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Assessment and Development of Cold Temperature Germination Tolerance in Oat and Hard Red Spring Wheat

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ASSESSMENT AND DEVELOPMENT OF COLD TEMPERATURE GERMINATION TOLERANCE IN OAT AND HARD RED SPRING WHEAT

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Plant Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

AMMI	Additive Main Effects and Multiplicative Interaction Models
ANOVA	Analysis of Variance
AOSA	Association of Official Seed Analysts
CTGT	Cold Temperature Germination Tolerance
GDD	Growing Degree Days
HRSW	Hard Red Spring Wheat
PC	Principle Component
SD	South Dakota
SDSU	South Dakota State University

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ABSTRACT

ASSESSMENT AND DEVELOPMENT OF COLD TEMPERATURE GERMINATION TOLERANCE IN OAT AND HARD RED SPRING WHEAT

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Oat (*Avena Sativa*) and hard red spring wheat (HRSW; *Triticum aestivum* L.) are both spring planted cereal crops that yield best when planted early in the spring. These crops would benefit from improvements in cold temperature germination tolerance (CTGT) by allowing them faster and more uniform germination and emergence early in the spring. This study aimed to (i) evaluate germination in oat genotypes over a range of sub-optimal temperatures and (ii) establish a methodology for selection of hard red spring wheat for CTGT. Seed samples of ten different oat genotypes were evaluated for CTGT in a germination chamber at the temperatures 5, 7, 9, 11, 13, and 15°C. Temperature was held constant for two weeks for all temperatures, and three and four weeks for the lowest temperatures five and seven degrees Celsius. Results show that time and temperature, as well as their interactions affect germination significantly at reduced temperatures. Genotypes Horsepower and Colt germinated the best across the range of temperatures used. HRSW genotypes were planted on towels in a germination chamber, and the first and last seeds to germinate were transplanted into greenhouse pots. Four genotypes were used: Forefront, SD4011/Barlow, SD4330, and SD4189/SD3997. The first seeds to germinate were successfully transplanted into the greenhouse without difficulty, so breeders should be able to use this methodology to perform selections on HRSW to improve CTGT.

INTRODUCTION

Oats and hard red spring wheat (HRSW; *Triticum aestivum* L.) are both spring-planted, cool-season cereal grains. The US produced nearly 65 million bushels of oats and 532 million bushels of spring wheat in 2016. The states of South Dakota, North Dakota, and Minnesota produced 24 million bushels of oats and 390 million bushels of spring wheat during that same time (USDA NASS, 2017). As cool-season crops, both require early spring planting to maximize yield. Ideal planting dates for each crop range from late March to late April, depending on latitude within SD (Karki, 2015 and Mueller, 2013). Ideal planting time for each crop is based on soil temperature. Cool soils may slow germination and emergence.

Seed germination and seedling establishment are considered the most sensitive stages of development for plants. Germination involves reactivation of substrates stored in seeds, which become new components for embryo development and establishment of a new plant. Germination is activated by conditions including temperature, soil aeration, available nutrients, available water, and allelopathy caused by outside toxins. In general, seeds need temperature, water, and oxygen to germinate. Each crop species has a range of temperatures at which germination can occur, including base, maximum, and optimum temperatures for germination (Hadas, 2004). Germination temperatures may also vary among varieties of a species. A variety capable of germinating and emerging at a lower temperature would offer a competitive advantage for farmers.

This study was designed to evaluate cold temperature germination tolerance (CTGT) in oats to help growers select varieties to plant in cool soils, and to assist breeders in making selections for CTGT and evaluating this trait in future studies.

Another goal of this study was to perform selections for CTGT in HRSW, to determine the heritability of this trait in future research (See Appendix).

LITERATURE REVIEW

Oat and hard red spring wheat are spring seeded cereal crops, each with their own benefits. As a food, oats are mainly used in breakfast cereals, cereal bars, bread, beverages and infant foods. Oats are a source of fiber, including β-glucan, a soluble fiber associated with decreased risks of cardio-vascular diseases. They are free of gluten, making them a good choice for those suffering from celiac disease (Gangopadhyay et al., 2015). Recently, oats have also gained some importance as a cover crop for nitrogen absorption and dry matter production capabilities (Clark, 2012). South Dakota was the highest oat producing state in the Unites States in 2016, producing over 9 million bushels of oats (USDA NASS, 2017). HRSW accounts for approximately 20 percent of all US wheat production and is grown mainly in the Northern Plains (South Dakota, North Dakota, Minnesota, Montana). It has high protein levels and is used for specialty types of bread and for mixing with lower protein wheat (USDA ERS, 2016).

Many studies have examined the importance of seeding oats early in the spring. Gooding and Lafever (1991) conducted an experiment in Ohio in 1986 and 1987 to determine the effects of planting date on two oat cultivars. The cultivars were planted on three different dates, approximately 21 days apart, for the two years of the study. Many different traits were recorded for comparison, including heading date, maturity date, thousand kernel weights, seeds per panicle, and total weight of seeds per panicle. The results showed that delayed seeding reduced oat growth duration by 22 days in 1986 and 21 days in 1987. Vegetative growth was reduced by 17 days in both years. Overall, a difference of 1 bushel per day of yield reduction on average was found comparing planting in early April to mid-May (Gooding and Lafever, 1991).

While it is recognized that early planting is beneficial, low soil temperature early in the spring might inhibit seed germination. Current SDSU planting recommendations are to seed oats as early as possible in spring for maximum yield, with a minimum germination temperature of around 35°F (1.6°C) (Karki, 2015). However, Michigan State University suggests a minimum germination temperature of 43°F (6.1°C) for oats (Isleib, 2012). University of Nebraska Lincoln also suggests oat minimum germination temperature of 43°F (Meyer and Dutcher, 1998). SDSU recommendations are to plant spring wheat during the first three weeks of April, or earlier if soil temperatures are between 34°F and 36°F (Clay et al., 2012). As with oats, it is recommended to plant spring wheat as early as possible, as late planting can reduce yields. Studies of global crop planting dates indicated that spring wheat is usually planted when air temperatures are between 6°C and 14°C (42.8°F and 57.2°F). (Sacks et al., 2010)

In Queensland, Australia, oats are grown in late summer and fall for forage to provide a high-quality feed supply over winter. Growers, there, have had trouble with seeding oats too early in summer when soil temperatures are warm, causing poor emergence. Radford and Key (1993) conducted experiments in Australia to determine optimum temperature for germination of oats as well as coleoptile and mesocotyl length of the seedlings. Adding coleoptile and mesocotyl lengths together gives the likely maximum seeding depth at that temperature. These experiments were performed by planting seeds of different genotypes in a mix of sand and peat moss and completely excluding light by covering with plastic. Exclusion of light allows measurement of potential seedling elongation below the soil surface. Temperatures of 10, 15, 20, 25, 30, and 35°C were used for this study. Their results showed that 15°C was the optimum

temperature for seedling elongation. Seedling length was similar between 10 and 20°C, but shortened between 20 and 30°C. Germination percentage was adequate (over 83 percent) at temperatures between 10 and 25°C, but reduced significantly at 30°C. No germination occurred at 35°C (Radford and Key, 1993). This indicates that oats do germinate and emerge better in cool soils. However, their experiments did not examine temperatures below 10°C, which are often observed in South Dakota soils in early spring.

A different set of experiments conducted in 2005 focused on Canadian oat production and rapid germination to compete with wild oat. Seeds of six Canadian oat genotypes were germinated at 5°C to determine the effects of seed size, genotype, and osmotic potential on germination time and final germination percentage. The fastest and slowest varieties had 1.5 days difference in the amount of time to reach 50% germination. Final germination percentage also varied by genotype, but only by 4% between the highest and lowest germination recorded (Willenborg et al., 2005).

Butler et al (2012) studied differences in germination of cool season grasses, including two winter oat varieties, from 5°C to 35°C. Their results showed that 20°C was the optimum temperature for germination of cool-season grass species. Germination was delayed from 5°C to 15°C and decreased at temperatures above 25°C (Butler et al., 2017). Experiments in Iowa in 1981 and 1982 focused on planting date and growing degree day (GDD) relationships among varieties to determine if GDD is a better predictor compared to days for oat maturity stages. Results showed that seeding date affected the amount of days to reach a given stage more than GDD's. However, the earliest seeding date of March 18 in 1981 needed more GDD's to reach a given maturity stage than later seeding dates. There were also differences in GDD's required for emergence among

varieties (Baltenberger and Frey, 1987). These experiments indicate that CTGT differences may exist among varieties of oats.

Soil temperature data from SDSU for 2017 indicate that average soil temperature for bare soil at 4 inches deep did not remain near or above 41°F (5°C) until March 27 of 2017 at the Baltic station. Temperature at the same depth first reached 50°F (10°C) on April 8, 2017 (South Dakota Agricultural Experiment Station, 2018). So, in 2017, an oat variety capable of complete emergence and establishment at 5°C would have gained approximately a 12 day, or nearly 2 weeks, advantage over a cultivar that did not fully establish until soil temperatures remained at or above 10°C.

To evaluate CTGT in oats and spring wheat, germination tests must be conducted either in field plots or in a laboratory germination chamber. Research conducted in Great Britain in 1986 tested two lots of spring wheat seed for field emergence and laboratory germination. The results showed that field emergence was less than laboratory germination, but that laboratory germination was found to be an effective indicator of field emergence potential because the reduction in germination with temperature was found to be similar between field and lab (Khah et al. 1986).

Seefeldt et al (2002) experimented with six different cultivars of spring wheat commonly grown in the US Pacific Northwest, trying to find base temperatures for germination and compare germination rates between cultivars and temperatures. No significant differences in base temperatures for germination were found between varieties, with base germination temperature around 2.6°C. Germination time was much slower from 6.4°C to 15.7°C for all varieties. Germination time only varied significantly among varieties at 15.7°C, 20.2°C, and 25.2°C (Seefeldt et al. 2002). Previous graduate

students at SDSU have tested CTGT in HRSW. Jenkins (2014) found increased differences in germination percentages as temperatures decreased from 11 to 5°C. Broad-sense heritability was estimated at 0.59 for temperatures 13-20 °C and 0.73 for temperatures 5-11 °C with respect to germination percentage. Genotype SD4330 performed the best considering all factors in this study, including low temperature germination, stability across temperatures, and vigor score (Jenkins, 2014).

MATERIALS AND METHODS

Ten different oat genotypes were tested for cold temperature germination tolerance (CTGT) at the SDSU Seed Testing Laboratory. Seed for germination testing was grown in Crop Performance Testing plots at Winner in 2016. Seed was harvested in July of 2016, weighed for yield and test weight, and stored for future use. Genotypes used for germination testing include: Hayden, Horsepower, SD110466, Colt, Stallion, Deon, Natty, SD120665, Shelby 427, and Goliath. Four replications of each genotype from field plots were subsampled and combined to achieve 1 kilogram of each genotype for germination testing.

The 1 kilogram of seed for each genotype was obtained to ensure that enough was available to perform 3 replications of 100 seeds of each genotype for six temperatures, twice. The six temperatures used were 5°C, 7°C, 9°C, 11°C, 13°C, and 15°C. Roughly 25 grams of each genotype was divided out for each temperature using a Dean Gamet Precision Divider at the SDSU Seed Testing Lab. For the first 3 replications, seed was divided and then cleaned with an E.L. Erickson Products column style blower to remove empty hulls and light chaff. Seeds were then final cleaned with forceps to remove broken seed and remaining chaff.

For the second set of 3 replications, seed were cleaned prior to dividing to facilitate faster cleaning and remove smaller chaff pieces that hindered germination testing in the first 3 replications. Seed was first cleaned using a Bill's Welding Blower set to remove approximately 5 percent of seed. This easily removed all empty hulls and small pieces of chaff. Seed was then divided and cleaned with forceps in the same manner as the first set of 3 replications. For the second set, all dehulled seeds were

removed, because they germinated faster at the coldest temperatures than the seeds with hulls still intact. The shoot also became visible faster with no hull present, complicating certain germination counts.

Germination tests were conducted at the SDSU Seed Testing Lab in accordance with Association of Official Seed Analysts Rules for Testing Seeds (AOSA, 2017). One hundred randomly selected seeds were placed evenly on two sheets of germination paper soaked with deionized water. A third sheet was then placed on top of the first two and the three sheets were rolled together to one fourth of their initial width. Three sets of 100 seeds in germination paper were banded together for each genotype and placed in a bucket with a plastic bag to hold in moisture. The bags were loosely tied shut and buckets placed in a Percival pre-chilled incubator set to the respective temperature. The incubator has two thermometers to verify temperature setting and a beaker of water was used to check temperatures with a digital thermometer.

For the temperatures 15°C, 13°C, 11°C, and 9°C, seeds were left in the incubator for two weeks before counting germination. For 7°C, germination was counted after two weeks and again at 3 weeks for the second reps, so that all seeds were fully germinated with first true leaves present. For 5°C, germination was counted after two and three weeks for the first replications and after 2, 3, and 4 weeks for the second replications. For two week counts at 5°C, all seeds with visible shoots and roots were counted as germinated. Shoots were just starting to appear at this time, so this was done to find which varieties germinated fastest at the coldest temperature.

After 3 and 4 weeks at 5°C, shoots of seedlings had an appreciable difference in length by visual observation. These differences were not recorded for the first two sets of

three replications in germination testing. A third set was planted at 5°C to measure the lengths of shoots. Shoot lengths were measured after 3 and 4 weeks with a ruler and pictures taken to display for shoot length differences. Germination was also counted to add to the data previously collected.

All data were entered into a comma-separated values file format, to enable analysis in R. Factors recorded include genotype, time, replication, sheet, temperature, abnormal seedlings, non-germinated seeds, germination rate, and length of seedlings for those which were measured. Time was the number of weeks spent in the germination chamber, 2 weeks for most data points. Replication denotes which set of 3 germination sheets were used, this is used as a blocking factor because seed was cleaned differently for the first two sets of germination tests. The amount of time for which seed has been stored may also affect germination, due to dormancy and storage conditions. Sheet was used as a sub-block factor and indicates which sheet in a set of three each data point was. This was done in case amount of light present would affect germination percentage.

Data analysis was all conducted using R statistical software (R Core Team 2016). First, time and temperature variables were combined using the "paste" function to create the new factor environment. This allows for a factor that reflects both the temperature and time spent in the chamber, because germination was counted multiple times for lower temperatures, with some very low counts. Boxplots were then run for various environment and genotype combinations, to see data distribution and which genotypes might be performing better with regards to CTGT. Next, ANOVA (analysis of variance) was run to determine which factors were most significant regarding germination percentage.

After exploratory models were run, linear mixed model analysis was run using the R package minque (Wu, 2014). Linear mixed model with the jackknife resampling technique was used to estimate variance components, genotype effects, and confidence intervals. Additive Main Effects and Multiplicative Interaction Models (AMMI) was also conducted on the data using the R package Agricolae (Mendiburu 2016). Bi-plot graphics were generated using PC (principle component) scores for the data as well as germination percentage to determine which genotypes, if any, were best adapted for germination at a particular environment and which are more stable, so less affected by temperature and time with regards to germination.

RESULTS

Entire Dataset Analysis and Environment Comparison

Figure 1 shows the distribution of germination counts by environmental condition. Germination percentages for 2 weeks at 5 ºC were very inconsistent (Figure 1). The first and third sets of replications had very low counts, less than 20 percent. The second set had some counts as high as sixty to seventy percent germination for varieties such as Colt, Hayden, Horsepower, and Natty (Figure 2). For this reason, data analysis was conducted both with and without the 2 week counts at 5 degrees due to large variation. The environment is denoted as weeks: temperature, indicating the number of weeks spent in the germination chamber and the temperature in degrees Celsius.

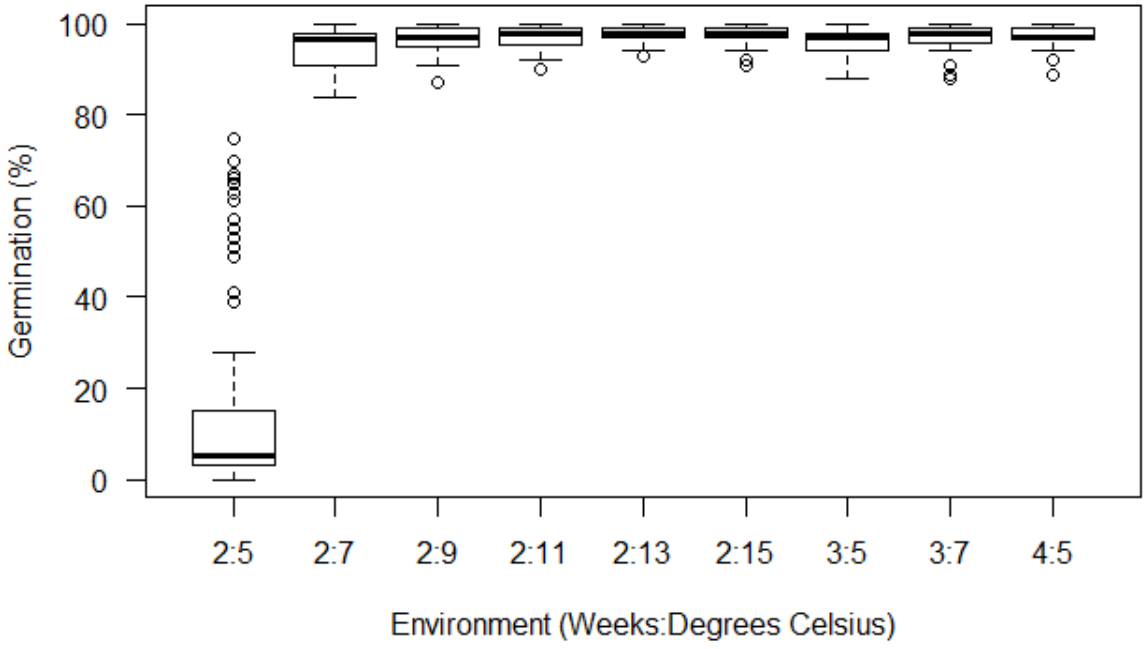

Figure 1: Oat germination by environment for ten different genotypes

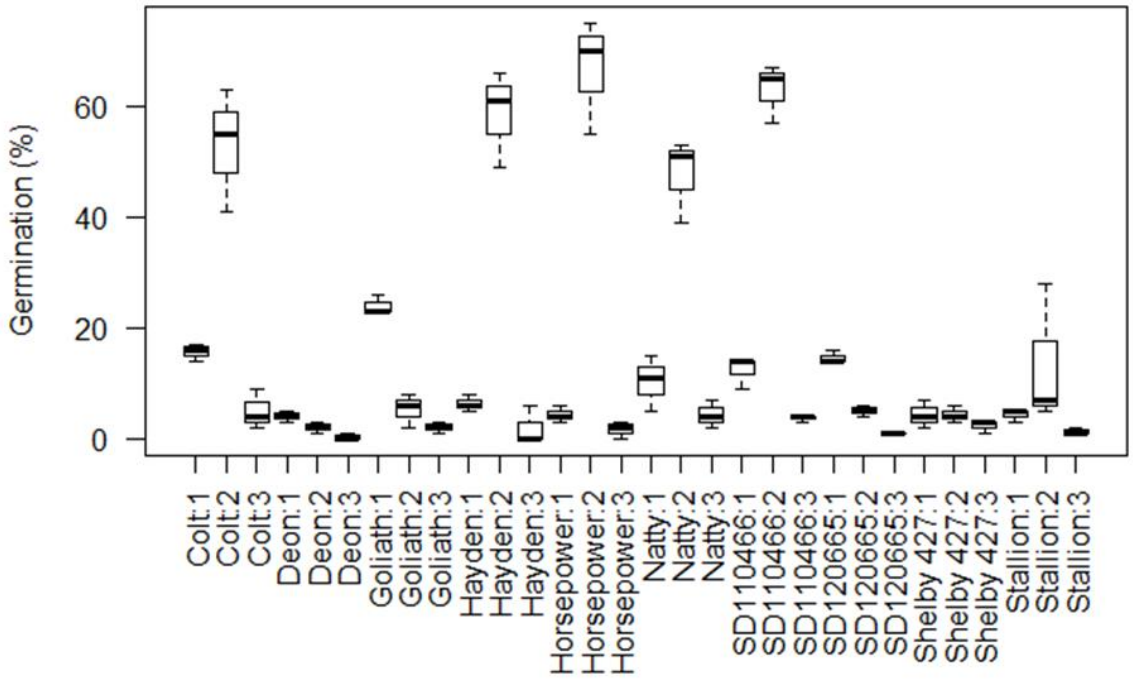

Figure 2: Oat germination by genotype and replication after two weeks at five degrees celsius

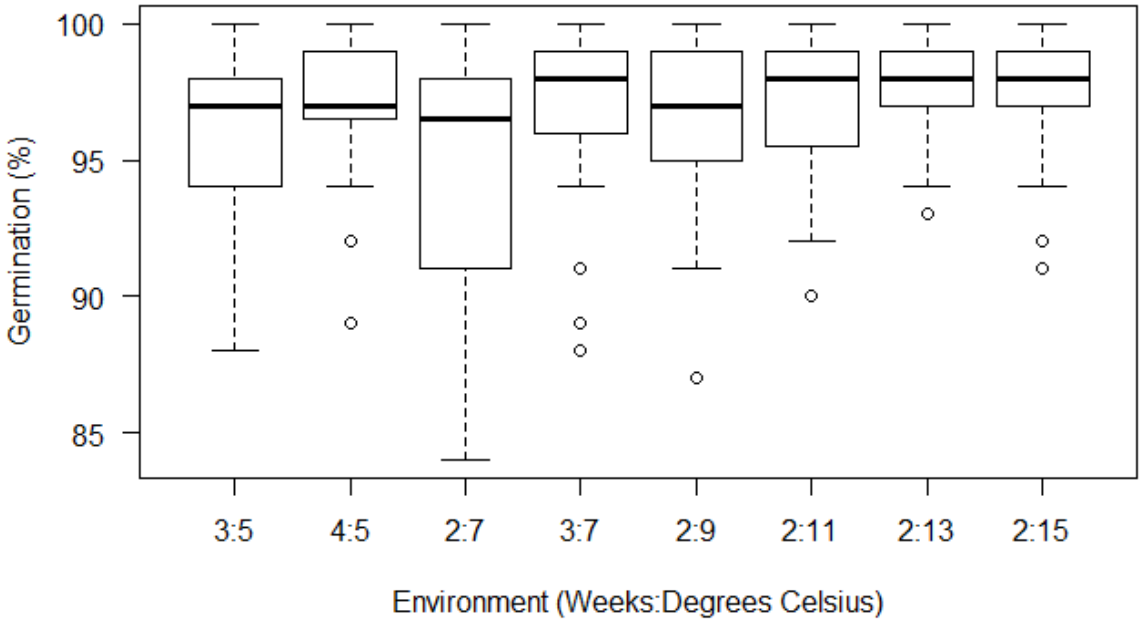

Figure 3: Oat germination by environment excluding two weeks at five degrees Celsius

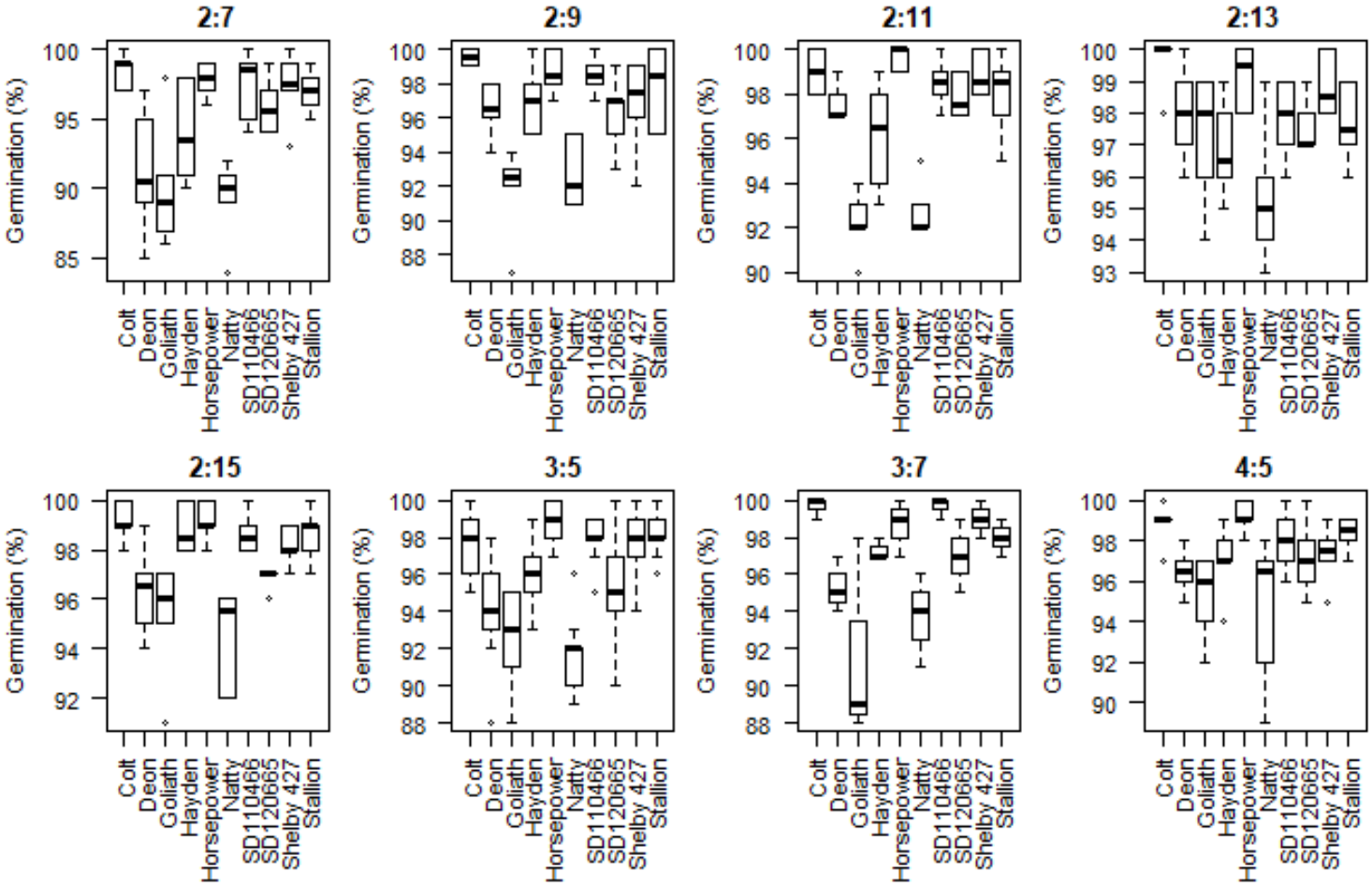

Figure 4: Oat germination by environment (weeks: degrees Celsius) and genotype

With two week counts at five degrees removed it becomes easier to compare data for all other environments (Figure 3). Two weeks at seven degrees Celsius shows the greatest overall distribution of germination percentage. Three weeks at five degrees also shows a wide spread of data. Two weeks at fifteen and thirteen degrees appear to have the highest germination percentages and tightest data clusters, with only some outliers having low germination percentage. Comparing all environments, it appears that Horsepower germinated consistently well across all time and temperature combinations, and Goliath consistently germinated poorly (Figure 4).

The variance component for environment changes drastically when the two week counts at five degrees are taken out, as indicated by the sum of squares and mean squares (Table 1 and Table 2). Either way the effects of genotype, environment, replication, and genotype by environment interaction are significant, so all of these were used in analyzing the data. Environment is the largest variance component when the entire dataset is used for ANOVA. However, without the 2:5 environment, genotype becomes the largest variance component.

Table 1: ANOVA of oat germination percentage for entire dataset

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Significance
Genotype	9	3523	391	6.82	***
Environment	8	509079	63635	1108.24	***
Replication	2	4177	2089	36.37	***
Sheet	2	23	11	0.198	
Genotype:Environment	72	6530	91	1.58	**
Residuals	476	27332	57		

Significance Codes for P Value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 2: ANOVA of oat germination percentage excluding two week counts at five degrees Celsius

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Significance
Genotype	9	2000.4	222.27	69.331	***
Environment	7	402.2	57.45	17.921	***
Replication	2	141.8	70.89	22.112	***
Sheet	2	29.1	14.53	4.531	*
Genotype:Environment	63	423.3	6.72	2.096	***
Residuals	396	1269.6	3.21		

Significance Codes for P Value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 3: Estimated mean and environmental predictors of oat germination percentage for entire dataset

				95% Confidence Interval	
	Estimate	Standard Error	Significance	Lower Limit	Upper Limit
Mean	86.063	0.080	0	85.781	86.345
4:05	11.352	0.209	***	10.614	12.090
3:05	9.901	0.176	***	9.280	10.521
2:13	9.655	0.116	***	9.245	10.065
2:15	9.402	0.090	***	9.085	9.720
2:11	8.864	0.194	***	8.179	9.549
2:09	8.390	0.092	***	8.064	8.716
3:07	7.036	0.180	***	6.402	7.670
2:07	6.679	0.193	***	5.999	7.360
2:05	-71.280	0.664	***	-73.627	-68.933

Significance Codes for P Value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

The greatest germination percentage was obtained when seed samples were placed at five degrees Celsius for four weeks, when analyzing the entire dataset (Table 3). The smallest environmental effect, suggesting the lowest potential germination, is 2:5. All confidence intervals do not include zero, so all environmental effects for germination are significantly different from the mean. Table 4 shows the same information when the environment 2:5 is excluded from the analysis.

Table 4: Estimated mean and environmental predictors on oat germination percentage excluding two weeks at five degrees Celsius

				95% Confidence Interval	
	Estimate	Standard Error	Significance	Lower Limit	Upper Limit
Mean	96.595	0.045	0	96.438	96.753
2:13	1.140	0.086	***	0.837	1.443
2:15	0.893	0.091	***	0.571	1.215
4:05	0.410	0.080	**	0.126	0.693
2:11	0.357	0.058	***	0.153	0.561
2:09	-0.109	0.059		-0.316	0.099
3:07	-0.243	0.166		-0.828	0.343
3:05	-0.627	0.065	***	-0.856	-0.398
2:07	-1.822	0.106	***	-2.197	-1.447

Significance Codes for P Value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Excluding the 2:5 environment from data analysis increases estimated mean germination percentage by about 10 percent. The highest temperatures used, 13 and 15 degrees Celsius, are now the top two predicted environmental effects. The 4:5 environment is still significantly higher than the mean, and in the top environmental effects. The 2:7 environment has the lowest effect for potential germination. Table 5 and Table 6 show the genotype effects for the complete dataset with and without the 2:5 environment, respectively. Both analyses show Colt, Horsepower, and SD110466 in the top three, so performing better than average with respect to CTGT. Deon, Natty, and Goliath perform the poorest in both analyses, with only the order rearranged.

Table 5: Genotype predictors on oat germination percentage and confidence intervals for all data

				95% Confidence Interval	
Genotype	Prediction	Standard Error	Significance	Lower Limit	Upper Limit
Colt	2.718	0.257	***	1.810	3.625
Horsepower	2.637	0.446	***	1.063	4.210
SD110466	2.401	0.312	***	1.299	3.503
Hayden	0.707	0.115	***	0.301	1.112
Stallion	0.063	0.174		-0.552	0.678
Shelby 427	-0.194	0.153		-0.736	0.347
SD120665	-0.829	0.162	**	-1.401	-0.256
Deon	-2.172	0.213	***	-2.926	-1.418
Natty	-2.218	0.274	***	-3.186	-1.250
Goliath	-3.112	0.150	***	-3.642	-2.582

Significance codes for P value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 6: Genotype predictors on oat germination percentage and confidence intervals excluding two weeks at five degrees Celsius

				95% Confidence Interval	
Genotype	Prediction	Standard Error	Significance	Lower Limit	Upper Limit
Colt	2.175	0.073	***	1.916	2.434
Horsepower	2.126	0.064	***	1.899	2.353
SD110466	1.522	0.066	***	1.288	1.756
Shelby 427	1.215	0.095	***	0.881	1.549
Stallion	1.189	0.082	***	0.899	1.478
SD120665	-0.028	0.072		-0.284	0.227
Hayden	-0.092	0.092		-0.415	0.232
Deon	-1.078	0.115	***	-1.484	-0.672
Goliath	-3.404	0.201	***	-4.113	-2.695
Natty	-3.624	0.161	***	-4.191	-3.057

Significance codes for P value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Environments Separated by Germination Potential

Environments were separated between higher and lower germination potential based on fixed effects, without including the two weeks, five degrees Celsius Environment. Once separated by germination potential, less environmental effects are significant (Table 7 and Table 8). Deon, Natty, and Goliath stay in the bottom three environmental effects for both low and high germination environments, so these varieties

germinated poorly across all temperature treatments used. Horsepower and Colt are the top two varieties for germination in both high and low germination environments. Hayden and SD120665 are not significantly different from mean germination percentage for either set of environments, so they germinate near average for these lines used regardless of environment (Table 9 and Table 10).

Table 7: Mean and environment predictors on oat germination percentage for low germination environments

				95% Confidence Interval	
	Estimate	Standard Error	Significance	Lower Limit	Upper Limit
Mean	95.781	0.055	0	95.587	95.975
2:09	-0.987	0.062	***	-1.205	-0.769
3:05	0.183	0.055	*	-0.011	0.378
3:07	0.076	0.067		-0.162	0.313
2:09	0.728	0.114	***	0.327	1.129

Significance codes for P value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 8: Mean and environment predictors on oat germination percentage for high germination environments

				95% Confidence Interval	
	Estimate	Standard Error	Significance	Lower Limit	Upper Limit
Mean	97.283	0.062	0	97.062	97.503
2:11	-0.447	0.063	***	-0.670	-0.225
4:05	0.021	0.121		-0.406	0.448
2:15	0.090	0.078		-0.186	0.365
2:13	0.337	0.044	***	0.182	0.491

Significance Codes for P Value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 9: Genotype predictors on oat germination percentage for low germination environments

				95% Confidence Interval	
Genotype	Prediction	Standard Error	Significance	Lower Limit	Upper Limit
Horsepower	2.537	0.064	***	2.311	2.764
Colt	2.534	0.158	***	1.975	3.092
SD110466	2.169	0.052	***	1.986	2.351
Stallion	1.764	0.080	***	1.480	2.047
Shelby 427	1.553	0.139	***	1.061	2.044
Hayden	0.032	0.126		-0.413	0.478
SD120665	-0.046	0.101		-0.402	0.311
Deon	-1.899	0.171	***	-2.504	-1.293
Natty	-4.321	0.133	***	-4.789	-3.853
Goliath	-4.323	0.220	***	-5.100	-3.547

Significance codes for P value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 10: Genotype predictors on oat germination percentage for high germination environments

				95% Confidence Interval	
Genotype	Prediction	Standard Error	Significance	Lower Limit	Upper Limit
Horsepower	1.791	0.048	***	1.621	1.961
Colt	1.676	0.063	***	1.452	1.900
Shelby 427	0.842	0.060	***	0.632	1.052
SD110466	0.797	0.049	***	0.625	0.968
Stallion	0.722	0.082	***	0.433	1.010
SD120665	-0.075	0.113		-0.474	0.324
Hayden	-0.212	0.188		-0.875	0.451
Deon	-0.318	0.056	**	-0.515	-0.121
Goliath	-2.274	0.173	***	-2.886	-1.662
Natty	-2.948	0.105	***	-3.320	-2.577

Significance Codes for P Value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

AMMI Analysis

Table 11: AMMI variance components of oat germination percentage

Component	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Significance
ENV	7	402.18	57.455	1.644	
REP(ENV)	8	279.65	34.956	11.805	***
GEN	9	2000.45	222.272	75.065	***
ENV:GEN	63	423.34	6.720	2.269	***
Residuals	392	1160.74	2.961		

Significance codes for P value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 11 shows variances from AMMI analysis. The high sum of squares for environment with the entire dataset is consistent with previous ANOVA tables, showing that including the 2:5 environment increases environmental variation considerably. Table 12 shows percentage of variation for the various PC scores in AMMI analysis. Because PC1 contributes 91.3% and 62.4% of the variation, genotypes which are located near the PC1 score of zero are considered most stable. Only bi-plot analysis was run, because PC3 score only contributes 1.4% and 11.3% of the variation.

Table 12: ANOVA of AMMI PC scores of oat germination percentage

	Percent of Variation	Accumulated d.f	DF	Sum Squares	Mean Squares	F-Value	Significance
PC1	62.4	62.4	15	134.713	8.981	3.03	***
PC2	16.1	78.5	13	34.753	2.673	0.9	
PC3	11.3	89.8	11	24.434	2.221	0.75	
PC4	5	94.8	9	10.698	1.189	0.4	
PC5	3.2	98	7	6.945	0.992	0.34	
PC6	1.4	99.4	5	3.078	0.616	0.21	
PC7	0.6	100	3	1.193	0.398	0.13	

Significance codes for P value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Figure 5 shows PC1 vs. PC2 score without the 2:5 environment. Goliath and Deon are far from the zero line for PC1 or PC2 score. Hayden is very near the intersection of the zero lines, suggesting it is very stable. Horsepower and SD120665 are

also near zero for PC1 and PC2 scores, suggesting they are also stable. No genotypes appear to be adapted well to a certain environment. Figure 6 shows PC1 score vs. germination percentage, excluding the 2:5 environment. Once again, Hayden is at zero for PC1 score and near mean germination percentage. SD120665 is also near the intersection of mean germination percentage and a PC1 score of zero. On this graph, Natty, Deon, and Goliath are far from PC1 score zero and all environments. All the remaining genotypes are clustered above mean germination percentage and PC1 score zero.

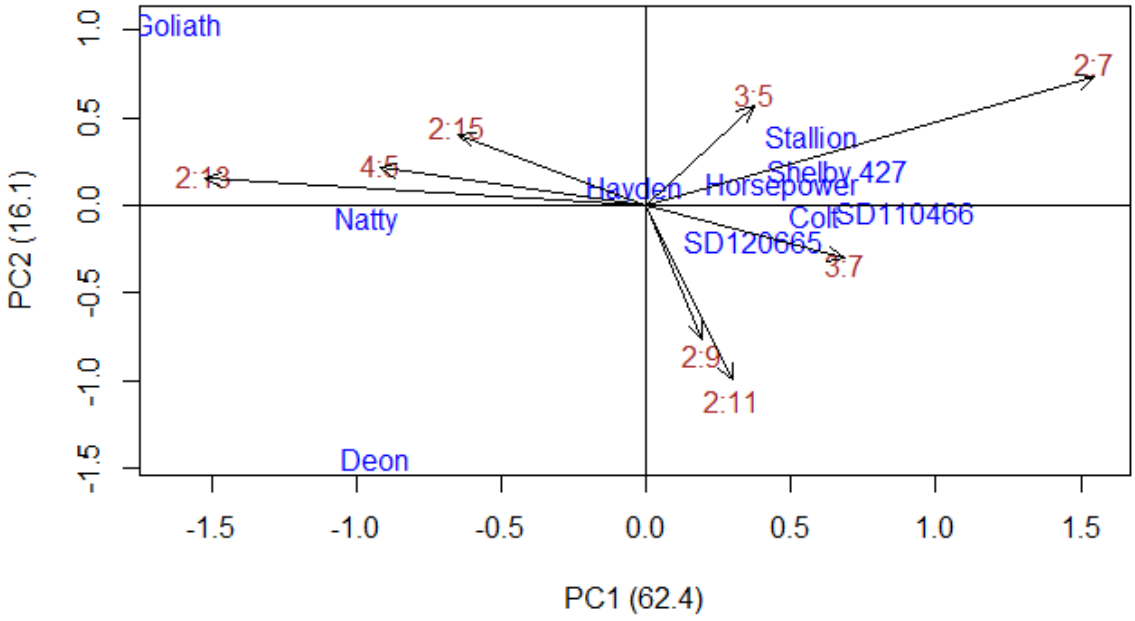

Figure 5: PC1 vs. PC2 score of oat germination percentages for genotypes and environments (weeks: degrees Celsius)

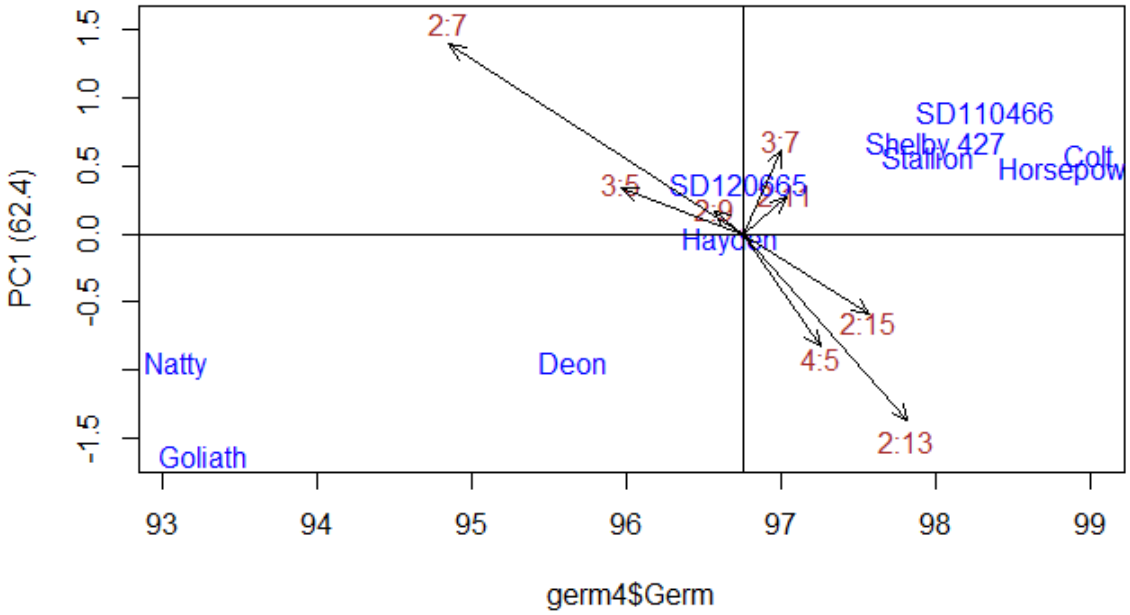

Figure 6: PC1 score vs germination percentage for oat genotypes and environments (weeks: degrees Celsius)

Seedling Length Analysis

Table 13 shows ANOVA of the length measurements of seedlings. This shows that time appears to be the most significant factor affecting length of seedlings, based on high sum of squares and low P value. Genotype and genotype-time interaction are also significant, as is sheet, the blocking factor in this analysis. Table 14 shows proportional variances from linear mixed model analysis. Time was treated as a fixed effect, so was not included in proportional variances. This shows that genotype accounts for over 50% of the variation in length measurements, so there are significant differences between genotypes.

Table 13: ANOVA of oat seedling length measurements

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Significance
Genotype	9	5.86	0.65	20.363	***
Time	1	61.41	61.41	1921.112	***
Sheet	2	0.27	0.13	4.15	*
Genotype:Time	9	1.28	0.14	4.461	***
Residuals	38	1.21	0.03		

Significance codes for P value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 14: Proportional variances of oat seedling length components

Source of Variation	Estimate	Standard Error	Significance	95% Confidence Interval Lower Limit	95% Confidence Interval Upper Limit
Genotype	0.532	0.034	***	0.4124	0.6524
Sheet	0.031	0.012		-0.0115	0.0733
Time:Genotype	0.235	0.034	***	0.1153	0.3556
Error	0.201	0.021	***	0.1256	0.2770

Significance codes for P value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 15 shows predicted genotype effects on seedling lengths. This suggests that SD110466 is significantly better than all other genotypes, due to a high confidence interval that is outside of all other confidence intervals. Horsepower and Natty also performed significantly better than the mean, with confidence intervals not including

zero. Shelby 427 and Deon performed significantly worse than the mean, indicated by negative confidence intervals far from zero. Table 16 shows the average length of seedlings at three and four weeks, and the average growth rate calculated by subtracting the mean lengths. This suggests that horsepower grew the best between three and four-week measurements, followed closely by Natty and SD110466.

Table 15: Genotype predictors on oat seedling length

				95% Confidence Interval	
Genotype	Prediction	Standard Error	Significance	Lower Limit	Upper Limit
SD110466	0.438	0.025	***	0.350	0.525
Horsepower	0.263	0.017	***	0.202	0.324
Natty	0.234	0.015	***	0.182	0.286
SD120665	0.100	0.017	***	0.041	0.159
Colt	0.028	0.035		-0.096	0.153
Stallion	0.028	0.011		-0.010	0.066
Goliath	-0.075	0.018	**	-0.140	-0.011
Hayden	-0.134	0.014	***	-0.184	-0.084
Shelby 427	-0.349	0.030	***	-0.457	-0.241
Deon	-0.532	0.043	***	-0.683	-0.382

Significance codes for P value: “***” 0.001 “**” 0.01 “*” 0.05 “.” 0.1

Table 16: Average oat seedling lengths at three and four weeks and growth rate

Genotype	Three Week Length	Four Week Length	Growth
Horsepower	1.1	3.7	2.5
SD110466	1.4	3.8	2.3
Natty	1.2	3.5	2.3
Hayden	0.9	3.0	2.1
Shelby 427	0.7	2.7	2.0
Stallion	1.2	3.1	1.9
Goliath	1.1	3.0	1.9
Colt	1.2	3.0	1.8
Deon	0.6	2.4	1.8
SD120665	1.5	3.0	1.5

DISCUSSION

One of the main objectives of this study was to identify a temperature at which to screen a larger pool of germplasm for CTGT in the future. The results suggest that not only temperature but also time should be considered for future evaluations. For the two weeks, five degrees Celsius environment, one replication had much higher germination for certain varieties than the other two. One possible explanation for this is the fact that timing of germination counts was only based on calendar day without time of day recorded. This could have caused a variation upwards of twelve hours in the amount of time different sets of seed samples spent in the germination chamber. There may be a critical time at around fourteen or fifteen days when certain varieties are germinating faster than others at five degrees Celsius. The data collected suggest that five or seven degrees Celsius with varying intervals of time would be the most effective method to screen larger amounts of germplasm in the future.

It appears that germination may only be delayed at low temperatures, rather than reduced. This is indicated by the high environmental effect for four weeks at five degrees Celsius. This environment has a consistently positive effect on germination regardless of how the data are split up for analysis. This is also apparent in the boxplots, as 4:5 is nearly as high as 2:13 or 2:15 (Figure 3). Experiments with Canadian oats found around a 1.5-day difference in germination time between fastest and slowest varieties, but only around 4% difference in final germination percentage (Willenborg et al. 2005). As previously mentioned, the distribution of two-week, five degrees Celsius counts suggests there may be a critical time when certain genotypes are germinating much faster than

others. Germination and emergence one or two days sooner may give an oat cultivar enough advantage to yield higher than another cultivar.

The genotypes Horsepower and Colt germinate better than average with confidence intervals far from zero for all data analyses. Natty, Goliath, and Deon are always the lowest three genotypic effects regardless of environment, with only order rearranged. Confidence intervals become farther from zero for lower germination environments. This suggests that germination is further reduced for the poorest performing genotypes as potential germination reduces with environment. For lower germination environments, confidence intervals for germination percentage of genotypes range by about five to seven percent. This could translate into an agronomic advantage for Horsepower, Colt, and SD110466 under the right environmental conditions in the field. Because Horsepower consistently has significant, positive effects for germination percentage, it may perform better than the other genotypes used when planted into cool soil in early spring.

AMMI analysis was run to investigate the stability of germination for genotypes across the range of sub-optimal temperatures. A genotype with more stable germination across temperatures may be less affected by soil temperature at planting time and yield more consistently regardless of planting date. Based on PC1 vs PC2 score, Hayden appears the most stable because it is near the intersection of the zero lines for each score. Deon, Natty, and Goliath are scattered on the left side of the graph, with negative PC1 scores (Figure 3). Because the environments 2:13, 2:15, and 4:5 are also on this side, these three genotypes show a positive interaction with these environments due to all having negative PC1 scores (Crossa 1990). The genotype Natty is near the PC2 score

zero. Because PC1 score accounts for a much larger percentage of variation (62.4% compared to 16.1%), it is more significant when comparing genotypes and environments.

Plotting PC1 score against germination percentage, Hayden is near the intersection of mean germination percentage and PC1 score zero (Figure 4). SD120665 is also near this intersection, only with a slight positive PC1 score. Once again, Deon, Natty, and Goliath have negative PC1 scores. The environments 4:5, 2:15, and 2:13 also have negative PC1 scores, but are on opposite sides of the line for mean germination percentages. This further confirms that Deon, Natty, and Goliath have consistently low germination, but may have a positive interaction with these three environments for germination percentage. All other genotypes are clustered above PC1 score zero and mean germination percentage, suggesting consistently high germination.

Seedling length analysis was conducted to determine which genotypes, if any, had longer shoots after three and four weeks at five degrees Celsius. A genotype with longer shoot elongation should emerge faster and perform better in colder environments than one with less elongation. Based on proportional variances, genotype accounts for over half of the variation (53%) of seedling measurements, so there are significant differences between genotypes for shoot length (Table 14). Genotypic effects for shoot length show SD110466 having the greatest positive effect, with a confidence interval greater than that of all other genotypes. Horsepower also has a positive effect for shoot length, as does Natty and SD120665 (Table 15). When comparing average lengths between three and four weeks and calculating growth rate, Horsepower is higher than SD110466, which is equal to Natty (Table 16). These calculations were performed without replication, so their significance cannot be verified.

In conclusion, the genotypes SD110466 and Horsepower consistently have high germination and shoot elongation over a range of sub-optimal temperatures in a germination chamber. The only exception is for higher germination environments, in which Horsepower germinates significantly better than SD110466 (Table 10). The genotype Colt germinated well for all data analyses but did not have a significant effect for seedling elongation (Table 15). Stability analysis shows that SD120665 and Hayden are more stable than other genotypes across environments, so germination may be less affected by temperature and time for these genotypes.

Future work should examine relationships between time and temperature in a germination chamber, as previously mentioned. Seed size should also be considered in future work. Seed size has been shown to affect final germination percentage by five percent, and germination time by 8% (Willenborg et al. 2005). Seed should be sorted by size in the future, to compare genotypic effects on germination based on seed sizes. Light may also be a factor in triggering germination of oat seed (Hadas, 2004). For this experiment, lights were on in the germination chamber for eight hours per day for all temperature and time treatments. A seed planted in the field would likely receive very little or no light during germination. Simulating light conditions in the field may affect the results in future research. All seed used in this experiment was grown in plots near Winner, SD in 2016. Using seed grown at different locations and/or years could also affect germination percentage, based on seed size and test weight.

APPENDIX

SPRING WHEAT TRANSPLANTING

Introduction

Many lines of hard red spring wheat have been previously screened for CTGT. Broad-sense heritability of CTGT from the temperatures 5-11°C has been calculated around 0.73, suggesting that CTGT should be able to be manipulated easily by wheat breeders (Jenkins, 2014). To demonstrate that CTGT can be manipulated within a population and improve this trait in HRSW, selections need to be made. This was performed by transplanting seeds from germination towels to pots of soil in the greenhouse. This way, new populations could be grown from the first seeds and the last seeds to germinate at a low temperature. These populations can then be re-screened for CTGT once enough seed is grown for a germination test.

Materials and Methods

Four different genotypes of HRSW were used for this study. Two of which, SD4011/Barlow, and SD4330, have been identified as having high CTGT in previous screening. The other two genotypes, SD4189/SD3997 and Forefront, were identified as having low CTGT (Jenkins, 2014). The objective was to create new populations of each genotype from the first and the last 10% of seeds to germinate. Seed was obtained from storage at the seed house. When needed, a Gamet divider was used to obtain a smaller sample of approximately 25 grams for the experiment. To start the experiment, two replications of 100 randomly selected seeds were planted on germination towels presoaked with deionized water, in the same manner as for oat germination testing. The germination towels with seed were placed in a germination chamber at 5°C and checked

every 4-5 days for germination.

At fourteen days after planting, shoots were starting to appear on most of the seeds for all varieties. The next day, planting was started in greenhouse pots filled with pre-moistened Pro-Mix BX Mycorrhizae General Purpose Growing Medium. One paper (100 seeds) for each variety was transferred to a sealed plastic bag to retain moisture and transported to the greenhouse. The ten seedlings with the longest shoot and well-developed roots, by visual observation, were selected for transplanting from each genotype. Each transplanted seed/seedling was given its own unique number and labeled with a stake. Seedlings were planted so the endosperm was about 1 inch into the soil. An attempt was made to place them with the shoot pointed upward and the roots downward. Seedlings were then loosely covered with pre-moistened soil and gently watered. A third set of 100 seeds was planted in the germination chamber for each genotype at this time, in case some seedlings would fail to emerge.

After twenty days in a germination chamber, the second replication of 100 seeds was removed for each genotype and taken to the greenhouse for transplanting. This time, the seedlings with very little or no germination were selected for transplanting. Different genotypes had varying levels of germination at this time, so many of the seeds planted were non-germinated. Seeds with mold or very soft seeds were not used for transplanting. Neither were seeds with either shoots or roots missing, as these were considered abnormal and may not develop into a full plant. The ten seeds or seedlings for each genotype with the least amount of germination, or no germination, were selected and transplanted in the same manner as the first ten seedlings few days earlier. The number of germinated seedlings and non-germinated seeds were recorded.

At eight days following the second transplanting, seedlings which had not yet emerged were excavated to check for growth. One seedling, #13 from SD4011/Barlow, was found with a live shoot that was pinched off at the soil surface. This shoot was pulled out of the soil by hand, with the goal of exposing it to light and the plant surviving. Because these later germinated seedlings were planted for comparison purposes in the future, this does not affect the results of this experiment.

Pots containing seedlings with no growth were re-used for the next transplanting, to save greenhouse space and resources. This time, seeds which were still non-germinated in the germination chamber were planted into greenhouse pots. This was to determine if less time in a germination chamber on moist towels (14 days compared to 20 days) would increase the germination of seeds once planted in warm soil. Non-germinated seeds from the third germination paper planted for each genotype were removed, secured in a sealed bag to retain moisture, and transferred to the greenhouse for planting. These seeds were planted in the same manner as all others, approximately 1 inch below the soil surface. Some seeds were planted in pots with no emergence from the last transplanting, re-using the stake and number. Five days later, the remaining seedlings from the third replication of germination testing were transplanted into the greenhouse. These were the seedlings that had germinated at 5°C but had the least amount of growth. These seedlings were transplanted into greenhouse pots in the same manner as all previous seedlings. Enough seedlings were transplanted for each variety to obtain at least 10 early and 10 late germinated plants.

Results

At four days after initial transplanting, shoots had emerged from soil and first

leaves were present for all transplanted seedlings. These plants were numbered and labeled one through ten for their respective genotypes. Six days after the second set of transplanting was conducted, many of the seedlings had begun to emerge from the soil with first true leaves present. Many had also not emerged yet, mainly those which were planted from non-germinated seeds. Notes were recorded indicating which seedlings had not yet emerged (Table 17). At seven days after transplanting, one additional seedling had emerged.

Table 17: First transplant of late germinated HRSW seedlings

Genotype	Seedling	Germinated	Date Emerged	Notes
SD4330	11	Yes	25-Jan	
SD4330	12	Yes	25-Jan	
SD4330	13	Yes	29-Jan	
SD4330	14	Yes	29-Jan	
SD4330	15	Yes	25-Jan	
SD4330	16	Yes	29-Jan	
SD4330	17	No	N/A	
SD4330	18	No	N/A	
SD4330	19	No	N/A	
SD4330	20	Partial	N/A	
SD 4011/Barlow	11	Yes	25-Jan	
SD 4011/Barlow	12	Yes	25-Jan	
SD 4011/Barlow	13	Yes	31-Jan	Shoot pulled out by hand, very short
SD 4011/Barlow	14	Yes	N/A	
SD 4011/Barlow	15	Yes	31-Jan	Very Short
SD 4011/Barlow	16	No	N/A	
SD 4011/Barlow	17	No	N/A	
SD 4011/Barlow	18	No	N/A	
SD 4011/Barlow	19	No	N/A	
SD 4011/Barlow	20	No	N/A	
Forefront	11	Yes	29-Jan	
Forefront	12	Yes	29-Jan	
Forefront	13	Yes	29-Jan	
Forefront	14	Yes	29-Jan	
Forefront	15	Yes	29-Jan	
Forefront	16	Yes	29-Jan	
Forefront	17	Yes	29-Jan	
Forefront	18	Yes	29-Jan	
Forefront	19	Yes	29-Jan	
Forefront	20	No	N/A	
SD4189/SD3997	11	Yes	29-Jan	
SD4189/SD3997	12	Yes	N/A	
SD4189/SD3997	13	Yes	29-Jan	
SD4189/SD3997	14	Yes	1-Feb	
SD4189/SD3997	15	Yes	30-Jan	
SD4189/SD3997	16	Yes	N/A	
SD4189/SD3997	17	No	N/A	
SD4189/SD3997	18	No	N/A	
SD4189/SD3997	19	No	N/A	
SD4189/SD3997	20	No	N/A	

Non-germinated seeds did not emerge well from the second transplanting either. The only exception is Forefront #20, which was a non-germinated seed that emerged three days after planting in the greenhouse. Table 18 shows the seeds/seedlings from the second transplanting.

Table 18: Second late transplanting HRSW seeds and seedlings

Genotype	Seedling	Germinated	Date Emerged	Notes
Forefront	20	No	9-Feb	only non-germinated seed to survive
Forefront	21	No	N/A	
SD 4011/Barlow	16	No	N/A	
SD 4011/Barlow	17	No	N/A	
SD 4011/Barlow	18	No	N/A	
SD 4011/Barlow	19	No	N/A	
SD 4011/Barlow	20	No	N/A	
SD 4011/Barlow	21	No	N/A	
SD4189/SD3997	16	No	N/A	
SD4189/SD3997	17	No	N/A	
SD4189/SD3997	18	No	N/A	
SD4189/SD3997	19	No	N/A	
SD4189/SD3997	20	No	N/A	
SD4189/SD3997	21	Yes	6-Feb	
SD4189/SD3997	22	Yes	6-Feb	
SD4330	17	No	N/A	
SD4330	18	No	N/A	
SD4330	19	No	N/A	
SD4330	20	Partial	N/A	

Most of the seedlings from the last transplanting survived and emerged, except for SD4330: number 23 and 25, Forefront number 23, and SD4189/SD3997 number 23. Table 19 shows which seedlings emerged from the last transplanting. Total late seedlings emerged for each variety from the second planting are as follows: SD4330: 9, SD4011/Barlow: 10, Forefront: 11, SD4189/SD3997: 9.

Table 19: Final late transplanted HRSW seeds and seedlings

Genotype	Seedling	Germinated	Date Emerged	Notes
Forefront	22	Yes	9-Feb	
Forefront	23	Yes	N/A	
SD 4011/Barlow	22	Yes	9-Feb	
SD 4011/Barlow	23	Yes	8-Feb	
SD 4011/Barlow	24	Yes	8-Feb	
SD 4011/Barlow	25	Yes	9-Feb	
SD 4011/Barlow	26	Yes	9-Feb	
SD 4011/Barlow	27	Yes	12-Feb	
SD4189/SD3997	23	Yes	N/A	
SD4189/SD3997	24	Yes	9-Feb	
SD4189/SD3997	25	Yes	12-Feb	
SD4189/SD3997	26	Yes	12-Feb	
SD4330	21	Yes	12-Feb	
SD4330	22	Yes	8-Feb	
SD4330	23	Yes	N/A	
SD4330	24	Yes	12-Feb	Very short, possibly underwatered
SD4330	25	Yes	N/A	

Discussion

Due to limited seed supply, no additional late seedlings were transplanted. The plants obtained will provide an adequate supply of individuals to test for inheritance of CTGT. Seed from each of the transplanted plants will be harvested and planted as its own population, to obtain enough seed for germination testing. These new lines will then be re-screened for CTGT. This study showed that after fourteen days in a germination chamber at 5°C, seedlings of HRSW with the most germination can be transplanted into greenhouse pots and grown easily. If these selections are proved successful in future testing, this methodology could be applied to more populations or possibly segregating populations of HRSW to improve CTGT.

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