Winter wheat nurseries were evaluated for cold tolerance in artificial screening trials. The Washington Hard Red Spring Wheat Trials were evaluated for cold tolerance.	June 2019 - June 2022.	<p>Presentation at grower meetings, Wheat commission meetings, Wheat Life and Research Review. Published on WSU small grains Web-site.</p> <p>This information is incorporated into the WSU small grains variety selection tool. https://varietyselection.cahnrs.wsu.edu/</p>
2. Evaluate cold tolerance of new breeding lines in US regional nurseries in order to identify germplasm to use in crossing for better winter survival.	Ratings for freezing tolerance for advanced wheat germplasm from the US that can be used as new sources of cold tolerance for the PNW.	The Western Regional Winter wheat nurseries was evaluated for freezing tolerance in artificial screening trials.	June 2019 - June 2022.	<p>Email results to regional nursery cooperators and publish on regional nursery web sites.</p> <p>Yates, Scott. "Wheat farmers never stop and neither does their wheat" Wheatlife. 2020. 63.11:43-45.</p>
3. Evaluate cold tolerance of advanced breeding lines contributed by regional winter wheat breeding programs, including the WSU and USDA-ARS wheat breeding programs.	Ratings for breeding lines contributed by regional wheat breeders that will facilitate their selection decisions.	We evaluated freezing tolerance for breeding lines from the WSU Winter wheat and the USDA club wheat breeding programs and reported the data to the breeders. These data were used for selection.	June 2019 - June 2022.	Several cultivars released with excellent cold tolerance, including Devote, Piranha, Castella and Pritchett.

4. Evaluate cold tolerance of F3-F5 (early generation) wheat populations that are segregating for cold tolerance and select resistant progeny.	Selections made for cold tolerance in early populations.	This was not accomplished due to less testing during the pandemic and equipment failures, but we did release Castella club wheat in 2019, which was selected using this strategy.	June 2019 - June 2022.	Presentation at Research Review. Peer reviewed publications. Direct communication with wheat breeders.
5. Identify genes controlling cold hardiness in winter wheat.	New molecular markers and genomic selection indices for cold tolerance in PNW winter wheat and Hard red spring wheat.	We analyzed several mapping populations and identified or confirmed consistent QTLs for markers on multiple chromosomes that are being used for marker assisted selection for cold tolerance. Our epigenetic work identified several genes that differ in expression between Norstar that is induced to flower based on cold, or based on photoperiod. We are following up on 30 of these genes.	June 2019 - June 2022.	Presentation at Research Review. Peer reviewed publications. Direct communication with wheat breeders.