Selection for Seedling Cold Vigor in Grain Sorghum

by

Jacobo Sanchez

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Approved

Glen Ritchie
Chair of Committee

Yves Emendack

John Burke

Mark Sheridan
Dean of the Graduate School

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Christ for all the people I've mentioned here, it is in HIS will and strength that we all operate.

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ABSTRACT

Sorghum is the fifth most widely grown cereal grown in the world, but one limitation to sorghum production is the restriction of cool temperatures on crop growth and development. The primary objective of the current study was to validate five previously identified markers of cold tolerance in sorghum by deriving a recombinant inbred line population from a cross between BTx623, a warm season elite sorghum line, and Hong Ke Zi, a Chinese sorghum landrace with increased cold tolerance. We conducted controlled environment screening testing of 307 recombinant inbred lines (RILs), followed by field-based phenotypic testing early-planted sorghum for 31 selected lines. Genotyping of the 31 F4 RILS using the KASP genotyping technology indicated that only two of the five markers tested showed segregation for the HKZ, BTx623, and the heterozygous genotype. Using the genotype data obtained, the genotype of each of the 31 RILS was compared to the various growth parameters measured by performing a one-way ANOVA analysis. Significant separation of the three genotypic groups (homozygous for HKZ allele, homozygous for BTx623 allele, and heterozygous for both alleles) was seen with the root biomass per plot (RBM/plot) phenotype only. That is, RILS that contained the homozygous HKZ (cold-tolerant) allele had significantly more root biomass than did RILS that were homozygous for the BTx623 allele or heterozygous for both alleles (Table 3.2). This result indicates that the two markers tested could be potential candidates in selecting for the seedling cold vigor trait using marker assisted selection (MAS). Though this result is promising, the markers need to be tested against a larger pool of diverse individuals to observe if the markers are truly viable as a selection tool.

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CHAPTER I

INTRODUCTION

Sorghum [Sorghum bicolor (L.) Moench] is the fifth most important cereal grown in the world, behind maize, rice, wheat, and barley (FAO 2008). Sorghum is well adapted to a great many areas for cultivation outside of Africa, where it was introduced. Across the developing world such as Asia and Africa, sorghum is used primarily for food, shelter, and fuel for cooking. In the United States, sorghum is mainly used as feed within the livestock industry, and it is beginning to emerge as a viable source for use in the production of bioenergy.

Sorghum production in the United States is mainly within the Sorghum Belt, which extends from South Dakota to Texas. Sorghum production within the Sorghum Belt has increased steadily from 2013 to the present in acreage planted and grain yield harvested (USDA, January 2016). The area of the U.S. where sorghum is grown is temperate in nature and is subject to cold soil temperatures during early season planting.

Since sorghum is of tropical origin, cold soil temperatures during early season planting in temperate areas lead to poor stand establishment, which in turn affects yield stability. The identification of cold vigor in sorghum seedlings would increase both the geographic area over which sorghum could be grown and the timings in which it could be planted. Thus, the need to improve the early season cold vigor of sorghum seedlings is evident. From its domestication about 8,000 years ago in northeast Africa until the present, sorghum has developed into a crop with significant diversity. Dillon et al. (2007) have reported that there exists vast diversity among sorghum races for many traits, making breeding and selection for desirable traits relatively easy. Burow et al. (2011) and Emendack et al. (2014) studied genetic diversity in a collection of Chinese sorghum landraces and found that there exists significant diversity within these landraces for the seeding cold vigor trait. These landraces can in turn be used as parental material for the introgression of the cold vigor trait into elite germplasm thus resulting in improved genetic gain for this important agronomic trait.

Sources of cold tolerance in grain sorghum originate from the highlands of Uganda, China, and Ethiopia (Singh, 1985). Four Chinese landraces (Gai Gaoliang, Hong Ke Zi, Shan Qui Red, and LiangTangAir) have been used to study the strength of the cold tolerance trait within these landraces in cold soil field tests (Kapanigowda et al., 2013), with the Chinese landraces having improved final percent emergence, improved per plant biomass, and improved rate of emergence (lower emergence index) compared to elite lines (RTx2737, BTx3042, BTx623, BTx398, TX430R, Redbine58B). Burow et al. (2006) studied 10 Chinese accessions from the working group Nervosum-Kaoliang, 10 U.S. inbred parental lines, and 10 U.S. commercial hybrids for cold tolerance and found that the Chinese Kaoliang lines were superior in field-based rates of emergence and laboratory rates of germination. Based on these studies, it appears that the Chinese landraces may be promising genetic sources for introducing seedling cold tolerance to elite sorghum breeding lines.

Research into the introgression of cold tolerance traits, such as germination and emergence rates, from Chinese landraces into elite breeding lines is ongoing. To accomplish the successful introgression of cold tolerant traits into elite breeding lines of sorghum, efficient and reliable physiological measures for the selection of cold tolerance traits within breeding lines are necessary. Previous studies into finding reliable measures of cold tolerance at the seedling level in sorghum used measures such as respiration rate, germination rate, emergence rate, and seedling shoot growth rate (Yu et al., 2004; Patanè et al., 2006; Balota et al., 2010; Tiryaki and Andrews, 2001; Fernandez et al., 2014). All of these methods were done in controlled laboratory conditions and verified with field studies.

A second method, complementary to the use of phenotypic measures used to predict cold tolerance in sorghum germplasm, is the use of QTL (quantitative trait loci) markers. This technique, also known as marker assisted selection (MAS), involves the creation of a hybrid cross between two distinct parents for the cold tolerance trait or the use of a population of germplasm with wide genetic diversity for the cold tolerance trait. MAS involves the generation of single sequence repeats (SSRs) or single nucleotide polymorphism (SNP) markers based on DNA sequence data from the two parents or from the population. These markers are then tested against phenotypic responses of the individuals for cold tolerance. A statistical analysis of the genotypic results against the phenotypic measures indicates which markers are strongly associated with a particular phenotypic measure that relates to seedling cold tolerance.

Knoll et al. (2008) tested this technique with a recombinant inbred population created from a cross between Shan Qui Red (cold tolerant Chinese landrace) versus SRN39 (a cold sensitive breeding line). Using germination percentage under controlled conditions and emergence percentage under cold field conditions, they were able to develop a genetic map that contained several QTL markers associated with early season cold tolerance. Three of the markers identified were subsequently tested in two more populations where one of the parents was Shan Qui Red. The results of the second study verified a significant relationship between two of the markers and early-season cold tolerance traits, allowing selection for early season cold tolerance in sorghum. A more recent study by Chopra et al. (Chopra et al. 2017) sought also to develop QTL markers (SNPs) for sorghum germplasm under thermal stress (cold and heat). Using a sorghum association panel that represented major races and working groups with diverse phenotypic responses to cold and heat, they were able to locate several quantitative trait loci (QTL) for seedling cold vigor.

In the current study, a recombinant inbred line (RIL) population was developed to generate germplasm that could be used to introgress seedling cold vigor into existing breeding lines used for sorghum production. BTx623, a tropical converted elite breeding line, was selected for its desirable agronomic traits (short plant, compact panicle and large diameter stem) (Miller, 1977) and also because its genome has been sequenced (Paterson et al., 2009) thus providing a platform for genetic analysis of cold tolerance. Hong Ke Zi, a Chinese landrace of the working group Kaoliang, was chosen because it has shown to have excellent cold germinability and high emergence rates in cold soils. Although the Chinese landraces are known to have undesirable traits for production and also for diseases (Qingshan and Dahlberg, 2001), the Hong Ke Zi landrace has large panicles (length and width) that could prove beneficial for improved yield potential.

The primary objective of the current study was to validate five of the markers identified by Chopra et al. using the following methods:

- Derive an RIL population from a cross between BTx623 (Miller, 1977) and Hong Ke Zi
- Conduct controlled-environment screening of cold tolerance using a growth chamber
- Verify controlled-environment findings through field-based phenotypic responses in early-planted sorghum

CHAPTER II

SELECTION AND CHARACTERIZATION OF THE RILS

Introduction

Predicted fluctuations in rainfall and temperature patterns within the Great Plains of the United States (EPA, Climate Impacts in the Great Plains, 2016), which contains the Sorghum Belt, pose challenges for sorghum production in this geographic area. Consequently, the development of populations of elite breeding lines with positive genetic gains for abiotic stress tolerance is imperative to maintaining yield stability. While abiotic stress tolerance can help maintain yield stability, especially during periods of environmental fluctuations, agro-morphological traits of sorghum breeding lines can also provide positive benefits for maintaining and improving productive yield.

Yield potential in grain sorghum is impacted by environmental factors such as temperature and moisture availability. In addition to these environmental factors, agro-morphological traits such as: stem diameter, plant height, number of leaves, panicle length, and panicle width also affect yield potential. By developing breeding lines with both improved abiotic stress tolerance and improved agronomic traits, yield stability and productivity of grain sorghum may be attainable.

In high elevation areas of production within the United States and around the world, cold soils during early season planting diminish stand establishment and ultimately yield (Cisse and Ejeta, 2003). To successfully introduce sorghum into these areas of production, it is necessary to develop sorghum cultivars that will be able to germinate, emerge, and grow under the cold soil temperatures often seen in temperate areas. To accomplish this task, sources of seedling cold tolerance need to be exploited and used to introduce the trait into the elite sorghum lines currently available for production. Here we describe the characterization of a recombinant inbred line (RIL) population of sorghum for both yield components and the seedling cold tolerance trait.

Materials and Methods

Development of the F2 Population

The F1 cross between BTx623 and Hong Ke Zi (HKZ) was made in the summer of 2010 at the USDA-ARS farm in Lubbock, Texas (33.6°N, -101.9°W) with BTx623 being the female and HKZ being the male. The resulting F1 seeds were planted the following year at the same location to develop the F2 generation. The harvested panicles from the F1 plants were kept as separate entities. F2 seed from a single panicle was planted at the USDA-ARS farm in Lubbock, Texas on June 1, 2015. The field layout was 2 rows and 20 ranges, totaling 40 plots of 4.7 m in length and 1.0 m row spacing. Each plot consisted of 100 seeds planted 3 cm deep with a John Deere MaxEmerge Planter modified for use in small plot research. The soil type was an Amarillo fine sandy loam. All plots were irrigated via sub-surface drip at 5 mm per day.

Selection of the RILs

To properly characterize the F2 population for both seedling cold vigor and improved yield potential, two populations (namely; Panel 1 and Panel 2) of individuals were selected. The first population, Panel 1, was selected to determine the presence of improved yield potential. Selection of Panel 1 was done at random by placing a measuring tape along the length of each plot and tagging those plants that coincided with the 1 foot markings. A total of 10 plants per plot were selected. The second population, Panel 2, was selected via targeted visual selection for thick stems and large panicles and subsequently evaluated for seedling cold vigor under controlled temperature conditions. From this initial evaluation, thirty-one RILs from Panel 2 were selected for further cold vigor characterization. These thirty-one RILs represented sixteen cold tolerant and fifteen cold sensitive types. Both Panel 1 and Panel 2 had RILs that were eliminated from the trial due to loss of panicles from bird damage, sugarcane aphid damage, or plant lodging due to high winds.

Agro-morphological Characterization: Panel 1

Stem diameter was measured with a digital caliper at 30 cm from the base of the plant. The numbers of leaves (NOL) were counted at the flowering stage of development. Plant height, panicle length, panicle width, and panicle weight were measured at maturity. Panicle length was measured from the base of the last rachis to the tip of the panicle. Panicle width was measured at the widest point of the panicle. Panicles were harvested and allowed to dry to standard moisture content by placing in a drying oven at 65˚C for 48 hours. Once dried, the panicles were weighed and threshed. The seed from each panicle was weighed and recorded as panicle seed weight (PSW).

Assessment and selection for cold tolerance: Panel 2

Evaluation under controlled environment

Ten seeds from each of the 307 RILs selected were treated with fungicide (Concep III – fluxofenim, 4’-chloro-2,2,2-trifluoroacetophenone O-1,3-dioxolan-2-ylmethyloxime; Novartis Crop Protection, Inc., Greensboro, NC and Apron XL –

metalaxyl-m. methyl N-(methoxyacetyl)-N-(2,6-xylyl)-D-alaninate; Syngenta Crop Protection, Greensboro, NC) and allowed to air-dry overnight. The seeds were then sown onto a piece of filter paper (Bio-Rad, USA) contained within a 100 x 15 mm plastic petri dish. The filter paper was then moistened with 6 ml of pre-chilled (15˚C) deionized water. The petri dishes were subsequently placed into a tabletop incubator (Percival Scientific, USA) set to constant 15˚C in the dark. The filter paper was kept moist as needed with pre-chilled water during the course of the experiment. The seed was allowed to germinate for 12 days and germination counts were taken every two days. On the 12th day, the roots of all ten seedlings were cut, excess water was wicked away with a Kimwipe (Kimberly-Clark, USA), and a combined fresh weight of the roots of all ten seedlings was measured and recorded as root biomass (RBM). Germination index was calculated by using the following equation:

$$GI = \frac{\sum(E_j x D_j)}{E}$$

where E_j = number of seedlings germinated on day j, D_j = days after planting, and E = final number of seedlings germinated (Smith and Millett, 1964). Seedlings were counted as germinated when the radicle had emerged approximately 2 mm from the seed coat (Tiryaki and Andrews, 2001). Final percent germination (FGP) was transformed by ASIN (arcsine) transformation (Franks et al., 2006) to minimize the effect of heterogeneity of error variances common in percentage data. Root biomass weights were standardized and expressed as proportions of experimental RBM_{max}-RBM_{min} using the following formula:

$$RBM_s = \frac{RBM - RBM_{min}}{RBM_{max} - RBM_{min}}$$

where RBM_s= Standardized root biomass, RBM = measured root biomass weight of RIL, RBM_{min}= minimum root biomass measured amongst all RILs, and RBM_{max} = maximum root biomass measured amongst all RILs.

Evaluation under field conditions

Thirty-one RILs from Panel 2, representing fifteen cold tolerant and sixteen cold sensitive types, were selected to perform a field study that would assess the seedling cold tolerance trait. Fifty seeds of each RIL and parents were planted on April 01, 2016 at the USDA-ARS farm at Lubbock, Texas. Single row plots per RIL of 4.7 m in length and 1.0 m row spacing was used. The experimental design was Randomized Complete Block with 3 replications per RIL. Germination counts were taken daily beginning at 8 days after planting (DAP). Vigor ratings were taken at 30 DAP using a visual rating on 1 to 5 scale; with 1 = robust vigor and 5 = poor vigor (Maiti et al., 1981). Root and shoot biomass were determined from 20 randomly selected plants at 30 DAP.

Statistical Analysis

Data were analyzed using JMP 12.1 (SAS Institute, 2014). A Pearson correlation matrix was used to identify correlations between measured traits in Panel 1 and Panel 2. Linear regression analysis was performed on traits that showed significant Pearson correlation coefficients. One-way ANOVA analysis was performed as genotype score vs measured field parameters of the RILs (total dry biomass per plant, shoot biomass per plant, root biomass per plant, total biomass per plot, shoot biomass per plot, root biomass per plot, emergence index, final emergence percent, and vigor rating). Biomass on a per plot basis were calculated by multiplying the biomass per plant by the total seedlings that emerged within a plot at 30 DAS (days after sowing). The genotype score was a nominal score of 0, 1, or 2 representing the BTx623, HKZ and Heterozygous alleles, respectively. Tukey's honest significant difference (HSD) test separated the means for the genotypes among the parameters measured and the observed trends in the ranking of genotypes were based on Tukey–Kramer HSD connecting the letters ranking report for the assessed parameters. Significance is stated at $p = 0.05$ or $p = 0.01$ where applicable.

Results

Field agro-morphological characterization: Panel 1

Agro-morphological measurements of Panel 1 RILs showed normal distributions for all the traits that were measured with the exception of panicle weight which showed a bi-modal distribution (Figure 2.1). The normal distribution suggests that the traits may be heritable. Transgressive segregation was present in the following measured traits: stem diameter (Figure 2.1B), panicle width (Figure 2.1C), panicle weight (Figure 2.1D), plant height (Figure 2.1E), and number of leaves (Figure 2.1F), suggesting that there is potential for improved on these traits within the population. Both parents showed similar panicle lengths (Fig 2.1A).

Correlation analysis of the measured traits for Panel 1 (Table 2.1) showed all the traits to be positively associated with each other. Additionally, all the traits were also positively correlated with panicle weight, which is a measure of grain yield. That grain yield may be measured by the panicle weight (PWT) was verified by the high R^2 of 0.99 between PWT (panicle weight) and PSW (panicle seed weight) (Figure 2.2). Finally, the three parameters used to visually select Panel 2 for improved yield components, namely, stem diameter (SDM), panicle length (PNL), and panicle width (PWD), proved to be positively correlated with panicle weight with correlation coefficients of 0.42, 0.48, and 0.83, respectively. Linear regression of each trait against PWT (panicle weight) showed that stem diameter and panicle length were low predictors of grain yield with R^2 coefficients of 0.18 and 0.22, respectively, and that panicle width (Figure 2.3) was a good predictor of grain yield with R^2 coefficient of 0.69. The interaction of SDM, PNL, and PWD combined predicted yield with an R^2 coefficient of 0.72.

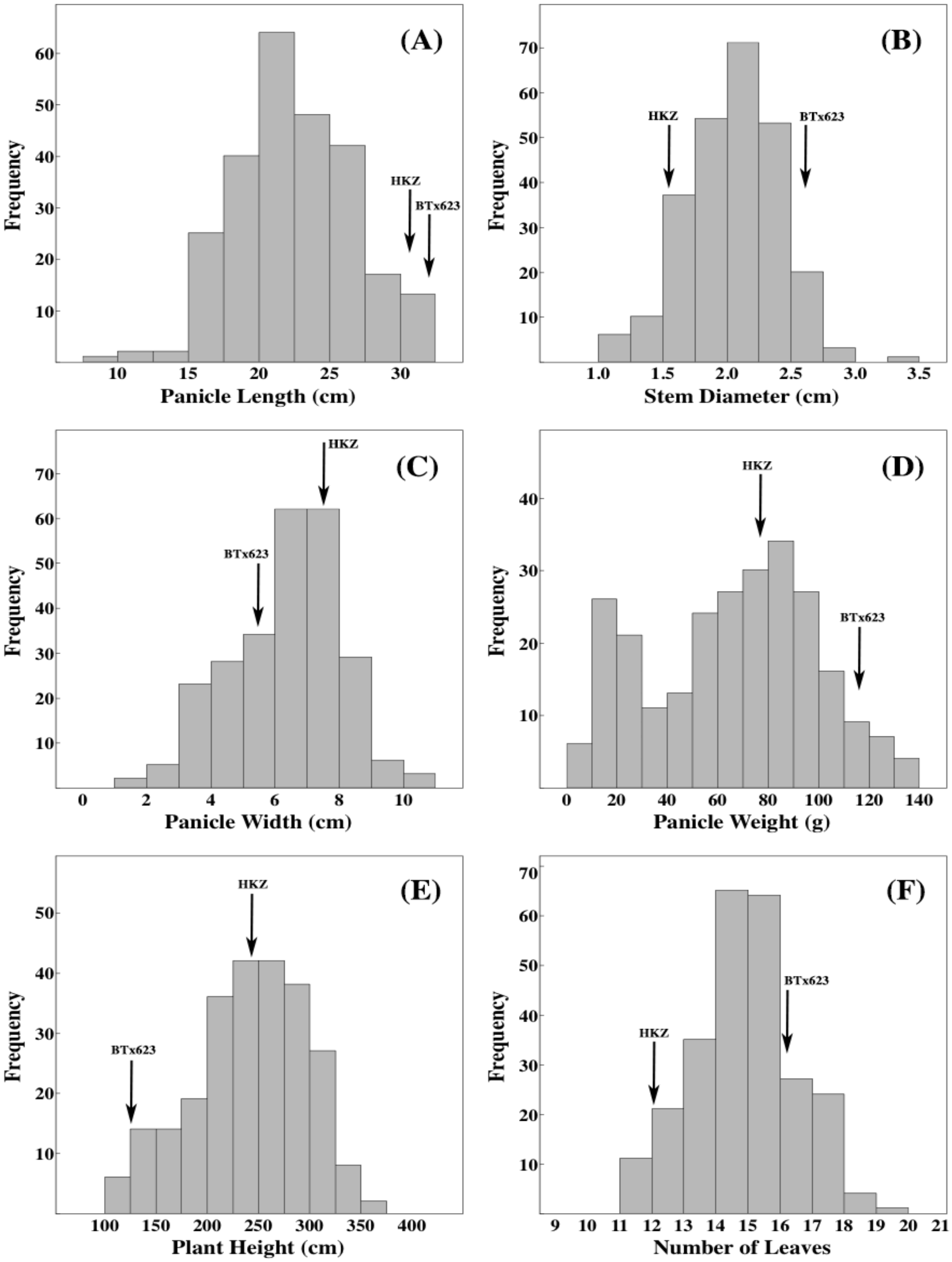

Figure 2.1 - Statistical distributions of measured traits for Panel 1 viz: (A) panicle length, (B) stem diameter, (C) panicle width, (D) panicle weight, (E) plant height, (F) number of leaves in Panel 1 of a BTx623*HKZ RIL population evaluated for early-season cold tolerance.

Table 2.1 - Significant Pearson relations between assessed traits in Panel 1 of a BTx623*HKZ RIL population evaluated for early-season cold tolerance

	§SDM	PNL	PWD	PWT	PSW	NOL	HGT
SDM	-	-	-	-	-	-	-
PNL	0.25	-	-	-	-	-	-
PWD	0.39	0.43	-	-	-	-	-
PWT	0.42	0.48	0.83	-	-	-	-
PSW	0.40	0.46	0.83	0.99	-	-	-
NOL	0.39	0.21	ns	0.25	.24	-	-
HGT	ns	.35	0.31	0.36	.33	.39	-

§SDM, stem diameter; PNL, panicle length; PWD, panicle width; PWT, panicle weight; PSW, panicle seed weight; NOL, number of leaves; HGT, plant height. “ns” indicates relation is non-significant at $p<0.05$, N=255.

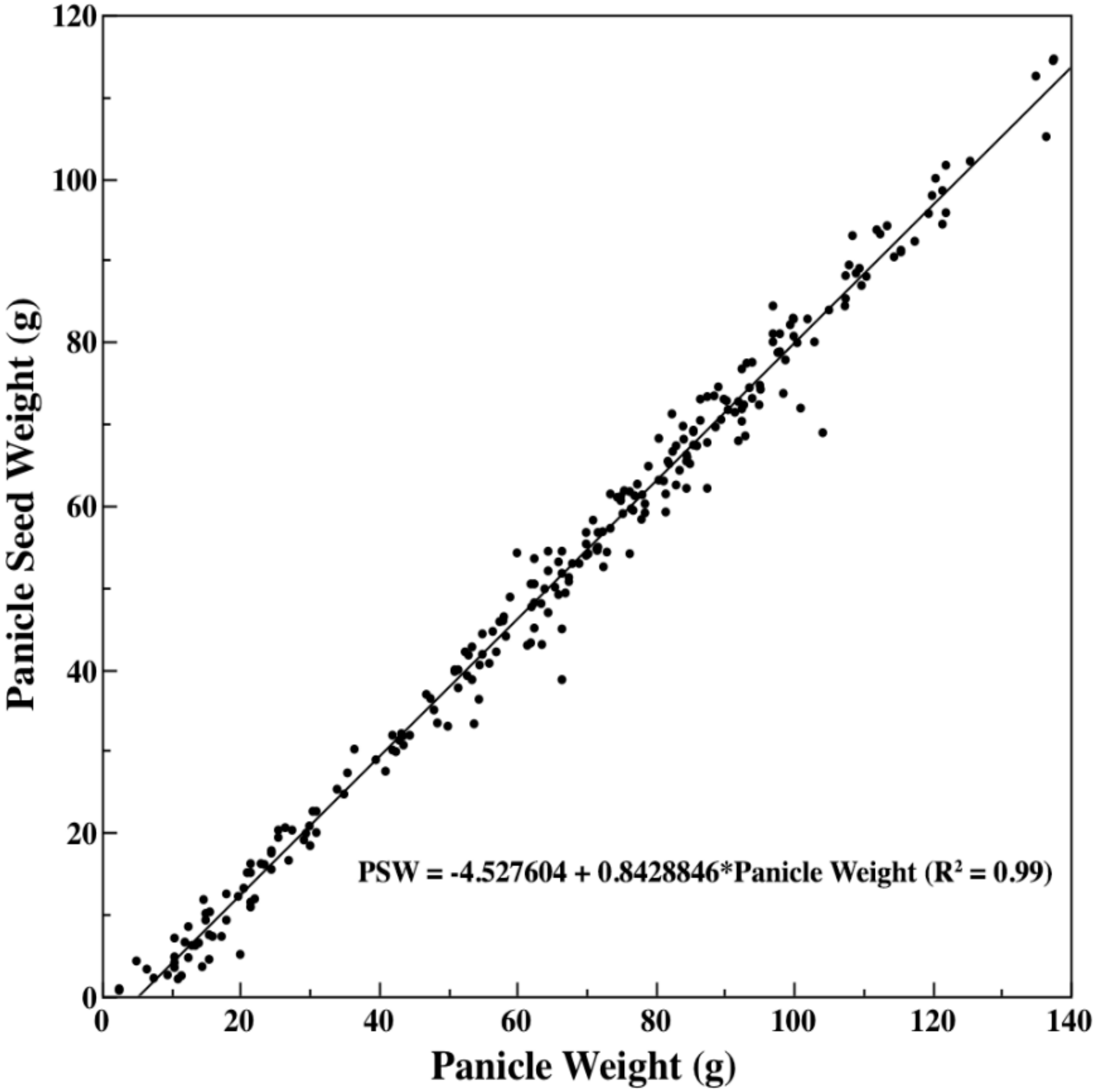

Figure 2.2 - Relation between panicle weight (PWT) and panicle seed weight (PSW) in an BTx623*HKZ RIL population evaluated for early-season cold tolerance.

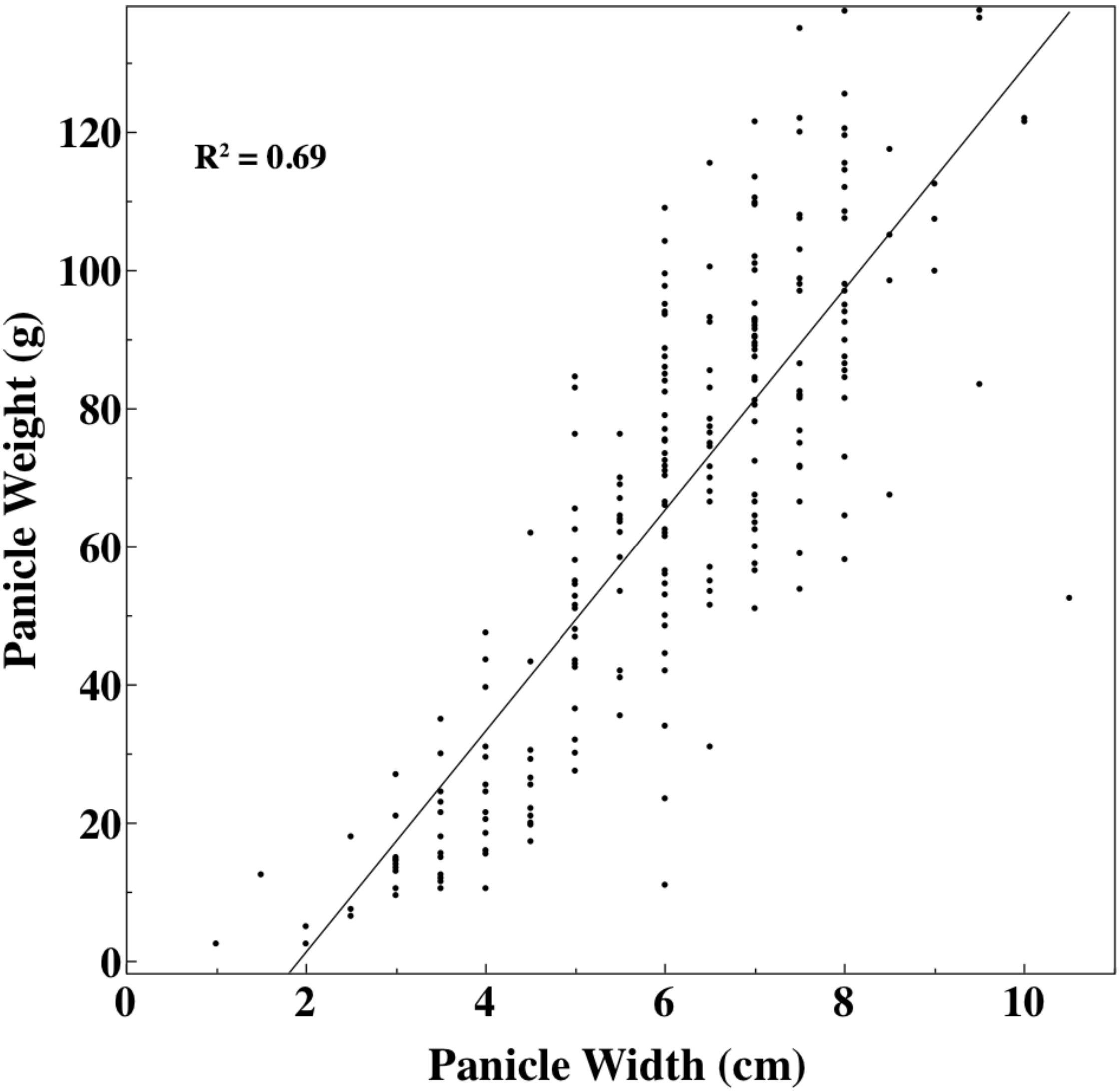

Figure 2.3 - Regression of panicle width versus panicle weight of a BTx623*HKZ RIL population evaluated for early-season cold tolerance.

Controlled environment cold tolerance assessment using Panel 2

Assessment of the seedling cold tolerance trait under controlled temperature conditions, as measured by final percent germination (FGP), germination index (GI), and root biomass accumulated (RBM) shows that there is variation in the population for these traits (Figures 2.4). Germination index (GI) measures the relative rate of germination, thus, lower values of GI would be desirable for selection. The GI distribution (Figure 2.4A) shows that the majority of the individuals selected performed much better than the cold-sensitive parent (BTx623) and only some performed better than the cold-tolerant parent (HKZ). In addition, when compared to the cold tolerant parent (HKZ), the distribution of the root biomass trait (Figure 2.4B) shows transgressive segregation suggesting that certain recombination events gave rise to improved genetics for this trait. Results of FGP (final germination percent) at 15˚C showed HKZ (FGP = 84%) to be superior to BTx623 (FGP = 48%). The RIL population showed transgressive segregation for this trait (Figure 2.4C).

A comparison of the three traits measured in the chamber study (Table 2.2) shows FGP and root biomass (SRBM) to be positively associated with each other ($r = 0.58$). Additionally, germination index; a relative measure of the rate of germination that is based on number of seedlings emerged per day, although weaker than FGP, also shows positive association with SRBM ($r = 0.24$). Regression analysis of FGP and GI as predictors of root biomass showed FGP to be the best predictor of the two for root biomass with a regression coefficient value of 0.32 ($p<0.0001$) compared to 0.05 for GI. When regressed in combination, the two traits accounted for 38% of the variability in the accumulated root biomass by weight.

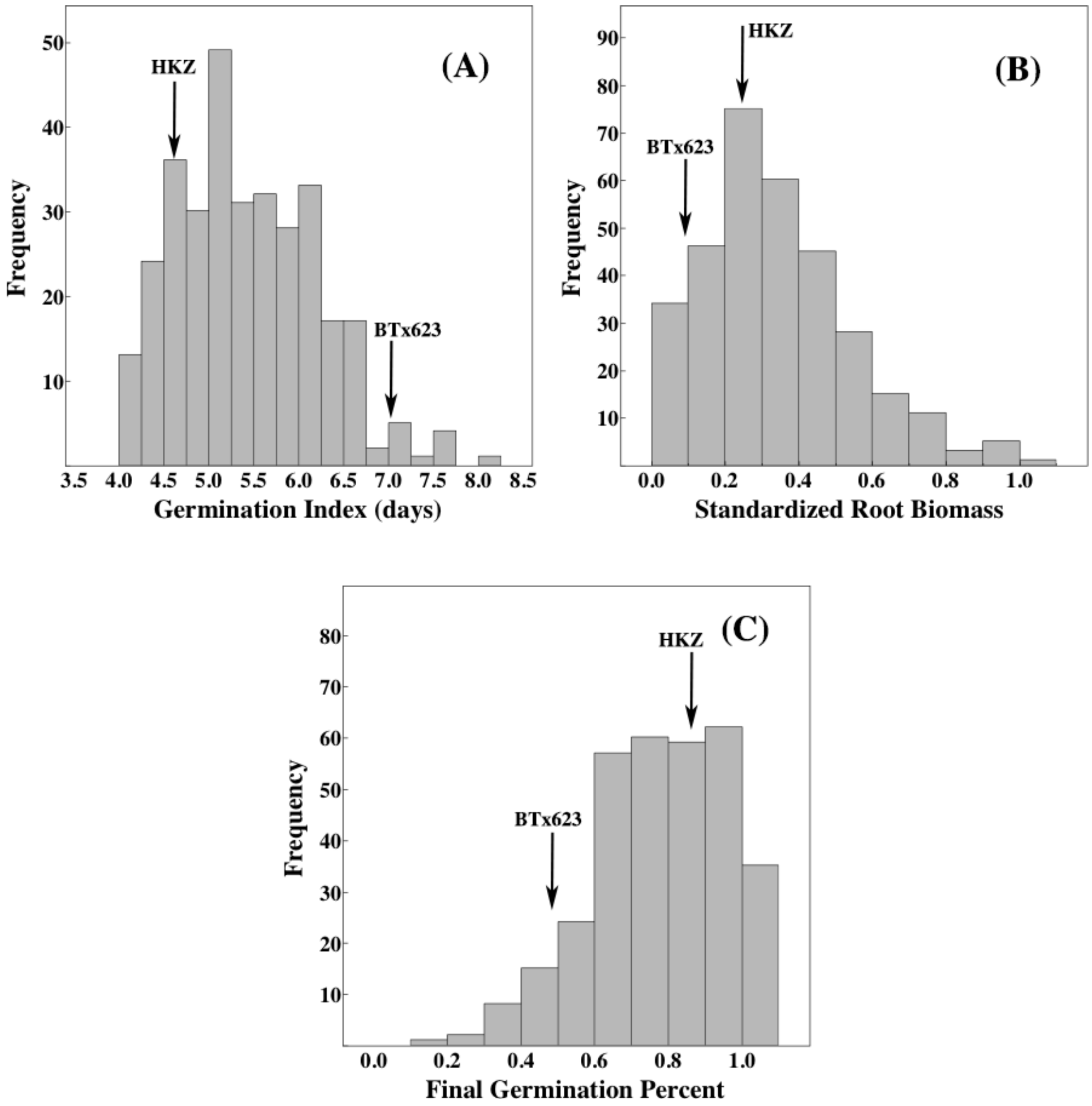

Figure 2.4 - Statistical distributions of measured traits for Panel 2; (A) Germination index, (B) Standardized root biomass, and (C) Final germination percent of a BTx623*HKZ RIL population seeds after 12 days of growth at constant 15˚C under dark conditions.

Table 1.2 - Significant Pearson relations between assessed traits in Panel 2 of a BTx623*HKZ RIL seeds evaluated in controlled chamber for cold tolerance at 12 days under 15˚C

	§FGP	GI	SRBM
FGP	-	-	-
GI	ns	-	-
SRBM	0.58	0.25	-

§FGP, Final Germination Percent; GI, Germination Index; SRBM, Standardized Root Biomass. "ns" indicates non-significant at $p<0.05$, N=307.

Field cold tolerance evaluation using Panel 2

The average daily soil and air temperatures during the field study of the 31 RILs were well below optimum as can be seen in Figure 5. The optimal temperature for sorghum is 27˚C. The soil temperature averaged 17˚C and the air temperature averaged 22˚C during the 30-day trial period indicating that conditions were sufficiently cold to assess seedling cold vigor.

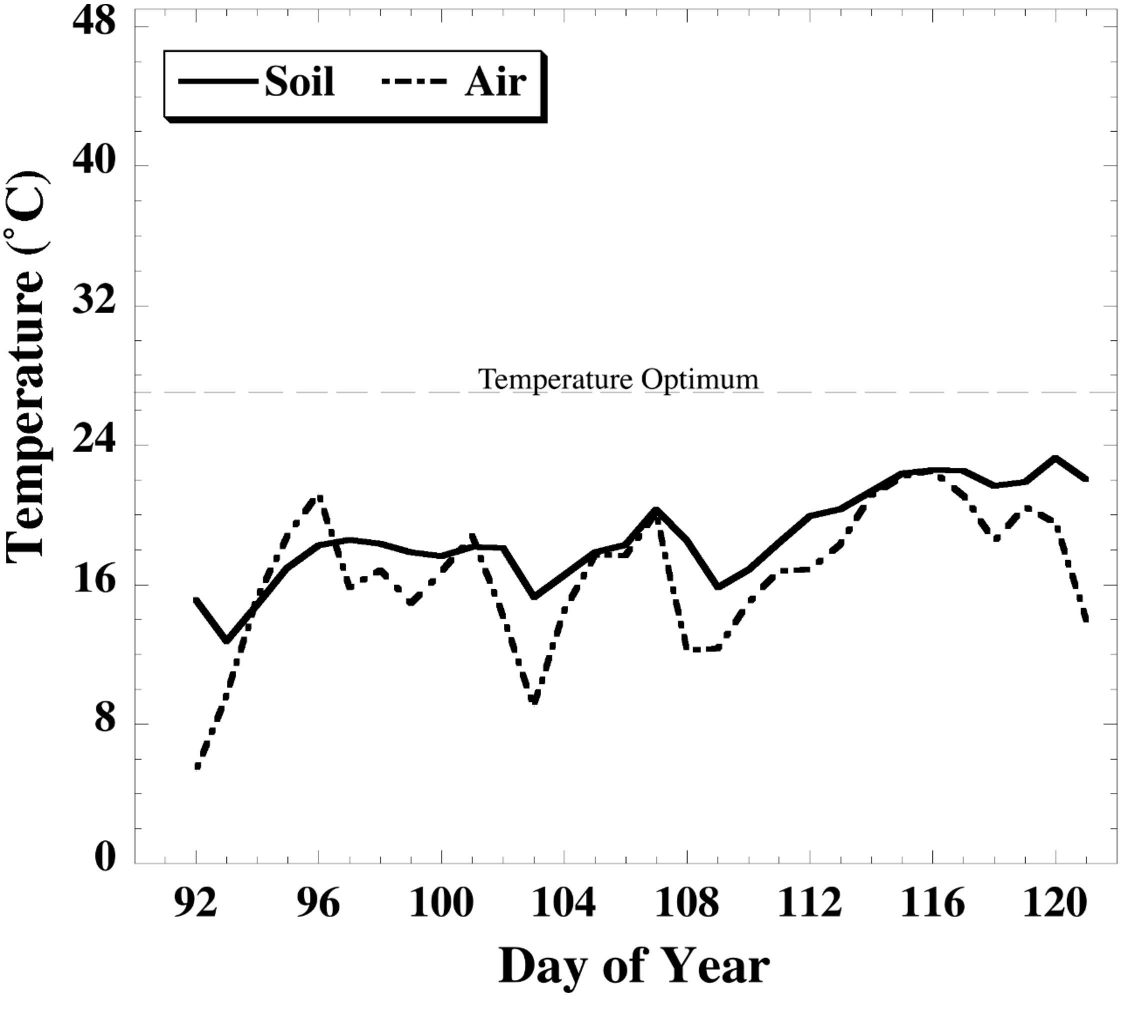

Figure 2.5 - Average daily soil and air temperatures from April 01, 2016 to April 30, 2016 at the USDA-ARS farm in Lubbock, Texas.

All of the traits assessed under the cold field conditions showed significant interactions with each other (Table 2.3); suggesting that seedlings that were sensitive to the cold soil conditions were growing and germinating at reduced rates compared to their cold tolerant counterparts. Additionally, analysis of the final germination percent (FGP), germination index (GI), and root biomass (RBM) results supported the findings of the controlled temperature (15˚C) study. FGP and GI both showed positive correlation with RBM of 0.47 and 0.33, respectively. These values were similar to the results of the cold chamber study for the two measured traits. Root biomass correlated well with total biomass (TBM) and shoot biomass (SBM), 0.93 and 0.74, respectively. Vigor rating (VGR), which is a visual and therefore subjective score of the growth of the seedlings at 30 DAP, also showed positive correlations with all the parameters measured. This is not surprising since several growth parameters are used in the visual scoring (e.g. overall seedling health, stand establishment, chlorophyll accumulation of the seedlings (based on visual assessment), and seedling height). Regression analysis of shoot biomass (SBM) and final germination percent (FGP) as predictors of root biomass (RBM) showed significant effects with SBM accounting for 54% of the variability in the response and FGP accounting for 19% of the variability. When the two traits were regressed together against root biomass, the R^2 value was 0.54.

Table 2.3 - Significant Pearson correlations between assessed traits in Panel 2 of a BTx623*HKZ RIL population evaluated in the field for early-season cold tolerance

	[§]GI	TBM	SBM	RBM	FGP	VGR
GI	-	-	-	-	-	-
TBM	0.42	-	-	-	-	-
SBM	0.45	0.94	-	-	-	-
RBM	0.33	0.93	0.74	-	-	-
FGP	0.46	0.56	0.56	0.47	-	-
VGR	0.50	0.42	0.44	0.33	0.46	-

[§]GI, Germination Index; TBM, Total Biomass; SBM, Shoot Biomass; RBM, Root Biomass; FGP, Final Germination Percent; VGR, Vigor Rating (1 = Excellent, 5 = poor). Significance at $p<0.05$, N=31.

CHAPTER III

EVALUATION OF THE QTL MARKERS

Introduction

The main objective in agricultural research is to increase production of a given crop within the environmental and resource constraints under which cultivation occurs. The global movement of sorghum to various regions outside of its original domestication has served to provide for vast genetic diversity within the crop. This genetic diversity gives current researchers the ability to move beneficial traits from exotic germplasm sources into new breeding lines in an effort to increase production. These traits include biotic and abiotic stress tolerance, agro-morphological traits such as panicle architecture, seed quality, seed size, seed color, plant stature, and maturity. The challenge that breeding programs now faces is the efficient and timely introgression of these desirable traits into hybrids.

The complex and random nature of inheritance patterns in genetics makes developing large populations a necessity. The modern breeder therefore encounters the problem of having to visually select elite germplasm from field populations that often number in the thousands. Variability in environmental conditions compounds to the difficulty in making field selections. Controlled temperature studies in growth chambers or greenhouses to select for a particular trait provide for more accurate screening and selection. However, screening large populations using controlled environment studies, while feasible, will necessitate the use of increased human resources to generate data sets that are reliable. Therefore, screening of large populations for a trait of interest could benefit from an additional component that could reduce the population numbers and thus make chamber and field studies more efficient. Given this paradigm, it is therefore essential that methodologies be developed to expedite and improve the efficiency of introgressing beneficial genetic traits into elite germplasm (Collard and Mackill, 2008).

The recent advances in high throughput DNA and RNA sequencing platforms has revolutionized the study of the genetic mechanisms that control and direct plant responses to the environment and thus impact plant productivity. Marker assisted selection (MAS) is one such methodology that has taken advantage of using high throughput DNA sequencing platforms. Breeding involves the mating of plants and consequently the transfer of genetic material from one plant to another, thus generating a vast array of genetic combinations. By being able to quickly, accurately, and efficiently detect changes in the genetic sequence of thousands of individual plant samples at a time, researchers can now harness the power of this information by statistically analyzing the correlation of the genetic information with many phenotypic responses of interest. This analysis results in finding particular regions of a chromosome that highly correlate with the phenotypic response of interest. These regions are termed quantitative trait loci (QTL). The actual region may be marked by the presence or absence of a simple sequence repeat (SSR) or by a single base change within the genetic sequence in that region. The latter is referred to as a single nucleotide polymorphism (SNP). By studying the correlation of the QTL marker to the phenotypic response, QTL markers are discovered that may be used to select for a specific phenotypic response or trait, a trait such as seedling cold tolerance. By combining controlled temperature studies, field studies, and QTL analysis, accurate selection for a particular trait of interest may be improved. The present study sought to test 5 QTL markers that were recently published by Chopra et al. (2017) and said to be putatively involved with seedling cold tolerance in sorghum. Testing of the markers involved performing simple statistical analysis of the segregation of the genotype of the 31 RILs previously mentioned versus various phenotypic responses under cold soil conditions, namely final germination percent (FGP), total biomass (TBM), shoot biomass (SBM), root biomass (RBM), and vigor rating (VGR). Our objective was to determine if any of these markers could in fact be used to select for seedling cold tolerance in sorghum.

Materials and Methods

Field Study and Measurement of Phenotypic Responses

31 RILs from Panel 2 were selected to perform a field study that would assess the seedling cold tolerance trait. 50 Seeds of each RIL and parents were planted on April 01, 2016 at the USDA-ARS farm at Lubbock, Texas. Single row plots per RIL of 4.57 m in length and 1.02 m row spacing was used. The experimental design was Randomized Complete Block with 3 replications per RIL. Germination counts were taken daily beginning at 8 days after planting (DAP). Vigor ratings were taken at 30 DAP using a visual rating on 1 to 5 scale with 1 = robust vigor and 5 = poor vigor (Maiti et al., 1981). Root and shoot biomass were determined from 20 randomly selected plants at 30 DAP. JMP 12.1 was used for statistical analysis.

Genotype Determination Using KASP (Kompetetive Allele Specific PCR)

DNA was extracted from dry seed of each of the 31 RILs. Briefly, 10 seed were placed into a 2.0 mL Safe-Lock micro-centrifuge tube (Eppendorf, USA). One tungsten bead was placed into the tube and the seed was ground by vigorous shaking of the tubes using a Tissue Lyser II (Qiagen, USA) unit set to 30 Hz for 1 minute, 3 cycles were performed to ensure seed was sufficiently ground. DNA was extracted using a modified commercial protocol that was developed in house at the USDA-ARS laboratory (Chopra et al., 2017). Kompetitive Allele Specific PCR; KASP (LCG Genomics, London, U.K.) genotype analysis was used to determine allele data for each marker for each of the 31 RILs. The assay was performed per the instructions given in the literature supplied by the kit. KASP is a simple PCR method that uses a fluorescence-based reporting signal to detect single nucleotide polymorphisms (SNPs) within a population (He and Anthony, 2014). The SNP markers used are published in Chopra et al. (2017). They include S6_43169342, S6_53848131, S6_54030245, S6_54057566, and S3_59699677. These five markers were tested with the two parents (HKZ and BTx623) and found to be polymorphic and therefore useful in screening our 31 RILs.

Results

Genotype Determination Using KASP (Kompetetive Allele Specific PCR)

KASP genotyping analysis of the 31 RILs showed only QTL markers S6_53848121 and S6_54057566 segregating homozygous for the HKZ allele, homozygous for the BTx623 allele and heterozygous for both alleles (Table 3.1). QTL markers S6_43169342 and S3_59699677 showed no segregation as all RILs showed to have the HKZ allele only. QTL marker S6_54030245 showed a dispersed and random segregation pattern that could be a function of the primer having non-specific binding to the template leading to the observed random segregation.

Table 3.1 - KASP genotyping result of 31 BTx623*HKZ RILs evaluated under cold field conditions

SNP Marker	HKZ allele	BTx623 allele	Heterozygous
S6_43169342	31	0	0
S6_53848121	**15**	**6**	**10**
S6_54030245	15	16	0
S6_54057566	**15**	**6**	**10**
S3_59699677	30	0	1

QTL marker list from Chopra et al. (2017)

Genotype x Phenotype Correlations

The two QTL markers that exhibited segregation for the 3 genotypic alleles, S6_53848121 and S6-54057566, were evaluated against the various phenotypic traits to determine if any one of the traits correlated with the presence of the allele from the cold tolerant parent (HKZ). One-way ANOVA analysis using JMP 12.1 Fit X by Y function was performed at $\alpha = 0.05$. Root biomass per plot (RBM/plot) and root biomass per plant (RBM/plant) showed significant separation of the means by genotype (Table 3.2). That is, higher root biomass correlated with the HKZ genotype allele and lower root biomass correlated with the BTx623 and heterozygous genotype alleles. Analysis of the means for RBM/plot and RBM/plant using Fisher's Student t-test showed the HKZ allele group to have higher root biomass per plot and higher root biomass per plant than the BTx623 and heterozygous allele groups. The other phenotypic responses measured did not have significant separation by genotype.

Table 3.2 - Variation of phenotypic traits across genotypic alleles serving as possible markers for early-season cold tolerance in a BTx623*HKZ population

Genotype	Root BM/plant	Total BM/plot	Shoot BM/plot	Root BM/plot
	(g)			
BTx623 allele	0.29b† ± 0.021	26.9 ± 2.7	16.6 ± 1.9	10.3b ± 0.91
HKZ allele	0.41a ± 0.025	32.3 ± 2.1	17.1 ± 1.1	15.1a ± 1.1
Het allele	0.34ab ± 0.030	25.6 ± 2.5	15.0 ± 1.3	10.9b ± 1.2
BTx623 parent	0.16 ± 0.045	1.64 ± 0.32	0.83 ± 0.27	0.81 ± 0.11
HKZ parent	0.44 ± 0.11	43.3 ± 9.6	22.9 ± 4.5	20.4 ± 5.1

Means ± standard error
†Letter values within a column with the same letter are not significantly different at $\alpha = 0.05$

DISCUSSION

Research towards improving the genetics of sorghum so as to enhance its productivity under a variety of abiotic stresses is ongoing. The goal of breeders is to improve the genetics that allow for improved morphological traits that affect productivity (i.e. grain yield) and also those that allow for abiotic stress tolerance and thus yield stability. Tesso et al. (2011) in their study of dhurra sorghums from Ethiopia, found that almost all of the phenological and morphological traits they measured among a diverse panel of 200 accessions across two environments had significant influence on yield, as measured by panicle weight and panicle seed weight. They suggested that targeted breeding for improved yield component traits such as panicle size would improve yield.

Within the U.S. Sorghum Belt, which extends form South Dakota to the southern tip of Texas, early season planting of sorghum would take advantage of winter precipitation and provide for less insect pressure during the reproductive period of the crop. Early season planting could also extend the growing season and potentially provide for higher yields in late maturing varieties. However, early season planting would subject the seedlings to cold soils during germination and to cold ambient temperatures that would affect growth. Chlorosis in young seedlings, poor establishment, and a restriction of growth are some of the symptoms of chilling injury in sorghum (Peacock, 1982). A study of the relative growth responses of sorghum to chilling stress found that chilling injury can lead to decreased photosynthesis and thus slower growth (Ercoli et al., 2004). Additionally, the study found that plant shoot growth was very sensitive to chilling exposure. Sorghum is a tropical plant by origin and as such suffers from sensitivity to cold temperatures during germination and growth. To make early season planting a reality, sorghum hybrids would need to be developed that could tolerate the cold soil temperatures but could also continue vegetative growth under cooler ambient temperatures. Thus, to improve germinability, emergence, and active growth under cold soil and ambient conditions, the introgression of the seedling cold tolerance trait into existing sorghum hybrids is necessary. Sources of seedling cold tolerance are present within the existing germplasm collections of sorghum that are available to breeders. In particular, the Chinese Kaoliang land race has been shown to have excellent cold germinability and growth (Franks et al., 2006, Kapanigowda et al., 2013). The development of RIL (recombinant inbred line) populations that contain the Kaoliang genetics for seedling cold tolerance could prove useful in improving stand establishment under the cold soil conditions present in temperature environments. Selected RILs that show enhanced seedling cold tolerance when compared to the Kaoliang parent could then be used to introgress the trait into existing elite hybrids. An additional component of the Kaoliang land race is that it has very large panicles that could prove beneficial in improving the productivity of sorghum. As mentioned earlier, panicle size has been suggested as a selection target for improving grain productivity in sorghum. In the present study, an RIL population was developed from a cross between BTx623 and Hong Ke Zi (HKZ). BTx623 is a tropical line of short stature, thick stem, and compact panicle architecture. HKZ is Chinese Kaoliang land race sorghum with excellent cold germinability and growth under cold soil and cool ambient conditions. The developed population was used to determine the presence of improved yield component traits within the population, to select individuals with improved seedling cold tolerance and improved yield components, and to test 5 QTL markers for their use in marker assisted selection for the seedling cold tolerance trait.

In our study, correlation analysis of Panel 1 for the importance of improved morphological components on productivity as measured by panicle weight (PWT) showed that stem diameter (SDM), panicle length (PNL), and panicle width (PWD) had significant effects on PWT (r^2=0.42, 0.48, and 0.83, respectively). Regression analysis of these three traits also showed that these traits, in combination, are good predictors of grain yield ($R^2 = 0.72$). Because yield is a complex trait under multi-genetic control, combining multiple morphological traits that contribute to yield could prove more beneficial and perhaps more stable than a single trait. Blum (1970, 2004), indicated that the most consistent and stable trait for exhibiting the presence of heterosis with respect to increased yield was an increase in the kernel number per panicle. In addition, Blum indicated that the effect of heterosis on increased panicle grain number, and therefore yields, is related to panicle structure, i.e. the number of florets per panicle. Tesso et al. (2011), in the study of dhurra sorghums of Ethiopia, also found a significant correlation between panicle weight and panicle width. Additionally, these researchers suggested that the use of panicle size as a focus of selection for improved yield in the dhurra sorghums could prove useful. Our study is in agreement with the studies mentioned as our results also showed significant correlation between the panicle structure parameters, PNL and PWD and panicle weight (PWT). Panicle weight (PWT) showed a highly significant correlation of 0.99 and linear regression of 0.98 with panicle seed weight (PSW) indicating that panicle weight can be used as a proxy measure of yield. Based on the correlation results of the effects of PNL, PWD, and SDM on panicle weight (PWT), our selection criteria for Panel 2 (i.e. thick stem and large panicle) turned out to be well founded for selecting individuals with increased productivity potential.

Evaluation of the cold tolerance trait under controlled temperature conditions for Panel 2 showed that root biomass had significant correlation with final germination percent (FGP) and germination index (GI); r=0.58 and 0.23, respectively. A correlation analysis of these same traits under field conditions also showed a similar correlation, r=0.47 for FGP and r=0.33 for GI. These results point to the importance of a proper root structure for a developing seedling. Blum (2004) indicated that the emergence of a sorghum seedling is dependent on the extension of both the mesocotyl and hypocotyl. He went on to state that in order for a seedling to develop into a growing plant, a transition from the dependence on the seminal root, which is only viable for about 30 days, to dependence on crown roots is essential for successful seedling establishment. Radford and Henzell (1990) found that elongation under the soil surface was more a consequence of the growth of the mesocotyl. Consequently, an actively growing seedling is necessary for proper emergence from the seedbed, especially under cold soil temperatures. Given this reality, an RIL with a low germination index (GI) under cold soil conditions would be desirable. A low GI value indicates that seedlings germinated at a relatively faster rate than those with a high GI value. These seedlings would be accumulating more root and shoot biomass and therefore be able to make the transition from seminal to crown root dependence much sooner. This can be seen by the significant correlation (r=0.42) of germination index and total plant biomass in our field study of the 31 RILs. Faster growing and emerging seedlings would then avail themselves to the available water and nutrients in the soil profile and continue growth. Yu et al. (2004) found significant correlation of shoot dry weight and root dry weight with emergence in a controlled temperature study. Additionally, when they compared results from a plate based chamber assay under cold conditions to field results for the same germplasm, they found significant correlation between root length (r^2=0.27), shoot length (r^2=0.49) to field emergence. This suggests seedlings with cold tolerance may emerge first and thus begin active growth compared to cold sensitive seedlings. McLaren and Rijkenberg (1991) found that cold soils often promote seedling disease and that by reducing the time from planting to emergence the incidence of disease was reduced. They recommended that early season planting should occur when soil temperatures are optimal for seedling growth to reduce the incidence of seedling diseases. By using cold tolerant seedlings that can grow under suboptimal soil and air temperatures, seedling disease can be avoided. Results from our field study indicate that those seedlings that carried the seedling cold tolerant trait had increased positive growth under the prevailing soil and air temperature conditions. This can be seen by the significant correlation between FGP and biomass measurements. These traits were direct measures of growth and thus seedling vigor. Vigorous seedlings exhibited improved total biomass, improved shoot biomass, improved root biomass, improved final germination percent, improved germination index, and low vigor ratings.

Genotyping of the 31 F_4 RILS using the KASP genotyping technology indicated that only two of the five markers tested showed segregation for the HKZ, BTx623, and the heterozygous genotype. Significant separation of the three genotypic groups (homozygous for HKZ allele, homozygous for BTx623 allele, and heterozygous for both alleles) was seen with the root biomass per plot phenotype only. That is, RILS that contained the homozygous HKZ (cold-tolerant) allele had significantly more root biomass than did RILS that were homozygous for the BTx623 allele or heterozygous for both alleles. This result indicates that the two markers tested could be potential candidates in selecting for the seedling cold vigor trait using marker-assisted selection (MAS). Though this result is promising, the markers need to be tested against a larger pool of diverse individuals to observe if the markers are truly viable as a selection tool.

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APPENDICES

Appendix A – Panel 1 Morphological Trait Data

Row-Range	Plant No.	# Leaves	Stem Diameter	Plant Height	Panicle Length	Panicle Width	Panicle Weight	Seed Weight per Panicle
			-----------------------------	(cm)	-------------------------		---------- (g)	---------
1-1	5	16	1.73	246.0	21.5	4.0	20.5	13.2
1-1	8	12	1.51	148.5	15.0	6.0	42.0	31.9
1-1	9	13	1.83	255.0	21.0	6.0	62.0	43.2
1-1	10	15	1.93	301.0	26.0	5.5	62.1	47.6
1-2	15	15	2.10	196.0	30.0	8.0	97.0	80.0
1-2	17	17	2.15	332.5	26.0	4.5	20.0	5.1
1-2	18	16	2.02	287.5	23.0	5.0	54.5	36.3
1-2	20	14	2.40	127.0	22.5	5.5	42.0	30.1
2-1	1	14	2.40	108.0	19.0	4.0	31.0	22.6
2-1	2	12	2.22	159.5	19.0	5.0	62.5	50.4
2-1	4	-	1.41	186.5	17.0	3.5	15.6	10.3
2-1	6	13	2.04	239.0	20.5	6.0	66.0	49.1
2-2	12	15	1.31	270.5	24.0	4.0	15.5	7.5
2-2	14	15	2.04	250.0	23.0	6.0	97.7	78.7
2-2	16	14	2.28	252.0	19.0	7.0	121.5	94.4
2-2	18	17	1.70	206.0	23.5	3.5	21.5	11.5
2-2	20	-	2.01	343.0	32.0	7.0	84.5	66.2
3-1	2	14	1.68	126.0	17.0	1.0	2.5	0.7
3-1	5	16	1.84	189.0	19.0	4.0	47.5	36.4

3-1	10	18	2.62	283.5	25.5	5.5	53.5	38.7
3-2	13	14	1.89	220.0	31.0	3.0	14.5	3.6
3-2	14	12	2.08	139.0	26.0	5.5	64.0	49.8
3-2	17	15	2.21	246.0	18.0	3.5	12.5	4.7
3-2	19	15	1.43	281.0	26.0	8.5	105.1	83.9
4-1	2	15	1.34	231.5	20.0	2.5	7.5	2.2
4-1	3	14	2.07	228.0	23.0	6.0	93.6	74.4
4-1	4	12	1.60	186.0	15.0	4.0	25.5	20.3
4-1	6	13	1.18	150.0	20.5	4.5	30.5	22.6
4-1	7	16	2.42	317.0	25.5	7.0	92.0	67.9
4-1	9	15	1.73	125.0	17.0	3.0	10.5	7.1
4-2	12	12	1.62	186.0	20.0	3.5	24.5	17.5
4-2	13	13	2.05	136.5	17.0	4.0	43.6	30.7
4-2	15	14	1.16	200.5	9.5	2.0	2.5	0.9
4-2	16	11	1.88	168.5	28.0	5.5	69.0	52.9
4-2	17	15	2.20	303.5	20.5	6.5	70.0	53.9
4-2	18	14	1.20	275.5	22.0	4.5	17.3	7.3
4-2	19	14	1.63	330.0	25.0	5.5	63.6	43.0
5-1	2	13	1.53	100.5	20.0	1.5	12.5	8.5
5-1	3	17	2.46	173.5	27.0	6.5	85.5	69.2
5-1	4	14	1.58	140.0	16.0	4.0	21.5	15.1
5-1	5	15	2.57	188.0	24.0	8.0	94.0	77.5
5-1	6	15	2.34	221.5	22.5	6.5	115.5	91.0
5-1	8	15	1.91	228.0	11.0	2.0	5.0	4.3
5-1	9	13	2.17	282.5	24.5	6.5	68.0	52.9

5-1	10	15	2.04	291.0	21.0	6.0	66.5	44.9
5-2	11	15	2.17	267.0	24.0	7.0	91.5	71.4
5-2	12	17	2.30	294.0	21.0	7.0	92.5	71.8
5-2	14	17	2.24	295.5	21.5	7.0	89.5	70.5
5-2	16	13	1.99	282.0	21.0	7.0	90.5	71.7
5-2	17	14	1.62	258.5	18.0	7.5	86.5	73.0
5-2	18	16	2.47	306.0	26.0	6.5	100.5	79.9
5-2	19	11	1.77	262.0	21.0	6.0	61.5	42.9
5-2	20	14	2.37	-	24.5	7.0	66.5	38.7
6-1	1	14	1.30	223.5	22.5	7.0	72.4	56.8
6-1	3	13	1.64	221.0	25.0	5.0	27.5	20.3
6-1	7	15	1.36	216.5	16.0	3.5	30.0	20.8
6-1	9	15	2.24	256.5	22.0	6.0	71.7	54.9
6-1	10	14	2.06	248.0	23.0	5.0	84.6	65.9
6-2	12	15	1.92	253.5	20.0	3.0	9.5	2.6
6-2	13	15	1.99	227.0	18.0	4.5	43.3	32.1
6-2	14	13	1.44	253.0	24.5	6.0	44.5	31.9
6-2	15	17	2.25	343.0	25.0	7.0	101.0	71.9
6-2	16	12	2.29	153.0	25.5	6.0	72.5	52.5
6-2	18	12	1.78	201.5	27.0	7.0	67.5	50.7
6-2	19	12	2.08	106.0	16.5	7.0	51.0	39.9
6-2	20	14	2.23	169.0	23.0	8.0	85.5	67.4
7-1	1	14	2.02	278.0	20.5	5.0	48.0	35.0
7-1	2	13	1.59	256.5	16.0	3.5	35.0	24.7
7-1	4	15	2.06	271.0	19.0	3.5	11.5	2.5

7-1	6	14	1.83	273.0	32.0	6.0	84.0	69.7
7-1	7	14	2.39	240.0	24.0	8.0	81.5	59.2
7-1	8	18	2.34	279.0	25.0	8.0	87.5	67.7
7-1	10	17	2.10	302.0	24.0	6.5	75.0	61.1
7-2	14	15	2.12	199.5	18.0	5.5	67.0	49.3
7-2	16	15	2.35	312.5	28.0	8.0	95.0	72.3
7-2	17	15	1.86	315.5	30.0	8.0	120.5	100.0
7-2	18	14	1.83	264.5	28.0	8.0	73.0	54.3
7-2	19	13	1.89	141.0	21.0	5.0	51.5	37.7
8-1	2	15	1.72	253.5	25.0	7.0	80.5	63.1
8-1	4	15	1.56	295.0	25.5	5.5	70.0	56.7
8-1	5	15	1.64	102.0	23.5	7.0	78.1	61.3
8-1	6	19	1.87	318.5	20.5	5.5	64.5	52.0
8-1	7	15	1.75	-	24.5	8.5	117.5	92.3
8-1	8	15	2.35	169.0	19.0	7.0	60.0	54.2
8-1	9	17	2.55	224.0	13.0	3.0	21.0	15.1
8-1	10	15	2.39	350.0	26.0	7.0	87.5	62.1
8-2	12	13	2.08	193.0	18.0	5.0	43.5	31.8
8-2	13	14	1.56	280.5	18.0	3.5	18.0	12.5
8-2	15	12	1.80	147.0	24.0	6.0	34.0	25.3
8-2	16	18	2.65	298.0	25.5	8.0	84.5	66.2
8-2	17	13	1.00	124.5	15.0	2.5	6.5	3.3
9-1	2	14	2.19	248.5	22.0	5.5	35.5	27.3
9-1	3	11	1.65	125.0	15.5	3.5	15.0	9.3
9-1	4	11	1.06	196.5	14.5	3.5	23.0	16.2

9-1	6	16	1.58	279.5	17.0	3.0	15.0	10.1
9-1	9	16	2.27	291.5	24.5	7.5	75.0	60.6
9-2	12	14	2.32	293.0	28.0	7.5	107.5	85.3
9-2	13	14	2.43	306.0	23.0	7.5	122.0	95.8
9-2	19	15	2.21	248.0	22.0	5.0	52.8	39.2
10-1	1	15	1.53	308.5	23.0	6.0	86.0	67.3
10-1	3	15	2.22	223.0	31.5	8.0	64.5	54.4
10-1	5	17	2.91	208.0	28.0	8.0	114.5	90.4
10-1	6	15	1.94	304.5	26.0	6.0	62.5	48.1
10-1	7	13	1.94	211.5	22.0	7.5	81.8	65.4
10-1	9	18	2.37	243.0	21.0	7.0	92.8	72.3
10-2	11	13	2.27	300.0	29.0	7.5	81.5	61.4
10-2	12	15	2.40	214.0	21.0	7.0	84.1	68.1
10-2	13	12	2.55	293.0	23.0	7.5	71.5	54.7
10-2	14	14	2.40	227.0	20.0	6.5	53.5	42.7
10-2	15	14	1.66	235.0	24.0	4.0	21.5	10.9
10-2	17	15	2.02	260.5	22.0	3.0	27.0	16.6
10-2	19	16	2.25	166.5	19.0	6.0	82.4	71.2
11-1	2	14	2.21	247.0	22.0	6.5	93.2	77.4
11-1	4	15	2.16	300.5	24.0	9.5	83.5	64.3
11-1	5	14	2.32	139.5	18.0	6.0	56.5	44.6
11-1	7	14	2.61	-	22.0	6.0	73.5	57.2
11-1	8	16	2.23	248.5	20.0	8.0	108.5	93.0
11-1	9	13	1.77	338.0	27.5	4.5	29.2	19.1
11-2	12	13	1.69	265.0	22.0	5.0	42.5	29.9

11-2	15	16	2.19	287.0	21.0	4.0	18.5	-
11-2	16	11	1.89	239.0	23.0	9.0	107.4	84.4
12-1	2	14	1.10	243.5	16.0	4.0	21.5	16.2
12-1	3	13	1.66	270.0	22.0	6.0	53.0	41.7
12-1	6	15	2.13	311.5	28.0	6.0	104.2	68.9
12-1	7	13	2.10	301.0	23.0	8.0	85.5	69.0
12-1	8	14	1.39	215.0	17.0	4.5	19.7	12.2
12-2	11	14	1.89	185.5	18.0	6.5	51.5	39.9
12-2	12	14	1.83	257.5	22.0	7.0	109.8	86.9
12-2	13	14	1.76	258.0	22.0	8.5	67.5	51.2
12-2	14	17	2.82	-	17.0	5.0	32.0	-
12-2	15	12	1.73	215.0	-	-	13.6	
12-2	19	16	2.70	141.0	21.0	5.5	76.3	61.7
12-2	20	17	3.48	-	26.0	4.5	21.0	-
13-1	1	14	2.17	229.5	18.0	4.5	62.0	50.4
13-1	2	15	2.24	263.5	19.0	7.0	64.5	46.9
13-1	3	14	1.62	270.5	28.5	3.0	13.5	6.2
13-1	4	14	2.30	292.0	25.0	7.5	97.0	84.4
13-1	5	15	2.20	229.5	21.5	7.5	66.5	51.7
13-1	8	16	2.14	342.0	29.0	7.0	100.0	80.7
13-2	11	13	1.83	303.0	24.0	8.0	89.9	73.0
13-2	12	15	1.91	275.0	26.0	9.0	99.9	82.9
13-2	14	16	2.32	268.0	17.0	4.0	39.6	28.9
13-2	15	14	2.07	244.0	18.5	8.0	98.0	81.0
13-2	16	14	2.12	239.0	22.0	7.5	59.0	48.8

13-2	18	16	2.35	216.0	27.0	9.0	112.5	93.2
13-2	19	15	2.20	189.0	21.0	4.0	15.5	4.5
13-2	20	15	2.16	277.0	23.0	7.5	82.5	66.6
14-1	2	11	2.24	129.0	19.0	6.5	55.0	44.3
14-1	3	17	2.52	309.0	21.0	8.0	97.0	81.0
14-1	4	16	1.99	304.0	26.0	5.0	30.1	18.4
14-1	5	16	1.97	310.5	27.0	6.0	99.5	82.1
14-1	6	17	2.35	241.5	31.5	6.0	79.0	64.8
14-1	7	12	1.60	283.5	25.0	7.0	88.5	73.4
14-1	8	14	2.33	205.0	27.0	6.5	66.5	54.4
14-2	12	15	1.82	273.0	18.0	7.0	56.5	44.5
14-2	13	13	2.44	189.5	19.0	8.0	58.1	46.4
14-2	14	15	1.81	223.5	26.0	2.5	18.0	9.3
14-2	15	14	2.47	198.0	23.0	7.5	76.8	59.4
14-2	16	15	2.04	231.5	30.0	5.0	65.5	50.0
14-2	17	17	2.51	-	31.0	7.5	103.0	80.0
14-2	18	17	2.91	261.0	24.0	10.0	121.5	98.5
14-2	19	14	1.97	290.0	20.0	7.5	53.8	33.3
15-1	2	12	2.45	174.5	27.0	8.5	98.5	73.7
15-1	4	15	2.10	240.0	20.0	6.0	88.7	69.6
15-1	5	17	2.38	353.5	26.0	7.0	93.0	68.5
15-1	6	15	2.11	231.0	18.0	7.5	71.7	56.7
15-1	7	16	2.43	325.0	21.0	7.0	62.5	45.0
15-1	9	14	2.09	284.0	20.0	6.5	74.5	61.0
15-1	10	17	2.08	252.5	16.5	3.0	14.0	6.5

15-2	11	14	2.59	201.5	25.0	7.5	82.0	65.1
15-2	12	11	1.86	114.0	18.0	6.0	23.5	16.1
15-2	13	13	2.58	140.5	26.0	8.0	107.5	88.1
15-2	14		2.39	321.5	23.0	9.5	136.5	105.1
15-2	18	14	2.12	274.5	27.0	7.5	108.0	89.4
15-2	19	15	1.71	252.5	32.0	7.0	80.5	68.2
15-2	20	17	1.91	288.0	25.5	6.0	75.5	61.8
16-1	1	12	1.83	243.0	19.0	3.0	13.0	6.2
16-1	2	13	2.11	241.5	20.0	7.0	81.2	63.0
16-1	3	13	1.90	217.0	31.0	6.0	95.1	74.7
16-1	4	15	2.14	338.0	28.0	6.5	78.5	60.2
16-1	5	14	2.14	307.0	24.0	9.5	137.6	114.6
16-1	6	15	1.92	171.0	25.0	10.5	52.5	42.1
16-1	7	14	2.64	174.0	22.0	6.0	109.0	88.4
16-1	8	15	1.77	284.0	25.0	7.0	110.5	88.0
16-1	9	12	1.61	259.5	18.0	3.5	10.5	4.8
16-1	10	12	1.64	223.0	20.0	3.5	12.0	6.6
16-2	11	11	1.60	219.0	17.0	6.0	75.3	59.0
16-2	13	15	2.27	192.5	32.0	6.0	48.5	33.4
16-2	15	14	2.45	214.0	31.0	7.0	102.0	82.8
16-2	16	14	2.51	288.0	21.0	5.0	83.0	62.5
16-2	18	16	2.59	266.5	19.0	7.0	63.5	48.0
16-2	20	14	2.12	252.0	18.0	8.0	92.5	76.7
17-1	2	15	1.79	301.5	29.0	7.5	98.0	78.8
17-1	3	13	1.91	295.5	24.0	7.5	98.8	77.8

17-1	4	14	1.92	232.0	21.0	5.0	51.0	39.7
17-1	5	14	2.53	245.0	20.5	5.0	55.0	41.8
17-1	7	14	2.10	253.0	22.5	8.0	112.0	93.7
17-1	8	13	2.27	232.0	19.0	4.0	16.0	7.3
17-1	9	13	2.22	199.0	23.0	6.0	73.5	61.4
17-1	10	15	1.89	240.0	21.0	7.0	89.1	74.5
17-2	12	17	2.14	253.0	27.5	6.5	78.5	59.1
17-2	13	14	1.78	307.0	26.0	8.0	137.5	114.4
17-2	16	14	1.69	225.5	16.0	5.5	58.4	44.0
17-2	17	16	2.33	231.0	25.5	7.5	120.0	97.9
17-2	18	13	1.78	250.5	19.0	5.5	41.0	27.5
17-2	20	14	2.29	183.0	25.0	3.0	14.7	11.8
18-1	2	14	1.45	-	18.0	4.0	24.5	15.5
18-1	3	12	1.91	248.0	21.5	7.0	78.0	58.3
18-1	4	17	2.10	289.0	19.0	7.0	84.5	62.1
18-1	5	13	2.07	216.0	28.0	6.0	66.0	53.1
18-1	9	15	2.38	284.0	29.0	6.0	94.0	73.1
18-1	10	16	2.58	234.0	24.0	5.0	84.5	65.4
18-2	11	17	2.48	158.0	17.5	5.0	58.0	45.9
18-2	14	14	2.24	202.0	29.0	6.0	87.5	73.3
18-2	15	15	2.37	309.0	23.5	4.5	22.1	11.9
18-2	19	12	1.95	256.0	25.0	4.5	25.5	19.4
18-2	20	15	2.29	272.0	21.0	6.0	50.0	33.0
19-1	1	15	2.04	283.0	20.5	6.5	76.5	59.6
19-1	2	17	2.13	250.0	19.5	7.0	100.0	82.8

19-1	3	15	2.34	283.0	22.5	7.0	113.5	94.2
19-1	4	15	2.27	284.0	23.0	7.0	90.3	72.8
19-1	7	16	2.32	247.5	24.5	8.0	125.5	102.1
19-1	8	14	1.99	297.0	21.0	6.5	57.0	42.1
19-1	9	16	2.43	288.0	23.0	7.0	95.2	74.2
19-1	10	14	1.98	274.5	21.0	6.0	56.0	40.7
19-2	12	15	2.19	277.5	29.0	7.5	135.0	112.5
19-2	13	13	2.07	168.0	16.0	3.5	24.5	17.8
19-2	16	13	2.43	224.0	25.0	6.5	70.0	55.3
19-2	17	16	2.44	310.0	27.0	10.0	122.0	101.6
19-2	18	15	2.16	252.0	22.0	5.0	76.3	54.1
19-2	19	15	2.01	255.0	19.5	6.5	77.4	62.6
19-2	20	17	1.92	273.0	19.0	6.5	83.0	67.3
20-1	1	13	1.65	197.0	15.0	4.5	26.5	20.6
20-1	3	14	1.67	219.0	23.0	6.0	77.0	61.2
20-1	4	16	1.86	300.0	22.0	8.0	115.5	91.2
20-1	5	13	2.01	200.0	19.0	5.0	46.9	36.9
20-1	6	16	2.15	179.5	22.0	6.0	71.0	58.2
20-1	7	13	2.16	212.0	17.0	7.0	57.5	45.8
20-1	8	11	1.76	227.0	21.0	4.0	10.5	4.1
20-1	9	14	1.92	213.0	22.0	8.0	86.5	70.4
20-1	10	12	2.36	163.0	11.0	5.0	43.0	31.3
20-2	14	14	1.93	201.0	17.0	4.0	10.5	3.5
20-2	15	16	2.42	234.5	24.0	7.0	109.5	89.0
20-2	16	15	1.93	210.0	21.0	6.0	11.0	2.1

20-2	17	11	2.22	231.0	23.0	7.0	92.0	72.7
20-2	18	12	1.72	205.0	18.0	6.5	71.6	54.5
20-2	20	14	2.52	215.5	23.0	6.0	70.3	54.1
21-1	1	13	1.61	223.0	21.0	7.0	62.5	53.5
21-1	3	16	2.59	258.5	23.0	8.0	119.5	95.7
21-1	7	11	1.45	224.0	19.0	4.0	29.5	19.9
21-2	12	12	2.38	239.0	16.0	6.5	31.0	20.0
21-2	14	15	1.80	177.5	19.0	5.0	36.5	30.2
21-2	16	14	1.59	228.0	21.0	6.0	54.6	40.5
21-2	17	14	2.75	283.0	20.5	6.0	85.0	65.1
21-2	20	17	2.73	310.5	19.0	6.5	92.5	70.3

Appendix B – Panel 2 Growth Chamber Data at 15˚C; GI – germination index; RFW – root fresh weight; SRFW – standardized root fresh weight (proportion of max minus min); FGP – final germination percent.

RIL No.	Day 4	Day 5	Day 6	Day 9	Day 9	GI	RFW	SRFW	FGP
	---- (daily germination counts) ----				(total)	(days)	(g)		
1	1	6	0	1	8	5.38	0.0893	0.4813	0.80
2	5	2	1	1	9	6.43	0.0635	0.3350	0.90
4	3	2	0	2	7	5.71	0.0678	0.3594	0.70
5	1	5	0	1	7	5.43	0.0485	0.2500	0.70
7	0	4	1	2	7	6.29	0.0670	0.3549	0.70
8	4	4	1	1	10	5.10	0.1586	0.8741	1.00
9	2	7	1	0	10	4.90	0.1137	0.6196	1.00
12	6	2	0	1	9	4.78	0.1293	0.7080	0.90
13	2	3	1	2	8	5.88	0.0918	0.4955	0.80
15	0	6	0	2	8	6.00	0.0504	0.2608	0.80
16	2	5	2	1	10	5.40	0.0635	0.3350	1.00
17	3	3	0	2	8	5.63	0.0496	0.2562	0.80
18	1	4	2	2	9	6.00	0.0642	0.3390	0.90
19	1	0	2	3	6	7.17	0.0503	0.2602	0.60
20	1	4	0	1	6	5.50	0.0525	0.2727	0.60
22	1	5	1	1	8	5.50	0.0966	0.5227	0.80
24	2	5	0	0	6	5.50	0.0259	0.1219	0.70
25	0	7	0	2	9	5.89	0.0922	0.4977	0.90
29	3	3	1	1	8	5.25	0.1129	0.6151	0.89
30	2	3	0	3	8	6.25	0.0301	0.1457	0.80

31	1	3	2	1	7	5.71	0.0201	0.0890	0.70
32	3	2	0	1	6	5.17	0.0505	0.2613	0.60
34	2	2	2	0	6	5.00	0.0444	0.2268	0.60
36	1	1	1	1	4	6.00	0.0088	0.0249	0.40
38	1	4	0	0	5	4.80	0.0469	0.2409	0.50
40	4	2	0	2	8	5.50	0.0287	0.1378	0.80
41	1	4	0	2	7	6.00	0.0765	0.4087	0.70
42	2	1	0	3	6	6.67	0.0519	0.2693	0.60
43	5	1	1	0	6	5.17	0.0547	0.2851	0.70
46	1	4	0	4	9	6.67	0.0683	0.3622	0.90
47	4	2	1	0	6	5.33	0.0612	0.3220	0.70
50	3	2	1	2	8	5.75	0.0358	0.1780	0.80
51	7	2	0	1	10	4.70	0.0794	0.4252	1.00
52	4	0	0	0	4	4.00	0.0251	0.1173	0.40
55	4	4	0	1	9	5.00	0.1361	0.7466	0.90
56	2	4	0	2	8	5.75	0.0582	0.3050	0.80
62	3	2	0	1	6	5.17	0.0249	0.1162	0.60
63*	4	3	0	3	10	5.80	0.1637	0.9031	1.00
65	2	4	0	1	7	5.29	0.0490	0.2528	0.70
67	3	0	2	1	6	5.50	0.0696	0.3696	0.60
69	4	3	0	1	8	5.00	0.0987	0.5346	0.80
74	5	3	1	1	10	5.00	0.1529	0.8418	1.00
75	4	1	1	2	8	5.63	0.0418	0.2120	0.80
76	2	1	3	2	8	6.13	0.0698	0.3707	0.80
77	4	4	0	0	8	4.50	0.0880	0.4739	0.80

78	3	1	0	1	5	5.20	0.0768	0.4104	0.50
79	2	4	0	1	7	5.29	0.1027	0.5573	0.70
82	1	2	1	0	4	5.00	0.0472	0.2426	0.40
87	1	2	3	2	8	6.25	0.0878	0.4728	0.80
89	3	2	4	0	9	5.11	0.1005	0.5448	0.90
91	7	0	0	0	7	4.00	0.0794	0.4252	0.78
92	2	5	1	2	10	5.70	0.0403	0.2035	1.00
93	3	2	0	1	6	5.17	0.0413	0.2092	0.60
94	3	4	0	1	8	5.13	0.0459	0.2353	0.80
95	1	3	0	2	6	6.17	0.0362	0.1803	0.60
96	2	5	2	1	10	5.40	0.0683	0.3622	1.00
98	0	5	0	2	7	6.14	0.0700	0.3719	0.70
99	3	3	0	2	8	5.63	0.0707	0.3759	0.80
100	4	2	0	3	9	5.89	0.0530	0.2755	0.90
101	4	1	1	0	5	5.40	0.0740	0.3946	0.60
102	0	4	0	0	4	5.00	0.0339	0.1672	0.40
103	3	2	1	1	7	5.29	0.0904	0.4875	0.70
104	1	1	1	3	6	7.00	0.0075	0.0176	0.60
105	0	3	1	4	8	7.13	0.0701	0.3724	0.80
106	4	3	1	1	9	5.11	0.1258	0.6882	0.90
107	8	1	0	0	9	4.11	0.1163	0.6344	0.90
109	5	4	0	1	10	4.90	0.0991	0.5368	1.00
111	0	4	1	3	8	6.63	0.0662	0.3503	0.80
112	4	0	1	1	6	5.17	0.0779	0.4167	0.60
113	3	5	0	0	8	4.63	0.0597	0.3135	0.80

114	4	1	2	1	8	5.25	0.0990	0.5363	0.80
116	7	2	0	0	9	4.22	0.1410	0.7744	0.90
117	6	1	0	3	10	5.60	0.0773	0.4133	1.00
119	2	3	1	2	8	5.88	0.0399	0.2012	0.73
120	6	3	1	0	10	4.50	0.0862	0.4637	1.00
121	4	0	0	2	6	5.67	0.0255	0.1196	0.60
122	2	0	2	1	5	5.80	0.0387	0.1944	0.50
123	3	2	1	2	8	5.75	0.0296	0.1429	0.73
128	7	2	0	0	9	4.22	0.0798	0.4274	0.90
129	5	2	2	0	9	4.67	0.0569	0.2976	0.90
131	0	3	0	4	7	7.29	0.0265	0.1253	0.70
132	1	6	1	1	9	5.44	0.1076	0.5850	0.90
133	5	0	3	0	8	4.75	0.0735	0.3917	0.80
138	1	7	0	1	9	5.33	0.0622	0.3277	0.90
141	1	4	0	2	7	6.00	0.0506	0.2619	0.70
142	1	5	1	3	10	6.20	0.0444	0.2268	1.00
143	7	0	0	1	8	4.63	0.0359	0.1786	0.80
144	2	1	0	3	6	6.67	0.0395	0.1990	0.60
145	1	2	0	1	4	5.75	0.0107	0.0357	0.40
146	2	3	2	2	9	5.89	0.0789	0.4223	0.90
147	6	2	2	0	10	4.60	0.0892	0.4807	0.91
149	3	2	0	0	5	4.40	0.0225	0.1026	0.50
151	8	2	0	0	10	4.20	0.0688	0.3651	1.00
153	1	4	1	0	6	5.00	0.0069	0.0142	0.60
154	4	3	1	1	9	5.11	0.0823	0.4416	0.90

156	6	3	0	0	9	4.33	0.0660	0.3492	0.90
157	5	0	0	3	8	5.88	0.0671	0.3554	0.80
158	3	5	0	0	8	4.63	0.0472	0.2426	0.80
159	1	5	1	2	9	5.89	0.0795	0.4257	0.90
160	4	0	1	2	7	5.71	0.0708	0.3764	0.70
161	6	0	1	0	7	4.29	0.0498	0.2574	0.70
162	1	1	2	1	5	6.00	0.0216	0.0975	0.50
165	1	1	5	1	8	6.00	0.0770	0.4116	0.80
166	2	3	0	0	5	4.60	0.0203	0.0901	0.50
167	3	0	1	2	6	6.00	0.0708	0.3764	0.60
168	4	2	1	1	8	5.13	0.1004	0.5442	0.80
169	1	1	1	1	4	6.00	0.0092	0.0272	0.40
170	3	3	1	1	8	5.25	0.1009	0.5471	0.80
171	5	2	0	2	9	5.33	0.0321	0.1570	0.90
172	1	4	2	0	7	5.14	0.0741	0.3951	0.70
174	4	0	2	0	6	4.67	0.0611	0.3214	0.60
176	5	2	1	1	9	5.00	0.0603	0.3169	0.90
177	4	0	2	3	9	6.11	0.0973	0.5266	0.90
178	4	1	2	2	9	5.67	0.0598	0.3141	0.90
180	5	3	0	1	9	4.89	0.1808	1.0000	0.90
185	1	1	0	4	6	7.50	0.0070	0.0147	0.60
187	4	1	0	2	7	5.57	0.1218	0.6655	0.70
189	3	0	0	2	5	6.00	0.0571	0.2988	0.50
190	2	0	0	2	4	6.50	0.0424	0.2154	0.40
191	4	1	0	2	7	5.57	0.1258	0.6882	0.70

192	4	0	1	3	8	6.13	0.0787	0.4212	0.80
195	7	1	0	1	9	4.67	0.0678	0.3594	0.90
196	4	3	0	2	9	5.44	0.0419	0.2126	0.90
197	4	4	1	0	9	4.67	0.0661	0.3498	0.90
200	4	4	0	1	9	5.00	0.0254	0.1190	0.90
201	5	1	0	0	6	4.17	0.0187	0.0811	0.60
202	3	3	0	1	7	5.14	0.0256	0.1202	0.70
203	5	0	1	2	8	5.50	0.0972	0.5261	0.73
204	4	2	1	0	7	4.57	0.0399	0.2012	0.70
207	5	3	0	1	9	4.89	0.0203	0.0901	0.90
208	5	1	0	1	7	4.86	0.0813	0.4359	0.70
211	3	0	1	3	7	6.43	0.0523	0.2715	0.70
215	2	2	1	1	6	5.50	0.0171	0.0720	0.60
216	4	2	0	0	6	4.33	0.0647	0.3418	0.60
217	5	2	1	0	8	4.50	0.0820	0.4399	0.80
218	4	2	0	0	6	4.33	0.0804	0.4308	0.60
219	1	3	4	1	9	5.78	0.0653	0.3452	0.90
223	2	6	0	1	9	5.22	0.0503	0.2602	0.90
224	4	2	3	0	9	4.89	0.1025	0.5561	0.90
225	0	1	2	2	5	7.00	0.0313	0.1525	0.50
226	4	2	1	1	8	5.13	0.0171	0.0720	0.80
227	1	3	0	0	4	4.75	0.0064	0.0113	0.40
228	0	5	0	4	9	6.78	0.0736	0.3923	0.90
229	2	3	1	0	6	4.83	0.0230	0.1054	0.60
230	3	3	2	1	9	5.33	0.0794	0.4252	0.90

231	4	1	0	1	6	5.00	0.0365	0.1820	0.60
233	3	1	0	3	7	6.29	0.0631	0.3328	0.70
235	3	4	2	0	9	4.89	0.1016	0.5510	0.90
236	1	3	2	2	8	6.13	0.0490	0.2528	0.80
237	1	2	2	0	5	5.20	0.0192	0.0839	0.50
238	1	2	2	1	6	5.83	0.0428	0.2177	0.60
239	2	1	0	1	4	5.50	0.0152	0.0612	0.40
240	2	3	0	4	9	6.56	0.0783	0.4189	0.90
241	0	6	1	0	7	5.14	0.0092	0.0272	0.70
243	1	0	1	1	3	6.33	0.0078	0.0193	0.30
244	2	4	1	2	9	5.78	0.0606	0.3186	0.90
245	1	5	0	0	6	4.83	0.0792	0.4240	0.60
246	2	3	0	1	6	5.33	0.0364	0.1814	0.60
247	1	1	2	2	6	6.50	0.0044	0.0000	0.60
248	3	3	0	0	6	4.50	0.0460	0.2358	0.60
251	2	3	1	2	8	5.88	0.0303	0.1468	0.80
252	3	1	0	4	8	6.63	0.0782	0.4184	0.80
253	8	2	0	0	10	4.20	0.1272	0.6961	1.00
254	4	4	0	1	9	5.00	0.1065	0.5788	0.90
259	6	1	0	1	8	4.75	0.0934	0.5045	0.80
262	5	0	0	1	6	4.83	0.0517	0.2681	0.60
263	5	2	2	1	10	5.10	0.0795	0.4257	1.00
264	4	0	0	4	8	6.50	0.0701	0.3724	0.80
265	5	1	2	1	9	5.11	0.0762	0.4070	0.90
266	3	0	4	2	9	6.00	0.0673	0.3566	0.90

268	1	4	2	3	10	6.30	0.1115	0.6071	1.00
270	7	0	2	0	9	4.44	0.1202	0.6565	0.90
271	3	1	2	0	6	4.83	0.0790	0.4229	0.60
272	2	2	2	3	9	6.33	0.0378	0.1893	0.90
273	2	1	4	-1	6	4.67	0.0314	0.1531	0.60
275	2	1	1	3	7	6.57	0.0317	0.1548	0.70
276	2	0	1	1	4	5.75	0.0178	0.0760	0.40
277	1	1	3	2	7	6.43	0.0641	0.3384	0.70
278	3	3	0	2	8	5.63	0.0461	0.2364	0.80
279	0	3	1	2	6	6.50	0.0258	0.1213	0.60
280	6	0	0	1	7	4.71	0.0617	0.3248	0.70
281	5	1	1	0	7	4.43	0.0224	0.1020	0.70
282	3	2	2	0	7	4.86	0.0524	0.2721	0.70
283	7	0	3	0	10	4.60	0.1165	0.6355	1.00
285	1	2	0	0	3	4.67	0.0468	0.2404	0.30
286	2	2	1	2	7	6.00	0.0498	0.2574	0.70
287	1	3	2	0	6	5.17	0.0143	0.0561	0.60
290	2	6	1	1	10	5.30	0.0744	0.3968	1.00
291	2	1	0	0	3	4.33	0.0130	0.0488	0.30
292	3	1	0	0	4	4.25	0.0211	0.0947	0.40
293	3	1	2	2	8	5.88	0.0872	0.4694	0.80
294	1	1	3	1	6	6.00	0.0523	0.2715	0.60
295	1	1	3	2	7	6.43	0.0265	0.1253	0.70
296	2	4	0	3	9	6.11	0.0448	0.2290	0.90
297	2	5	0	0	7	4.71	0.0734	0.3912	0.70

299	1	5	0	0	6	4.83	0.0367	0.1831	0.60
301	2	0	0	0	2	4.00	0.0242	0.1122	0.20
303	0	4	2	1	7	5.86	0.0122	0.0442	0.70
304	2	0	1	3	6	6.83	0.0245	0.1139	0.60
305	2	2	1	2	7	6.00	0.0744	0.3968	0.70
310	3	2	0	3	8	6.13	0.0164	0.0680	0.80
311	1	2	1	1	5	5.80	0.0156	0.0635	0.50
312	1	3	1	2	7	6.14	0.0466	0.2392	0.70
313	3	3	1	0	7	4.71	0.0685	0.3634	0.70
314	1	4	0	3	8	6.38	0.0401	0.2024	0.80
315	3	5	1	1	10	5.20	0.1069	0.5811	1.00
316	2	0	2	0	4	5.00	0.0075	0.0176	0.40
317	1	1	1	3	6	7.00	0.0219	0.0992	0.60
318	1	4	0	1	6	5.50	0.0417	0.2115	0.60
319	3	0	3	0	6	5.00	0.0522	0.2710	0.60
320	0	3	3	3	9	6.67	0.0072	0.0159	0.90
321	2	3	3	0	8	5.13	0.1066	0.5794	0.80
322	4	1	0	0	5	4.20	0.0119	0.0425	0.50
323	2	3	1	1	7	5.43	0.0151	0.0607	0.70
324*	6	2	0	2	10	5.20	0.1309	0.7171	1.00
325	2	0	4	1	7	5.86	0.0287	0.1378	0.70
326	6	0	1	3	10	5.70	0.1151	0.6276	1.00
330	3	2	0	0	5	4.40	0.0362	0.1803	0.50
333	3	1	3	1	8	5.50	0.0655	0.3464	0.80
335	2	3	0	1	6	5.33	0.0562	0.2937	0.60

336	0	6	1	2	9	6.00	0.0522	0.2710	0.90
339	4	2	0	0	6	4.33	0.0794	0.4252	0.60
342	0	4	1	3	8	6.63	0.0819	0.4393	0.80
343	3	4	2	1	10	5.30	0.0582	0.3050	1.00
344	2	1	0	3	6	6.67	0.0702	0.3730	0.60
345	3	4	0	2	9	5.56	0.1110	0.6043	0.90
347	2	3	0	2	7	5.86	0.0421	0.2137	0.70
348	6	1	1	1	9	4.89	0.0862	0.4637	0.90
349	3	4	0	0	7	4.57	0.1122	0.6111	0.70
350	0	3	0	0	3	5.00	0.0070	0.0147	0.30
351	6	2	0	0	8	4.25	0.1375	0.7545	0.80
352	4	3	0	0	7	4.43	0.0317	0.1548	0.70
353*	3	6	1	0	10	4.80	0.1375	0.7545	1.00
354	4	2	0	1	7	5.00	0.0468	0.2404	0.70
355	1	2	0	2	5	6.40	0.0197	0.0867	0.50
359	5	5	0	0	10	4.50	0.1606	0.8855	1.00
360	3	0	0	3	6	6.50	0.0767	0.4099	0.60
361	2	4	0	0	6	4.67	0.0401	0.2024	0.60
362	3	3	0	1	7	5.14	0.0308	0.1497	0.70
364	5	2	1	0	8	4.50	0.0499	0.2579	0.80
365	5	1	1	0	7	4.43	0.0684	0.3628	0.70
366	3	4	0	1	8	5.13	0.1035	0.5618	0.80
367	3	2	0	1	6	5.17	0.1127	0.6139	0.60
369	8	1	0	0	9	4.11	0.1396	0.7664	0.90
370	1	2	2	3	8	6.63	0.0163	0.0675	0.80

372	5	1	2	1	9	5.11	0.0578	0.3027	0.90
373	5	0	2	1	8	5.13	0.0843	0.4529	0.80
377	5	3	0	0	8	4.38	0.0384	0.1927	0.80
379	6	1	1	0	8	4.38	0.0830	0.4456	0.80
380	4	4	2	0	10	4.80	0.0423	0.2149	1.00
381	2	3	2	1	8	5.50	0.0887	0.4779	0.80
382	1	5	0	1	7	5.43	0.1016	0.5510	0.70
383	2	4	1	1	8	5.38	0.0824	0.4422	0.80
384	1	2	2	1	6	5.83	0.0177	0.0754	0.60
386	0	1	1	0	2	5.50	0.0083	0.0221	0.20
388*	7	3	0	0	10	4.30	0.1512	0.8322	1.00
390	1	4	2	0	7	5.14	0.0377	0.1888	0.70
391	0	4	0	2	6	6.33	0.0402	0.2029	0.60
392	1	4	0	1	6	5.50	0.0164	0.0680	0.60
393	2	5	2	1	10	5.40	0.0628	0.3311	1.00
395	2	1	1	2	6	6.17	0.0119	0.0425	0.60
396	6	2	1	0	9	4.44	0.0991	0.5368	0.90
397	2	0	1	0	3	4.67	0.0535	0.2783	0.30
398	3	2	2	0	7	4.86	0.0700	0.3719	0.70
399	4	2	1	2	9	5.56	0.0202	0.0896	0.90
400	0	5	1	2	8	6.13	0.0457	0.2341	0.73
401	3	3	0	1	7	5.14	0.0516	0.2676	0.70
402	2	4	2	1	9	5.44	0.1718	0.9490	0.90
403	0	4	1	1	6	5.83	0.0293	0.1412	0.60
404	1	3	2	3	9	6.44	0.0324	0.1587	0.90

405	4	2	3	0	9	4.89	0.1006	0.5454	0.90
406	3	3	0	4	10	6.30	0.0919	0.4960	1.00
408*	9	0	0	1	10	4.50	0.1589	0.8759	1.00
409	1	2	1	1	5	5.80	0.0523	0.2715	0.50
411	4	4	1	1	10	5.10	0.0612	0.3220	1.00
412	2	0	0	1	3	5.67	0.0246	0.1145	0.30
413	3	0	2	0	5	4.80	0.0449	0.2296	0.50
414	2	2	0	0	4	4.50	0.0423	0.2149	0.40
417	0	3	1	2	6	6.50	0.0717	0.3815	0.60
418	6	3	1	0	10	4.50	0.1332	0.7302	1.00
419	0	6	1	1	8	5.63	0.0899	0.4847	0.80
420	3	3	0	0	6	4.50	0.0513	0.2659	0.60
421	2	1	0	2	5	6.20	0.0377	0.1888	0.50
422	2	3	1	1	7	5.43	0.0416	0.2109	0.70
423	2	8	0	0	10	4.80	0.0670	0.3549	1.00
424	0	3	4	1	8	6.00	0.0570	0.2982	0.80
425	2	2	0	0	4	4.50	0.0569	0.2976	0.40
426	2	5	1	1	9	5.33	0.1000	0.5420	0.90
427	2	2	1	0	5	4.80	0.0667	0.3532	0.50
428	2	3	1	2	8	5.88	0.0340	0.1678	0.80
430	2	3	0	2	7	5.86	0.0648	0.3424	0.70
431	3	1	0	1	5	5.20	0.0592	0.3107	0.50
432	5	2	1	0	8	4.50	0.0577	0.3022	0.80
434	4	2	0	0	6	4.33	0.0592	0.3107	0.60
435	3	1	1	1	6	5.33	0.0547	0.2851	0.60

436	3	2	0	3	8	6.13	0.0627	0.3305	0.80
437	6	3	0	0	9	4.33	0.0434	0.2211	0.90
438	4	3	0	0	7	4.43	0.0811	0.4348	0.70
439	3	0	1	2	6	6.00	0.0944	0.5102	0.60
440	8	2	0	0	10	4.20	0.1354	0.7426	1.00
442	5	0	0	1	6	4.83	0.0611	0.3214	0.60
443	6	0	1	0	7	4.29	0.0273	0.1298	0.70
446	2	1	2	2	7	6.14	0.0422	0.2143	0.70
448	3	1	1	0	5	4.60	0.0384	0.1927	0.50
450	0	1	0	0	1	5.00	0.0089	0.0255	0.10
451	3	0	1	1	5	5.40	0.0548	0.2857	0.50
452	1	1	2	1	5	6.00	0.0076	0.0181	0.50
BTx623	0	1	1	3	5	7.60	0.0411	0.2080	0.50
BTx623	0	2	0	6	8	8.00	0.0105	0.0346	0.80
BTx623	0	1	0	2	3	7.67	0.0041	-0.0017	0.30
BTx623	0	1	0	2	3	7.67	0.0079	0.0198	0.30
BTx623	1	2	0	2	5	6.40	0.0158	0.0646	0.50
BTx623	2	1	1	1	5	5.60	0.0409	0.2069	0.50
HKZ	4	2	3	0	9	4.89	0.0468	0.2404	0.90
HKZ	5	2	0	0	7	4.29	0.0101	0.0323	0.70
HKZ	6	1	1	1	9	4.89	0.0192	0.0839	0.90
HKZ	8	1	0	0	9	4.11	0.0409	0.2069	0.90
HKZ	7	2	0	1	10	4.70	0.0465	0.2387	0.91
HKZ	6	2	1	0	9	4.44	0.0604	0.3175	0.90
HKZ	7	1	1	1	10	4.80	0.0453	0.2319	1.00

HKZ	3	5	0	0	8	4.63	0.0327	0.1604	0.80
HKZ	5	5	0	0	10	4.50	0.0707	0.3759	1.00
HKZ	3	0	0	1	4	5.25	0.0248	0.1156	0.40

Appendix C – Field Trait Data of 31 F_4 RILs Evaluated Under Cold Temperature Conditions; DAP – days after planting; EI – emergence index; TBM – total biomass; SBM – shoot biomass; RBM – root biomass; FEP – final emergence percent; VGR – vigor rating.

Entry	8 DAP	9 DAP	10 DAP	11 DAP	14 DAP	30 DAP	30 DAP	EI	TBM per Plant	SBM per Plant	RBM per Plant	TBM per Plot	SBM per Plot	RBM per Plot	FEP	VGR
	------- (daily germination counts) -----						(total)	(days)	------------------------ (g) --------------------------							
5	2	3	22	6	8	7	48	13.1	0.405	0.218	0.186	19.42	10.47	8.95	0.96	3.5
5	0	3	11	8	7	14	43	16.3	0.480	0.235	0.245	20.64	10.11	10.54	0.86	3.5
5	0	2	15	2	6	11	36	15.9	0.795	0.440	0.355	28.62	15.84	12.78	0.72	3
29	12	16	4	1	2	2	37	10.1	0.867	0.538	0.329	32.07	19.91	12.16	0.74	2.5
29	1	4	11	3	6	4	29	13.1	1.225	0.950	0.275	35.53	27.55	7.98	0.58	3.5
29	21	15	5	0	3	2	46	9.8	0.345	0.210	0.135	15.87	9.66	6.21	0.92	1
42	0	0	1	1	4	1	7	14.9	0.571	0.357	0.214	4.00	2.50	1.50	0.14	3
42	7	10	11	9	3	0	40	9.9	1.445	0.765	0.680	57.80	30.60	27.20	0.80	2.5
42	2	22	12	3	3	0	42	9.7	1.043	0.505	0.538	43.80	21.20	22.60	0.84	1
55	13	10	5	4	3	1	36	9.9	0.655	0.395	0.260	23.58	14.22	9.36	0.72	1.5
55	11	14	6	4	5	5	45	11.6	0.405	0.258	0.147	18.24	11.61	6.63	0.90	2.5
55	15	15	6	1	2	2	41	10.0	1.375	0.800	0.575	56.38	32.80	23.58	0.82	1.5
74	25	13	5	2	4	0	49	9.1	-	0.410	-	-	20.09	-	0.98	1.5

74	7	5	20	9	0	0	41	9.8	1.145	0.560	0.585	46.95	22.96	23.99	0.82	3
74	19	13	10	3	2	2	49	9.9	0.695	0.405	0.290	34.06	19.85	14.21	0.98	1.5
82	6	11	11	5	0	7	40	12.5	-	0.445	-	-	17.80	-	0.80	3
82	6	12	12	7	5	3	45	11.2	0.505	0.310	0.195	22.73	13.95	8.78	0.90	3
82	8	13	7	2	2	3	35	10.9	1.075	0.610	0.465	37.63	21.35	16.28	0.70	1.5
89	7	16	11	2	3	5	44	11.6	0.740	0.515	0.225	32.56	22.66	9.90	0.88	2.5
89	3	14	8	3	3	0	31	9.8	0.715	0.465	0.250	22.17	14.42	7.75	0.62	3
89	0	6	2	5	6	2	21	12.7	0.722	0.411	0.311	15.17	8.63	6.53	0.42	3
93	10	12	6	2	7	-1	36	9.5	0.610	0.367	0.243	21.94	13.20	8.74	0.72	1.5
93	12	11	10	0	6	1	40	10.2	0.950	0.445	0.505	38.00	17.80	20.20	0.80	3.5
93	4	12	9	3	1	1	30	10.1	0.867	0.500	0.367	26.00	15.00	11.00	0.60	3
103	6	12	9	2	4	0	33	9.8	1.033	0.533	0.500	34.10	17.60	16.50	0.66	2
103	12	6	8	1	1	2	30	10.3	0.571	0.357	0.214	17.14	10.71	6.43	0.60	2
103	15	7	8	0	2	0	32	9.1	1.220	0.685	0.535	39.04	21.92	17.12	0.64	2
107	16	14	8	1	6	2	47	10.3	0.357	0.243	0.114	16.79	11.41	5.37	0.94	3
107	7	17	9	0	2	3	38	10.7	0.770	0.365	0.405	29.26	13.87	15.39	0.76	3
107	10	15	9	5	5	2	46	10.5	0.800	0.470	0.330	36.80	21.62	15.18	0.92	2
111	0	0	1	3	2	1	7	14.0	0.960	0.530	0.430	6.72	3.71	3.01	0.14	3

111	7	13	12	3	3	1	39	10.1	0.983	0.483	0.500	38.35	18.85	19.50	0.78	2
111	2	14	11	3	4	4	38	11.8	1.335	0.615	0.720	50.73	23.37	27.36	0.76	3
123	11	10	13	4	6	0	44	9.9	0.343	0.276	0.067	15.09	12.15	2.93	0.88	2
123	0	6	9	7	6	0	28	10.9	0.825	0.525	0.300	23.10	14.70	8.40	0.56	3.5
123	1	5	9	5	4	2	26	11.8	0.740	0.420	0.320	19.24	10.92	8.32	0.52	3
191	0	5	10	2	5	0	22	10.8	0.517	0.389	0.128	11.37	8.56	2.81	0.44	3.5
191	27	9	4	1	1	1	43	9.0	1.232	0.827	0.405	52.97	35.57	17.40	0.86	1.5
191	35	5	3	1	3	0	47	8.7	0.980	0.620	0.360	46.06	29.14	16.92	0.94	1.5
202	0	11	14	5	11	1	42	11.3	0.750	0.500	0.250	31.50	21.00	10.50	0.84	3
202	0	5	4	1	17	1	28	12.9	0.383	0.239	0.143	10.71	6.70	4.02	0.56	4
202	19	9	7	4	3	0	42	9.3	0.500	0.125	0.375	21.00	5.25	15.75	0.84	2
208	0	8	11	7	5	2	33	11.6	0.605	0.300	0.305	19.97	9.90	10.07	0.66	2.5
208	2	11	14	3	4	6	40	12.7	0.730	0.315	0.415	29.20	12.60	16.60	0.80	2.5
208	9	10	3	10	5	4	41	11.7	1.350	0.625	0.725	55.35	25.63	29.73	0.82	2
231	10	7	13	4	3	0	37	9.7	1.215	0.690	0.525	44.96	25.53	19.43	0.74	1.5
231	6	14	14	5	5	0	44	10.0	0.800	0.475	0.325	35.20	20.90	14.30	0.88	2.5
231	2	19	10	3	3	2	39	10.7	0.705	0.445	0.260	27.50	17.36	10.14	0.78	2
259	0	12	23	4	4	0	43	10.2	0.925	0.465	0.460	39.78	20.00	19.78	0.86	3

259	1	14	14	2	7	0	38	10.4	0.650	0.300	0.350	24.70	11.40	13.30	0.76	3
259	0	9	6	3	6	2	26	12.0	0.590	0.310	0.280	15.34	8.06	7.28	0.52	3.5
273	0	1	7	4	3	9	24	17.0	0.532	0.316	0.216	12.76	7.58	5.18	0.48	3
273	1	13	7	5	8	2	36	11.6	0.875	0.395	0.480	31.50	14.22	17.28	0.72	3
273	2	11	6	7	5	7	38	13.4	1.065	0.545	0.520	40.47	20.71	19.76	0.76	2
303	0	3	3	3	2	1	12	12.1	0.917	0.567	0.350	11.00	6.80	4.20	0.24	3
303	8	3	7	2	3	1	24	10.5	1.520	0.895	0.625	36.48	21.48	15.00	0.48	2
303	10	5	4	6	0	0	25	9.2	1.030	0.630	0.400	25.75	15.75	10.00	0.50	2
321	5	3	7	9	5	3	32	12.1	-	-	-	-	-	-	0.64	2.5
321	11	12	10	3	2	0	38	9.4	0.615	0.420	0.195	23.37	15.96	7.41	0.76	2.5
321	0	0	0	0	0	0	0	-	0.510	0.338	0.171	0.00	0.00	0.00	0.00	-
326	11	14	7	5	1	0	38	9.3	0.748	0.443	0.304	28.42	16.85	11.57	0.76	2
326	21	8	11	1	3	0	44	9.2	0.686	0.400	0.286	30.17	17.60	12.57	0.88	2
326	3	7	18	4	4	1	37	10.6	1.026	0.632	0.395	37.97	23.37	14.61	0.74	1
330	8	8	6	2	2	0	26	9.5	0.774	0.411	0.363	20.12	10.67	9.44	0.52	2.5
330	10	7	3	1	4	0	25	9.6	0.863	0.416	0.447	21.58	10.39	11.18	0.50	3.5
330	2	8	8	2	3	0	23	10.1	0.605	0.347	0.258	13.92	7.99	5.93	0.46	3.5
347	0	15	12	7	1	3	38	11.2	0.580	0.270	0.310	22.04	10.26	11.78	0.76	1.5

347	18	6	2	5	3	4	38	11.1	0.895	0.395	0.500	34.00	15.00	19.00	0.76	2
347	2	2	5	6	8	1	24	12.0	1.100	0.545	0.555	26.40	13.08	13.32	0.48	4
366	5	7	11	3	2	2	30	10.9	0.650	0.420	0.230	19.50	12.60	6.90	0.60	3
366	0	6	6	3	9	1	25	12.0	0.975	0.515	0.460	24.38	12.88	11.50	0.50	2.5
366	9	11	3	3	6	1	33	10.5	1.158	0.737	0.421	38.21	24.32	13.89	0.66	1.5
369	9	4	12	3	6	1	35	10.6	0.755	0.325	0.430	26.43	11.38	15.05	0.70	2.5
369	11	13	9	5	2	2	42	10.3	1.475	0.775	0.700	61.95	32.55	29.40	0.84	2.5
369	0	21	2	11	3	2	39	10.9	1.110	0.443	0.667	43.27	17.27	26.00	0.78	2
396	7	16	11	1	4	0	39	9.7	0.770	0.474	0.296	30.01	18.48	11.53	0.78	2
396	30	8	3	1	3	0	45	8.8	1.380	0.830	0.550	62.10	37.35	24.75	0.90	2.5
396	24	8	4	1	1	1	39	9.1	0.665	0.360	0.305	25.94	14.04	11.90	0.78	1
403	3	5	7	5	6	0	26	10.7	0.553	0.316	0.237	14.37	8.21	6.16	0.52	3
403	2	18	4	5	0	3	32	11.1	0.855	0.520	0.335	27.36	16.64	10.72	0.64	2.5
403	0	0	0	0	1	0	1	14.0	0.800	0.300	0.500	0.80	0.30	0.50	0.02	3
404	27	10	8	1	1	0	47	8.7	0.545	0.310	0.235	25.62	14.57	11.05	0.94	1
404	6	9	8	5	2	0	30	9.7	1.240	0.685	0.555	37.20	20.55	16.65	0.60	3
404	14	13	14	2	3	1	47	9.8	1.115	0.530	0.585	52.41	24.91	27.50	0.94	2.5
409	14	13	5	3	6	1	42	10.1	0.985	0.585	0.400	41.37	24.57	16.80	0.84	1

409	12	7	17	1	7	3	47	11.0	0.710	0.500	0.210	33.35	23.50	9.85	0.94	1.5
409	4	6	15	1	10	3	39	12.0	0.742	0.432	0.311	28.94	16.83	12.11	0.78	2.5
418	0	8	11	10	6	2	37	11.6	0.271	0.167	0.105	10.04	6.17	3.88	0.74	4.5
418	16	8	10	7	2	0	43	9.4	0.848	0.524	0.324	36.45	22.52	13.92	0.86	2.5
418	19	14	9	5	3	0	50	9.3	0.820	0.480	0.340	41.00	24.00	17.00	1.00	1
425	1	2	10	5	6	3	27	12.8	1.260	0.570	0.690	34.02	15.39	18.63	0.54	3
425	6	10	6	7	2	1	32	10.3	1.160	0.540	0.620	37.12	17.28	19.84	0.64	2
425	8	9	7	9	2	3	38	11.1	0.414	0.229	0.186	15.74	8.69	7.06	0.76	2.5
BTx623	0	0	0	0	2	7	9	24.1	0.325	0.225	0.100	2.93	2.03	0.90	0.18	4.5
BTx623	0	0	0	0	1	2	3	22.7	0.189	0.078	0.111	0.57	0.23	0.33	0.06	4.5
BTx623	0	0	0	0	1	5	6	24.8	0.267	0.144	0.122	1.60	0.87	0.73	0.12	5
BTx623	0	0	0	0	3	7	10	23.1	0.200	0.100	0.100	2.00	1.00	1.00	0.20	4.5
BTx623	0	0	0	0	0	3	3	27.0	0.500	0.125	0.375	1.50	0.38	1.13	0.06	4.5
BTx623	0	0	0	2	2	1	5	15.4	0.250	0.100	0.150	1.25	0.50	0.75	0.10	4.5
BTx623-bmr	0	1	6	7	9	2	25	13.0	0.308	0.177	0.131	7.69	4.42	3.27	0.50	4
BTx623-bmr	0	0	4	13	11	2	30	13.0	0.357	0.143	0.214	10.71	4.29	6.43	0.60	4.5
BTx623-bmr	0	0	9	3	15	0	27	12.3	0.361	0.167	0.194	9.75	4.50	5.25	0.54	4
BTx642	0	0	1	3	4	1	9	14.0	0.500	0.235	0.265	4.50	2.12	2.39	0.18	3.5

BTx642	0	1	1	3	4	2	11	14.7	0.933	0.350	0.583	10.27	3.85	6.42	0.22	3.5
BTx642	0	0	2	6	3	4	15	15.7	0.689	0.256	0.433	10.33	3.83	6.50	0.30	3
BTx642	0	0	3	1	4	7	15	19.1	0.567	0.200	0.367	8.50	3.00	5.50	0.30	4.5
BTx642	1	0	3	5	9	5	23	15.4	0.591	0.227	0.364	13.59	5.23	8.36	0.46	3.5
BTx642	0	0	2	1	3	1	7	14.3	0.310	0.150	0.160	2.17	1.05	1.12	0.14	3.5
GGL	31	3	6	1	2	0	43	8.7	0.286	0.133	0.152	12.29	5.73	6.55	0.86	3.5
GGL	2	3	4	0	2	0	11	10.1	0.317	0.167	0.150	3.48	1.83	1.65	0.22	3.5
GGL	13	11	7	3	0	0	34	9.0	0.308	0.192	0.117	10.48	6.52	3.97	0.68	4
GGL	6	1	1	4	0	0	12	9.3	0.200	0.100	0.100	2.40	1.20	1.20	0.24	3
GGL	3	5	6	3	0	0	17	9.5	1.000	0.500	0.500	17.00	8.50	8.50	0.34	4
GGL	0	2	4	3	2	1	12	12.2	0.080	0.060	0.020	0.96	0.72	0.24	0.24	4.5
HKZ	39	2	5	0	2	0	48	8.5	0.614	0.329	0.286	29.49	15.77	13.71	0.96	3
HKZ	29	7	5	2	2	0	45	8.8	0.857	0.481	0.376	38.57	21.64	16.93	0.90	2.5
HKZ	36	2	9	0	0	0	47	8.4	1.314	0.667	0.648	61.77	31.33	30.44	0.94	1
NSL51071	13	17	7	3	0	0	40	9.0	0.320	0.170	0.150	12.80	6.80	6.00	0.80	3
NSL51071	0	17	13	0	4	0	34	10.0	0.417	0.217	0.200	14.17	7.37	6.80	0.68	3
NSL51071	0	8	6	6	0	0	20	9.9	0.533	0.238	0.295	10.67	4.76	5.90	0.40	4
NSL51071	22	9	3	1	2	0	37	8.8	0.820	0.395	0.425	30.34	14.62	15.73	0.74	3

NSL51071	20	15	4	0	1	0	40	8.7	0.725	0.325	0.400	29.00	13.00	16.00	0.80	3
NSL51071	8	12	8	2	0	0	30	9.1	0.448	0.271	0.176	13.43	8.14	5.29	0.60	3.5
P8500	0	0	7	1	3	0	11	11.2	0.536	0.207	0.329	5.89	2.28	3.61	0.22	3.5
P8500	0	3	4	3	2	2	14	13.0	0.800	0.369	0.431	11.20	5.16	6.04	0.28	3.5
P8500	0	0	4	8	3	0	15	11.3	0.778	0.278	0.500	11.67	4.17	7.50	0.30	4.5
P8500	0	3	6	3	4	0	16	11.0	0.713	0.320	0.393	11.41	5.12	6.29	0.32	3
P8500	0	0	13	1	2	0	16	10.6	0.443	0.193	0.250	7.09	3.09	4.00	0.32	4
P8500	0	2	7	1	4	0	14	11.1	0.992	0.442	0.550	13.88	6.18	7.70	0.28	3.5
P85Y34	3	6	13	2	5	2	31	11.4	0.390	0.245	0.145	12.09	7.60	4.50	0.62	4
P85Y34	2	6	10	6	7	2	33	11.8	0.491	0.243	0.248	16.21	8.03	8.18	0.66	3.5
P85Y34	0	7	11	8	5	0	31	10.7	0.781	0.395	0.386	24.21	12.25	11.96	0.62	4
P85Y34	0	6	14	7	4	0	31	10.5	0.800	0.375	0.425	24.80	11.63	13.18	0.62	3.5
P85Y34	0	8	14	6	4	2	34	11.4	0.505	0.257	0.248	17.16	8.74	8.42	0.68	3.5
P85Y34	0	10	11	5	4	1	31	10.9	0.875	0.450	0.425	27.13	13.95	13.18	0.62	3