EXAMINING THE RELATIONSHIP BETWEEN BIOMASS SORGHUM HYBRIDS AND THEIR PHOTOPERIOD INSENSITIVE INBRED PARENTS

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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December 2020

Major Subject: Plant Breeding

ABSTRACT

In an effort to speed up the breeding process it would be beneficial to predict the performance of biomass sorghum photoperiod sensitive (PS) hybrids without having to make test crosses with the photoperiod insensitive (PI) inbred parents first. However, due to the differences in harvest index between biomass hybrids and grain type inbreds, and the confounding effects of maturity genes with heterosis, the ability to identify a relationship between biomass hybrid performance and photoperiod insensitive inbred parents is challenging. Thus, the objectives of this research were to analyze phenotypic traits related to biomass in an effort to identify a potential relationship between hybrid yield, heritability, heterosis and correlation of inbred performance on the performance of the related biomass hybrids, and lastly to conduct a QTL analysis on the inbreds and hybrids to attempt to identify QTL or markers for traits related to biomass yield.

Examination of both photoperiod sensitive inbred parents and their resulting biomass hybrids revealed few trends between the two populations. Statistical difference of phenotypic traits existed in all populations. Despite this, correlations of phenotypic traits between the PI inbreds and their PS hybrids were inconsistent across years with the exception of 3rd internode diameter and hybrid stalk weight per plant.

The range of high parent heterosis (HPH) for the hybrid populations varied greatly depending on the trait. Biomass yield HPH had a range of 1000% and is mostly attributed to the presence of maturity genes. When examining inbreds and hybrids grown in the same year, we report that inbred heritabilities were generally higher for phenotypic traits than their corresponding hybrids. It is speculated that the effects of the maturity genes and heterosis are

not only confounded with each other, but that their biomass increasing effects reduced the heritability and increased the heterosis for most of the phenotypic traits in the hybrids.

A genetic map was developed for both populations and both inbred and hybrid phenotypic and NIR trait data was analyzed using these genetic maps. Mapping of QTL was conducted, and several QTL were identified in both the inbreds and hybrids of both populations. Overall, most of the QTL identified were related to stalk characteristics and found on chromosome 7, the chromosome where *Dw3*, a major height gene, is located. These results suggest chromosome 7 plays a large part in both stalk traits and height. The small population size utilized herein made identification of QLT difficult and greatly over-estimated the explained phenotypic variation. No QTL were identified for biomass yield, which was expected due to the quantitative nature of biomass yield – it is unlikely that such a quantitative trait is controlled by a single gene(s).

DEDICATION

To my parents Roger and Carol Harvey

ACKNOWLEDGEMENTS

First and foremost, I would like thank Dr. William Rooney for allowing me the opportunity to conduct my research under his guidance; without him, none of this would be possible. He embodies everything that an advisor should be. I would also like to thank my other committee members for their help; Dr. Patricia Klein, Dr. Jackie Rudd, and Dr. Isabel Vales.

My project required the harvest and phenotyping of a significant amount of plant material and it couldn't have been done without a lot of assistance. I have a long list of people from the sorghum lab to thank for that assistance – Dr. Leo Hoffman Jr., Dr. David Horne, Dr. Nicholas Pugh, Dr. Francisco Gomez, Dr. Geraldo Carvalho Júnior, Dr. Brian Pfeiffer, Dr. Lloyd Mbulwe, Ammani Kyanam, Jales Fonseca, Mitchell Kent, Daniel Crozier, Fabian Leon, Steve Labar, Vickie Horne, and Delroy Collins. I would also like to thank Dr. Bob Klein, Dr. Patricia Klein, Lauren Fedenia, Dr. Nolan Bentley, Dr. Nikhil Patil, and Julie McCollum for their assistance and guidance when it came to genotyping my populations and conducting QTL mapping.

Thank you to my friends outside of the Soil and Crop Sciences Department for making my time in graduate school an enjoyable experience – Chris and Stephanie Dermody, Dustin Wilkerson, Lance Snodgrass, Brandon Treu, Josh Treu, and Matt and Lindsey Hageman. I have shared some great memories with these friends, and they have encouraged and supported me over the last four years of grad school. I truly value their friendship.

Most importantly I would like to thank my parents, Roger and Carol, and my wife, Kelsey, for all their encouragement and support. Graduate school is a long tunnel where you can't see the light at the end, but they were by my side each step of the way. I could have never

achieved this without them, and I am so grateful for everything they have given me, taught me, and sacrificed for me.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professor William Rooney of the Department of Soil and Crop Sciences, Professor Jackie Rudd of Texas A&M AgriLife Research, and Professors Patricia Klein and Isabel Vales of the Department of Horticultural Sciences.

The genomic data analyzed for Chapter IV was provided by Professor Patricia Klein. The custom R code used to identify QTL in Chapter IV was provided by fellow graduate student, Lauren Fedenia.

All other work conducted for the thesis (or) dissertation was completed by the student independently.

Funding Sources

Graduate study was supported by an assistantship from Dr. Rooney and Texas A&M University.

This work was also made possible in part by funding from the Sorghum Checkoff Program, and by ARPA-E's Transportation Energy Resources from Renewable Agriculture (TERRA) program.

NOMENCLATURE

RIL	Recombinant Inbred Line
HPH	High Parent Heterosis
QTL	Quantitative Trait Loci
BMR	Brown Mid Rib
NIR	Near-Infrared Spectroscopy

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CHAPTER I

INTRODUCTION

Sorghum bicolor L. Moench is a cereal grain crop that originated from Northeast Africa and is grown across tropic and temperate regions for grain, forage, and bio-energy production. In the United States during the 2016 and 2017 growing seasons, there were 6.1 million acres and 5.3 million acres harvested for grain, respectively (USDA, 2018). Exact statistics for acres devoted to sorghum bio-energy/mass and forage production are not available, but acres dedicated to sorghum silage production range from 305,000 acres in 2015 to 298,000 acres in 2016 (USDA, 2018) and seed sales for forage sorghum indicate significant acreage devoted to these hybrids. Advances have been made in the development of energy sorghum hybrids and they have progressed at a faster rate than those in other dedicated energy crops (i.e., switchgrass, miscanthus) (Rooney et al., 2007). However, the breeding and development effort trails far behind other crops grown as a bioenergy resource such as maize and sugarcane (Smith et al., 1987). While there are multiple reasons for this disparity, a major reason is that both corn and sugarcane are considered first-generation bioenergy feedstocks. First-generation bioenergy feedstocks include the conversions of sugar and starch to ethanol from corn grain and sugarcane juice. Consequently, selection and breeding focus on the same trait that is desirable in the regular crop. This is not the case with sorghum or other dedicated bioenergy crop species.

Sorghum is a self-pollinated C4 grass crop that is grown as both a pure-line cultivar and a hybrid crop depending on the infrastructure available and the economics of a particular production region. Hybrid production predominates in countries with established mechanized agriculture where growers can take full advantage of the benefits of heterosis (Duvick, 1999) while pure-line cultivars are common in smaller, subsistence production systems (Smith and Frederiksen, 2000).

The benefits of heterosis manifest as increased productivity of the hybrid offspring over the inbred parents. Heterosis was first documented in maize (*Zea mays* L.) in the early 20th century (Crow, 1998) and was found to increase yields by at least 15% when compared to open-pollinated varieties. After elucidating methods to reliably produce hybrids, hybrids in maize were initially commercialized in 1930; by 1965 nearly 100% of all U.S. planted maize were hybrids (Duvick, 1999). Several studies have looked at the rate of gain over time and found that heterosis has increased steadily over the years and ranges from 50% to 65% (Duvick, 1984; Meghji et al., 1984). More recently, researchers have seen that in certain crosses the hybrid yield increased by up to 185% over the parents (Flint-Garcia et al., 2009).

Heterosis also exists in sorghum with a heterotic grain yield advantage of 35-40% over pure-line cultivars (Duvick, 1999). However, the logistics of hybrid production were problematic because sorghum has a complete flower, which meant that economic hybrid seed production systems had to be developed (Stephens and Holland, 1954). The discovery of cytoplasmic male sterility systems led to their development in 1955, and sorghum hybrids were rapidly adopted, occupying more than 90% of all sorghum acres in the U.S. within four years. Similar to the rates of increase in heterosis in maize, sorghum has also had increasing yield heterosis over time. However, the rate of increase for sorghum yield heterosis has been much lower than that of maize. Current studies suggest that sorghum yield heterosis values of the hybrids result in about a 30% yield advantage over the parents (Mahdy et al., 2011).

Maize and sorghum are not the only crops that benefit from heterosis – other species are also grown as commercial hybrids. In rice (*Oryza sativa* L.) hybrids showed a yield advantage of 30% over pure-line varieties (Virmani, 1994). Tomatoes (*Solanum lycopersicum* L.) boast a hybrid yield advantage of 60% over inbreds (Krieger et al., 2010). Canola (*Brassica napus* L.) hybrids were seen to have a 30-60% yield advantage over inbred lines (Riaz et al., 2001).

Sunflower (*Helianthus annuus* L.) hybrids showed a 300% increase in seed weight per plant over inbreds (Karasu et al., 2010). In wheat (*Triticum aestivum* L.), hybrids showed a modest yield advantage of 10% over inbred lines, but hybrid wheat was not adopted due to limited feasibility of hybrid seed production (Martin et al., 1995). More recent efforts to produce hybrid wheat are now underway with better hybrid production systems, but the relatively low levels of heterosis may still limit adoption.

In an effort to speed up the breeding process, reduce the number of test crosses required, and conserve resources, there has been motivation to examine the relationship between inbred parent performance and hybrid performance. However, previous studies reveal an inconsistent relationship between inbred parent performance and the performance of their hybrids. Early studies showed that the correlation between the inbred and the hybrid in complex yield traits in corn was very low ($r = 0.11$) (Gama and Hallauer, 1977). More recently, Knoll and Anderson (2016) found that the correlation in biomass yield between inbred and hybrid parents was $r = 0.752$, however, the research was conducted using sweet sorghum lines, so biomass yield was a secondary factor in original line selection. Gama and Hallauer (1977) suggested that there is no better indicator of hybrid performance than the hybrid itself. Many other factors impact predictability of the hybrid such as the level of inbreeding depression in the inbred parents, environmental effects, and sample size. Despite the substantial advances in breeding and development of hybrids, breeding programs spend a significant portion of their time and resources identifying and selecting parental inbred lines to produce improved hybrids, primarily using traditional approaches.

Each hybrid system is adopted based on the amount of heterosis and cost to produce hybrid seed of the crop. Therefore, different systems for seed production are adopted based on the value of the hybrid seed. For example, hybrid tomato seed is commonly produced using hand-emasculation which is justified based on the high value of the hybrid seed. Alternatively, all

commercial hybrid sorghum seed production relies on cytoplasmic male sterility (CMS) systems to facilitate hybridization.

In sorghum, the CMS system is the result of a mutation in the mitochondria resulting in male sterility (Schertz and Ritchey, 1978). In sorghum, inbred parents are selected and then crossed to tester inbred parents; these testcross hybrids are then evaluated for hybrid performance and combining ability. Selected 'elite' inbred parents are then hybrids in many combinations to identify the best hybrids for commercialization. Extensive research has been done to aid in predicting hybrid performance in an attempt to reduce the number of crosses that are required to be made and evaluated (Schrag et al., 2010). From the discovery and development of different molecular markers, to the implementation of different statistical methods such as BLUPs used to predict hybrid performance the goal of these tools is to help develop and identify superior hybrid combinations faster and more efficiently.

Forage and energy sorghums are also grown as hybrids, but the amount of heterosis and the logistics of production and testing differs between these two types of hybrids. Forage sorghums can be very diverse but they are commonly very high in total biomass yield; they can also have high grain yields (i.e., silage hybrids) or minimal grain yield (greenchop hybrids). Hybrid seed production uses the same CMS system that is used in grain sorghum programs. Most forage sorghum hybrids are produced using a grain seed parent and a forage sorghum pollinator. In some cases, for grazing or hay-type hybrids, a sudangrass female is combined with a sudangrass pollinator for a higher tillering, thinner stalk and leafy hay. In these types, levels of heterosis vary because the emphasis is now based on total biomass production. The harvest index is also different because the entire plant is harvested for grain and forage as opposed to just the grain.

Forage or energy sorghums can be delimited into groups by their response to photoperiod. In the first class, photoperiod-insensitive (PI) genotypes flower in a defined number of days from

planting; daylength does not influence the time of flowering. In temperate and subtropical environments all grain sorghum hybrids are PI (Murphy et al., 2011). The second class is photoperiod-sensitive (PS) sorghums wherein the induction of reproductive growth is determined by a minimum daylength; once daylengths are reduced below that time, flowering is induced (Murphy et al., 2011). In temperate environments, this can result in a genotype that will not flower until late in the fall season well beyond the normal growing season. Naturally, only forage and energy sorghums can be of this type.

In PS energy hybrids, the delayed flowering is a desirable trait because it allows a longer duration of biomass accumulation and increased drought tolerance (Rooney et al., 2007). The specific daylengths necessary to induce reproductive growth vary based on the genetics of the variety, but they are derived from landraces with very specific requirements based on their specific region/latitude of adaptation (Rooney and Aydin, 1999).

The genetic control of photoperiod sensitivity is relatively well understood in sorghum (Murphy et al., 2011, 2014). The flowering pathway is set to initiate anthesis as soon as the juvenile stage of growth is completed; regulatory genes are responsible for the suppression of flowering until a certain daylength period is reached (Yang et al., 2014). These genetic loci responsible for this regulation are collectively known as maturity (*Ma*) genes that are highly heritable and epistatic. The *Ma* genes are responsible for time to floral initiation, with lateness being dominant to earliness (Rooney and Aydin, 1999).

Understanding the genetic mechanisms controlling this photoperiod response is essential to utilizing this trait because seed production of such PS material is impossible without it. Three maturity loci are responsible for the major photoperiod sensitive response in sorghum. Collectively, the loci Ma_1, Ma_5, and Ma_6 are regulatory genes that influence the photoperiod sensitive response. Their effect on flowering date is dependent on planting date and day length

during the growing season (Murphy et al., 2014). Genotypes that possess the dominant allele (i.e., *Ma*$_{1-}$, *Ma*$_{5-}$, and *Ma*$_{6-}$) at all three loci are highly photoperiod sensitive.

The genes underlying *Ma*$_1$, *Ma*$_5$, and *Ma*$_6$ are known. The maturity gene *Ma*$_1$ which codes for PRR37 modulates flowering time and during long days, PRR37 activates expression of the floral inhibitor CONSTANS and represses the expression of floral activators and floral induction. Expression of *Ma*$_1$ is light dependent and regulated by the circadian clock (Murphy et al., 2011). *Ma*$_5$ is a phytochrome B, which inhibits flowering in long days by activating the main response proteins of *Ma*$_1$, which ultimately results in the repression of floral initiation (Yang et al., 2014). *Ma*$_6$ is a strong repressor of flowering in long days; this gene was identified as *Ghd7*, which increases photoperiod sensitivity and delays flowering by inhibiting the expression of the floral activator proteins and genes controlling flowering time (Murphy et al., 2014).

If either the *Ma*$_1$ or *Ma*$_5$ locus is homozygous recessive, that genotype is photoperiod insensitive. Consequently, it is possible to produce two genotypes that are both photoperiod insensitive that, when hybridized produce a PS hybrid (Rooney et al., 2007). As an example, Tx2909 is a forage pollinator line developed specifically to produce photoperiod sensitive hybrids with existing male sterile seed parents (Rooney et al., 1998). Using this complementary gene action system to develop photoperiod sensitive hybrids is important because they not only gain the yield advantage of heterosis, they also allow the logistical and economic production of hybrid seed in temperate environments.

While this system is essential to the breeding of energy sorghum, it results in a dramatic difference in phenotypes between the parental inbred lines and the PS hybrids created therefrom. Consequently, selection among the inbreds is not likely to be manifested in the hybrid. Therefore, selection relies heavily on the phenotype of the hybrid. Specifically, photoperiod insensitive inbred parents cannot effectively be phenotyped for heterosis in photoperiod sensitive hybrids for

two specific reasons: the harvest index is different between the two plants, the inbreds produce seed and the hybrids produce biomass, and the effects of heterosis are confounded with the effects of the *Ma* genes, both of which boost biomass yields.

To date, energy sorghum research has focused on phenotyping existing PS inbred lines. However, as these lines cannot functionally be used for hybrid seed production, the research is not directly relevant to energy sorghum breeding programs and the overall improvement of the commodity. Further, inbreds in this situation are likely not good predictors of the biomass hybrids produced from them. As such, there is a real need to develop methods to evaluate the best approaches to continue the improvement and breeding of PI lines to produce PS hybrids.

Therefore the overall objectives of this dissertation are to i) examine the distribution of traits and the correlation of yield related traits between the inbreds and the hybrids of two populations to identify trends that could be useful for breeding purposes; ii) examine the heritability and heterosis of traits between the inbreds and hybrids to see if a selectable trait can be used to breed for yield; and iii) construct a genetic map of two populations and conduct QTL analysis in an attempt to identify large effect QTL related to yield and to identify genomic regions related to biomass yield.

CHAPTER II

ANALYSIS OF BIOMASS RELATED TRAITS IN BOTH INBRED AND HYBRID POPULATIONS AND THEIR POTENTIAL USE IN PREDICTING HYBRID PERFORMANCE

Introduction

In an effort to speed up the breeding process, reduce the number of test crosses required, and conserve resources, there is motivation to examine the relationship between inbred parent performance and hybrid performance. If there is a relationship, then the need to make test crosses is eliminated which can speed up the breeding cycle by at least one year, ultimately allowing growers access to superior genetics more rapidly.

Initial studies reveal an inconsistent relationship between the performance of the inbred parents and their hybrids. For example, Gama and Hallauer (1977) reported a low correlation (r = 0.11) between inbreds and hybrids for grain yield in corn. These authors used randomly derived lines to reduce sampling error and compared eight plant and ear traits between inbred parents and their single cross hybrids. However, these traits were not good predictors of hybrid performance, and the authors concluded that making testcross evaluations is the only valid way to test the potential of inbred lines in single cross hybrids (Gama and Hallauer, 1977).

Contrary to the previous report, Prado et al. (2013) reported correlations between inbreds and their hybrids in maize of 0.63 and 0.71 for grain weight and grain moisture at maturity, respectively. Moreover, Knoll and Anderson (2016) reported a high correlation between parents and hybrids for biomass yield in sweet sorghum hybrids (r = 0.75). However, Rani et al. (2013) found that only 12 out of 30 sorghum hybrids demonstrated positive high parent heterosis values for biomass yield.

Ultimately, the level of heterosis is intrinsic to a specific hybrid combination; thus, the ability to predict levels of heterosis using phenotypic correlations varies for different crops, different traits, and between environments (Flint-Garcia et al., 2009; Li et al., 2018). Given that yield is a quantitative trait with many loci interacting and controlling it, it is unlikely that a single phenotypic measurement will be a sufficient indicator of hybrid performance (Bernardo, 2001). Additionally, many other variables impact predictability of the hybrid, including the level of inbreeding depression in the inbred parents, environmental effects, and sample size. Despite the substantial advances in breeding and development of hybrids, breeding programs spend a significant portion of their time and resources identifying and selecting parental inbred lines to produce improved hybrids, primarily using traditional approaches.

Given the inconsistency in correlations between inbred and hybrid performance, it is even less likely that the performance of an energy sorghum inbred will be predictive of hybrid performance. Due to the photoperiod sensitivity that is manifest in the hybrids produced from these inbreds, major differences exist between the parents and the hybrid. As such, it is very unlikely that major traits such as maturity and biomass yield will have any correlation between parents and hybrids. However, it is possible for other non-obvious traits to potentially identify these correlations. Therefore, it is important that correlations between phenotypic traits be examined for the potential of predicting hybrid biomass yield and yield related traits.

Within that context, the objectives of this chapter are i) to assess the relationship between plant-based phenotypic traits and biomass yield and ii) assess the correlations between inbred lines and their respective hybrids. The purpose of this study is to identify any unique traits in inbreds that are associated with hybrid performance, be they phenotypic traits or the inbred performance *per se*. A significant correlation between a phenotypic trait in the inbred and biomass yield of the hybrid would satisfy the criteria of identifying a predictor of yield. The underlying hypothesis is

that performance of any trait in the inbred is unlikely to be predictive in the hybrid due to the significant change in phenotype between the photoperiod insensitive inbred and the photoperiod sensitive hybrid.

Materials and Methods

Population Development

Two recombinant inbred line (RIL) populations developed for QTL mapping purposes were used for this study. Their use meets the requirement to minimize sampling error as described by Gama and Hallauer (1977). The first population is derived from the cross of Tx2910/R10712 and the second population is derived from the cross of R10709/F08331*bmr12* (Rooney et al., 1998). The parent Tx2910 is a restorer in the A1 cytoplasmic genetic male sterility system and is one of two lines that are the origin of the *Ma* complementary gene action that infers photoperiod sensitivity (Rooney et al., 1998). The parent R10712 is an experimental bioenergy pollinator line that is short and photoperiod insensitive with the genotype Ma_1 ma_5 Ma_6. The parent R10709 is derived from the cross of Tx2909/DMR Sudangrass. Tx2909 is also a restorer in the A1 cytoplasmic genetic male sterility system and is the other line that is the origin of the *Ma* complementary gene action. The parent F08331*bmr12* is derived from a cross between lines containing the brown midrib (BMR) trait for forage quality and downy mildew resistance.

Inbreds of both RIL populations are photoperiod insensitive, flowering between 66 and 88 days because they are homozygous for maturity genes in the allelic configuration of Ma_1 ma_5 Ma_6. In the F_2 generation of each population, the RIL populations were advanced through generations head-to-row until the $F_{2:5}$. Each population consisted of 90 RILs.

Each individual RIL was testcrossed to the seed parent line ATx2928 (Rooney, 2004). Tx2928 is grain-type seed sorghum parent used because its relative maturity is similar to the RIL

populations. Tx2928 is homozygous for the *Ma* genes in the configuration $ma_1\ Ma_5\ ma_6$. As such, the resulting testcross hybrids are photoperiod sensitive because they are heterozygous at Ma_1, Ma_5 *and* Ma_6.

Experimental Design

Both RIL populations, parental lines, and testcross hybrids were planted in College Station, TX in 2017 and 2018. Because of the large differences in maturity and harvest date, the RILs were planted separately from the testcross hybrids. In 2017, the hybrid population was planted on March 24th and the RIL population was planted on April 5th. In 2018, both blocks were planted on April 19th. A randomized complete block design with two replications was used throughout the test. In 2017, plot size consisted of 2 adjacent rows 5.48 m in length spaced at 0.762 m. In 2018, the same length and width were used but each plot consisted of three adjacent rows. For both tests, the plots were planted to achieve a stand density of 56,000 plants per acre.

The soil type at College Station was Ships Clay Loam. Agronomic practices followed standard practices for the environment and did not include supplemental irrigation due to adequate rainfall. In 2017, the inbreds were harvested from July 27-30, and the hybrid populations were harvested from August 3 - 6. In 2018, both the inbred and hybrid populations were harvested during the week of July 30 – August 3. These harvest dates occurred approximately 30 days after anthesis in the inbred line trials and as the hybrids were entering the lag phase of growth as a photoperiod sensitive crop. Harvest at this time allowed comparison between the inbred and hybrids as well as avoiding significant losses due to lodging, especially in the inbred lines.

Agronomic Data

The following phenological and morphological traits were measured on standing plants in the field in both populations prior to biomass harvest: days to anthesis; leaf angle, leaf length, and leaf width. Leaf angle measurement was defined as the angle of each leaf from a plane defined by the stalk below the node subtending the leaf (Mickelson et al., 2002). Leaf angle was measured on the leaf from the sixth internode of three different plants using a digital protractor. The average of these measurements was recorded for each plot. For leaf width and length, measurements were taken on the leaf from the sixth internode of three different plants, the average of these measurements were recorded for each plot. Leaf width was measured at the widest part of the leaf and length was measured from the leaf tip to the point of contact at the sixth internode. Days to flower was recorded only in the RIL populations because the photoperiod sensitive testcross hybrids did not flower in College Station. Days to flower was recorded as the day where 50% of the panicles within a plot were at mid-anthesis.

Just prior to mechanical harvest of the test, six plants from each plot were randomly harvested to measure panicle length (pollinator population only), stem diameter, internode length, plant height, and sample biomass yield. Panicle length was measured from the base of the first rachis of the panicle to the apex of the panicle. An average panicle length was recorded from all six measurements. Stem diameter and internode length were measured using a digital caliper and ruler, respectively, at the third and sixth internode from the base of the plant and recorded as an average of six stalks from each plot. In the pollinator populations, plant height was recorded as the length of each plant from the base of the plant to the top of the panicle. In the hybrid populations, plant height was recorded as the distance from the base of the plant to the bottom of the vegetative whorl (there was no reproductive growth). After measurements were completed, sample biomass weight was recorded as the total weight of all six plants. Following this measurement, leaves were

stripped, and the stalks were weighed again and recorded as total stalk yield. Leaf yield was determined by subtracting stalk yield from recorded total biomass yield. For the pollinator populations, panicles were removed, and stalk yield was adjusted by subtracting panicle weight from the total biomass yield.

After sampling, plots were harvested using a tractor attached New Holland single row silage harvester in 2017; in 2018 all three rows were harvested using a John Deere 7300 series forage harvester (John Deere, Moline, IL). Both systems recorded whole plot weights and a sub-sample of each plot was collected for bagasse measurements. The bagasse samples were collected and weighed in grams (g) and then dried in a Grieve model SC-400 (The Grieve Corporation, Round Lake, IL) forced convection dryer at 57-60 °C for 6 days. Dried samples were then re-weighed, and dry sample weight was recorded. Moisture content was calculated using the fresh and dry weights of each sample.

To measure plant composition, dried bagasse samples were ground using a Wiley knife mill (Thomas Scientific, Swedesboro, NJ). Biomass composition was estimated using near infrared spectroscopy (NIR) in which dried, ground samples were scanned using a FOSS XDS NIR spectrometer (Foss North America, Eden Prairie, MN). Predictions for biomass composition were based on a calibration curve developed by Wolfrum et al. (2013). The compositional traits that were estimated as a percentage using this calibration were: ash content, structural inorganics, nonstructural inorganics, protein, sucrose, water, ethanol extractives, lignin, whole starch, structural starch, cellulose, glucan, xylan, galactan, arabinan, and acetyl.

Statistical Analysis

For all measured traits, data was analyzed using JMP 14.3 PRO software using an all random model. All dependent variables were analyzed by year for both inbreds and hybrids in each population using the model of $Y = \beta ij + yk + yik + \varepsilon$ where β = repetitions (j = 1, 2), y = genotypes (k = 1… 86), and ε = error.

Following the assessment of main effects, the mean, max, min, standard deviation, and standard error were calculated for each population and year combination.

Tests of normality confirmed the normal distribution of the data for most traits. After individual environment analyses, tests of homogeneity were run and for most traits the error heterogeneity was significantly different between environments (Tables 7 & 8). In an attempt to normalize the data, different data transformations were attempted, but none improved the heterogeneity. Due to this, combined analysis was not completed.

Results and Discussion

Growth conditions for 2017 were uneventful as rainfall and daily temperatures fell within the yearly averages for College Station during the growing season. While the area was affected by Hurricane Harvey, all trials had been harvested prior to its arrival and it had no effect on the test. Growth conditions for 2018 were less favorable, with rainfall for the growing season coming in 7 inches lower than the yearly averages for College Station and daytime temperature was 4 °F higher than the monthly averages for the growing season. Despite these differences in precipitation during the growing season, the crop was planted into a full moisture profile in the soil (partially due to Hurricane Harvey) and this, combined with rainfall, provided sufficient moisture for biomass crop production through harvest.

R10709/F08331bmr12 Parental and RIL Means

Between parents R10709 and F08331*bmr12,* statistical differences were detected for five of the 25 phenotypic and composition traits evaluated (Table 1). For phenotypic traits; plant height, panicle length, 3rd internode length, and leaf width were greater ($P < 0.05$) for F08331*bmr12* compared to R10709. No differences were detected between the two parents R10709 and F08331*bmr12* for NIR traits, with one exception, lignin. The lignin concentration was greater ($P = 0.02$) in R10709 compared to F08331*bmr12* (Table 1). This outcome is likely attributed to the brown midrib phenotype expressed in the F08331*bmr12* parent, conditioned by the *bmr12* gene, given that brown midrib phenotypes in sorghum are consistently lower in lignin concentrations (Sattler et al., 2010).

Table 1. Average trait values and p values for the parents R10709 and F08331*bmr12* grown in 2017 in College Station, Texas.

Trait	R10709	F08331*bmr12*	Pedigree p value
Plant height (cm)	194.3	220.3	0.04
Panicle length (cm)	9.1	11.0	0.001
3rd internode length (cm)	15.3	22.4	0.001
3rd internode diameter (mm)	10.0	10.5	0.39
6th internode diameter (mm)	8.5	8.4	0.78
6th internode length (cm)	21.1	20.7	0.85
Stalk weight per plant (g)	113.1	122.3	0.64
Weight of leaves per plant (g)	27.5	25.2	0.46
% Moisture	80.4	79.9	0.53
Yield (t/ha)	4.5	4.3	0.80
Leaf length (cm)	76.3	76.3	1.00
Leaf width (cm)	3.8	4.9	0.03
Leaf angle (degrees)	29.6	38.5	0.32
Acetyl (%)	0.7	1.1	0.16
Arabinan (%)	1.9	1.7	0.19
Ash (%)	10.3	9.4	0.10
Cellulose (%)	29.0	31.0	0.20
EtOH (%)	4.2	3.9	0.06
Galactan (%)	1.0	0.9	0.09
Glucan (%)	30.6	33.3	0.06
Lignin (%)	21.3	15.9	0.02
Protein (%)	6.0	4.1	0.16
Sucrose (%)	4.6	4.6	0.99
Whole starch (%)	10.0	8.0	0.44
Xylan (%)	16.2	16.2	0.96

Compared to the limited differences among the parents, significant variation among the RIL entries in the R10709/F08331*bmr12* population was detected for all phenotypic traits and the compositional trait lignin (Table 2). The range observed between the minimum and maximum entries averaged slightly above 2X, although this was skewed somewhat by the wide range of variation for leaf weight (Table 2). Nevertheless, a wide range of phenotypes were detectable among these genotypes for all measured traits.

This transgressive segregation demonstrates that there remains selectable variation within the inbred populations that could be utilized for breeding purposes. Given that the RIL entries are in the $F_{2:5}$ generation, most of this variation is likely heritable and implies that improvements in the inbred lines are possible.

Table 2. P values for genotype and rep, and LSD values as well as the population mean, minimum, maximum, and standard error for the R10709/ F08331*bmr12* RIL population grown in 2017 in College Station, Texas.

	2017 R10709/ F08331*bmr12* RIL population						
Trait	pedigree	rep	LSD	Min	Max	Mean	Std Err
Plant height (cm)	<.0001	0.4828	32.8	126.6	262.9	212.2	2.02
Panicle length (cm)	<.0001	0.7411	1.4	5.0	12.8	9.3	0.13
3rd internode length (cm)	<.0001	0.0069	5.1	8.7	33.6	21.6	0.33
3rd internode diameter (mm)	<.0001	0.3877	2.1	7.7	18.2	10.7	0.13
6th internode diameter (mm)	<.0001	0.7981	1.7	4.9	14.1	9.6	0.14
6th internode length (cm)	<.0001	0.3155	7.9	18.1	50.8	26.5	0.48
Stalk weight per plant (g)	<.0001	0.1356	34.9	39.2	260.8	125.4	3.91
Weight of leaves per plant (g)	<.0001	0.2556	16.4	4.2	89.2	30.7	1.05
% Moisture	<.0001	0.1320	4.8	60.4	82.1	75.6	0.41
Yield (t/ha)	<.0001	<0.0001	1.8	1.0	9.9	4.5	0.12
Leaf length (cm)	<.0001	0.0464	9.9	59.0	97.3	81.0	0.69
Leaf width (cm)	<.0001	0.0006	1.0	2.8	7.8	5.3	0.06
Leaf angle (degrees)	0.0312	<0.0001	10.0	20.6	60.3	37.5	0.56
Lignin (%)	<.0001	0.0447	4.7	10.9	27.3	17.0	0.28

Tx2910/R10712 Parental and RIL Means

Between the parents Tx2910 and R10712, statistical differences were detected for 9 of the 25 phenotypic traits evaluated; most of the differences were observed in plant structural traits and none of the compositional traits differed between these parents (Table 3). Plant height, panicle length, 3rd internode length, and 6th internode length were greater ($P \leq 0.001$) for Tx2910 compared to R10712. Alternatively, 3rd internode diameter, 6th internode diameter, stalk weight per plant, leaf weight per plant, and leaf width were greater ($P \leq 0.01$) for R10712 compared to Tx2910 (Table 3). These differences are consistent with the phenotypes and pedigrees of the parents; Tx2910 is a very leafy, thin stalked, tall sudan-type sorghum whereas R10712 is of durra origin and has a shorter and thicker stalk with fewer leaves. Given these morphological differences it is somewhat surprising that no compositional differences were detected.

Table 3. Average trait values and p values for the parents Tx2910 and R10712 grown in 2017 in College Station, Texas.

Trait	Tx2910	R10712	Pedigree p value
Plant height (cm)	205.74	135.52	0.001
Panicle length (cm)	9.87	6.68	0.001
3rd internode length (cm)	27.36	12.48	0.001
3rd internode diameter (mm)	8.69	15.52	0.01
6th internode diameter (mm)	7.07	13.82	0.001
6th internode length (cm)	29.98	12.89	0.001
Stalk weight per plant (g)	86.12	155.2	0.001
Weight of leaves per plant (g)	18.45	43.33	0.001
% Moisture	80.31	78.7	0.71
Yield (Mg/ha)	3.04	4.35	0.23
Leaf length (cm)	74.16	71.75	0.62
Leaf width (cm)	3.95	6.58	0.001
Leaf angle (degrees)	27.08	35.51	0.07
Acetyl (%)	0.30	0.69	0.08
Arabinan (%)	1.75	1.67	0.65
Ash (%)	11.46	10.56	0.06
Cellulose (%)	29.57	31.16	0.14
EtOH (%)	4.08	3.96	0.67
Galactan (%)	0.93	0.93	0.99
Glucan (%)	31.06	31.17	0.95
Lignin (%)	21.48	17.69	0.24
Protein (%)	6.08	5.03	0.29
Sucrose (%)	5.46	4.56	0.67
Whole starch (%)	13.50	7.60	0.22
Xylan (%)	15.52	16.03	0.57

Significant variation existed among the entries in the Tx2910/R10712 RIL population for all the phenotypic traits, but no variation was detected for the compositional traits (Table 4). In the Tx2910/R10712 RIL population, transgressive segregation was observed in both directions, with an average range between minimum and maximum phenotypes for each trait of 2X. Like in the R10709/F08331*bmr12* population, the greatest ranges were observed for leaf weight, stalk weight and biomass yield. Most other differences were less than 2X in range (Table 4) and were lower

than observed in the R10709/F08331*bmr12* population. This transgressive segregation herein indicates selectable variation exists between the inbred parents that could be used to improve the lines.

Table 4. P values for genotype and rep, and LSD, mean, minimum, maximum, and standard error for the Tx2910/R10712 inbred population grown in 2017 in College Station, Texas.

	2017 Tx2910/R10712 inbred population						
Trait	pedigree	rep	LSD	Mean	Min	Max	Std Err
Plant height (cm)	<.0001	0.7189	23.9	208.4	129.3	254.4	1.99
Panicle length (cm)	<.0001	0.106	1.5	9.3	6.2	13.1	0.13
3rd internode length (cm)	<.0001	0.5421	6.0	21.4	11.8	33.0	0.37
3rd internode diameter (mm)	<.0001	0.1238	1.7	10.0	6.7	14.2	0.11
6th internode diameter (mm)	0.0064	0.3168	3.3	9.3	5.3	23.7	0.18
6th internode length (cm)	0.0003	0.1872	8.2	25.8	11.0	51.8	0.50
Stalk weight per plant (g)	<.0001	<.0001	37.6	110.3	39.2	229.2	3.59
Weight of leaves per plant (g)	0.0005	0.0007	16.7	27.9	6.7	68.3	1.02
% Moisture	<.0001	0.0318	4.4	75.3	62.8	83.6	0.41
Yield (Mg/ha)	<.0001	0.8836	2.2	4.3	1.5	10.4	0.13
Leaf length (cm)	<.0001	0.3421	7.7	78.7	54.0	104.0	0.89
Leaf width (cm)	<.0001	0.0137	1.1	5.2	2.8	7.3	0.08
Leaf angle (degrees)	0.0018	<.0001	6.8	34.3	19.2	47.5	0.43

The variation detected in these populations indicates that sufficient variation exists to improve the performance of the inbred lines for either a forage and or bioenergy sorghum. This is likely true because these are inbred lines wherein the genetic variation detected between the lines is primarily additive in nature and thereby should be responsive to selection. However,

given that the performance of the hybrids is the primary determinant of the value of any parental line, it is essential to assess whether this variation translates in any way to the phenotypes observed in testcross hybrids.

Testcross Hybrid Population Analysis – R10709/F08331bmr12 and Tx2910/R10712 populations

Analysis of variance of the hybrid populations produced from ATx2928 and the RILs from the R10709/F08331*bmr12* population revealed significant differences among genotypes in both 2017 and 2018 for 9 of 13 traits (Table 5). Of note, variation among hybrids was detected for plant height, panicle length, all measured internode diameters, 3rd internode length, and weight of leaves per plant (Table 5). Traits were generally consistent in their expression in both environments. For example, leaf weight per plant was highly significant in both years while stalk weight per plant was not significant. Alternatively, internode lengths differed in response across years with greater variation existing in 2018 than 2017.

In the hybrid population of ATx2928 testcrossed to the Tx2910/R0712 RILs, significant differences were detected among hybrids in 2017 and 2018 for plant height, panicle length, all internode diameters, 3rd internode length, weight of leaves per plant, percent moisture and yield (Table 6). Like the R10709/F08331*bmr12* testcross hybrids, traits were generally consistent in response across both environments but exceptions did occur; 3rd internode length and diameter were not variable in 2017 yet highly significant in 2018.

Within each environment the replication effect differed between the 2017 and 2018 growing seasons in both populations. In 2017, the replication effect was significant for most of the traits in both populations, while it was not significant in 2018 (Tables 5 and 6). This rep effect is likely due to spatial variation within the fields. It is possible that the field used in 2018 was more

uniform across the test, whereas the field used in 2017 had low areas that held water and impacted plant growth or the soil type was not the same across all reps.

Combined analysis revealed that the main effects of genotype and environment were both significant; environment was significant for all traits in both populations while genotype was significant for most traits (Tables 5 and 6). The genotype effect was significant for all traits except leaf weight, yield and leaf width in the ATx2928/(R10709/F08331*bmr12*) hybrids and leaf weight per plant, yield, and leaf angle in the ATx2928/(Tx2910/R0712) hybrids. Of note, stalk weight was not significant in either population. This was somewhat surprising given that stalk weight in the inbreds was one of the traits that showed the greatest range in values among RIL lines in both populations. This disconnect between the inbred line and subsequent hybrid implies that genetic effects in the hybrid are minimizing these differences.

The significant effect of environment in both hybrid populations is often expected in field evaluations as environment is typically the largest and most consistent effect in a combined analysis. Both hybrid populations were taller and had longer 3rd and 6th internodes in 2017 than in 2018. Furthermore, both hybrid populations had greater moisture content and yields in 2018. For traits such as biomass yield and moisture content, some of the differences are associated with the environment, however, biomass yield specifically can be associated with different harvest methods; in 2017 a single row harvester was used while in 2018 a new four row silage harvester was used. However, harvest method did not influence traits that were measured via sampling (Tables 5 and 6).

Table 5. Significance values for 2017 ATx2928(R10709/F08331*bmr12*) hybrids, 2018 ATx2928(R10709/F08331*bmr12*) hybrids and combined years for ATx2928(R10709/F08331*bmr12*) hybrids.

	2017 Hybrids		2018 Hybrids		Combined Years Hybrids			
Trait	pedigree	rep	pedigree	rep	pedigree	environment	rep	GxE
Plant height (cm)	0.0037	<.0001	0.0003	0.0004	<.0001	<.0001	<.0001	0.0116
Panicle length (cm)	0.0096	<.0001	0.0006	0.0034	<.0001	<.0001	<.0001	.0328
3rd internode length (cm)	0.0078	0.7001	<.0001	0.2799	<.0001	<.0001	0.5031	.0123
3rd internode diameter (mm)	0.0005	0.4709	0.0003	0.455	<.0001	<.0001	0.3024	.0351
6th internode diameter (mm)	0.0247	<.0001	<.0001	0.0901	<.0001	<.0001	<.0001	0.2153
6th internode length (cm)	0.1722	<.0001	0.0014	0.3012	.0230	<.0001	<.0001	0.3923
Stalk weight per plant (g)	0.1722	<.0001	0.1523	<.0001	0.3826	<.0001	<.0001	0.1767
Weight of leaves per plant (g)	<.0001	0.3243	<.0001	0.7739	<.0001	<.0001	0.4356	<.0001
% Moisture	0.0227	<.0001	0.0066	0.7371	0.1138	<.0001	<.0001	0.1531
Yield (Mg/ha)	0.0236	<.0001	0.0431	0.0022	<.0001	<.0001	<.0001	0.0679
Leaf length (cm)	0.2645	<.0001	0.0936	0.6321	0.1028	<.0001	.0299	0.2911
Leaf width (cm)	0.0195	0.3427	0.0733	0.0003	.0440	<.0001	0.0865	.0234
Leaf Angle (degrees)	0.0037	<.0001	0.0003	0.0004	<.0001	<.0001	<.0001	0.0116

Table 6. Significance values for 2017 ATx2928(Tx2910/R10712) hybrids, 2018 ATx2928(Tx2910/R10712) hybrids and combined years for ATx2928(Tx2910/R10712) hybrids.

	2017 Hybrids		2018 Hybrids		Combined Years Hybrids			
Trait	pedigree	rep	pedigree	rep	pedigree	environment	rep	GxE
Plant height (cm)	0.0451	<.0001	0.0044	0.001	<.0001	<.0001	<.0001	0.238
Panicle length (cm)	<.0001	<.0001	0.0017	0.149	<.0001	<.0001	0.1903	0.295
3rd internode length (cm)	0.0057	0.0495	0.0205	0.588	<.0001	<.0001	0.4376	.0448
3rd internode diameter (mm)	0.0004	0.0175	0.0047	0.858	<.0001	<.0001	0.2106	.0105
6th internode diameter (mm)	<.0001	<.0001	<.0001	0.536	<.0001	<.0001	<.0001	0.341
6th internode length (cm)	0.0565	<.0001	0.0032	0.020	0.0166	<.0001	.0013	0.683
Stalk weight/plant (g)	0.2537	<.0001	0.0193	0.092	0.2063	<.0001	<.0001	0.638
Leaf weight/plant (g)	<.0001	0.0069	<.0001	0.017	<.0001	<.0001	0.318	0.002
% Moisture	0.0257	<.0001	0.2641	0.048	0.1647	<.0001	<.0001	0.394
Yield (Mg/ha)	0.0111	<.0001	0.4049	0.112	<.0001	<.0001	<.0001	0.837
Leaf length (cm)	0.1058	0.0191	0.0299	0.982	0.0112	.0078	0.0833	0.112
Leaf width (cm)	0.0395	0.5721	0.7030	0.024	0.0631	<.0001	0.3761	0.168
Leaf Angle (degrees)	0.0451	<.0001	0.0044	0.001	<.0001	<.0001	<.0001	0.238

While there were strong differences in the environments, the genotype x environment interactions were minimal, indicating the responses of the genotypes were consistent across years. However, if a GxE interaction was detected it was usually highly significant and was associated with traits of significant importance such as yield and plant height (Tables 5 and 6). Based on these interactions, further analysis and interpretations were made using individual environment analysis.

None of the compositional traits were statistically significant in either hybrid population in either year or in the combined analysis (data not shown). While this was somewhat surprising, the absence of differences in the RIL lines implied that differences would be difficult, at best, to detect in the hybrids. In the R10709/F08331*bmr12* RILs, a difference in lignin concentration was detected; however, that variation was not expected to transmit to the hybrid because Tx2928 is a non-bmr parent and *bmr12* is a homozygous recessive phenotype that could not exist in the hybrids.

With the exception of the brown midrib phenotype being associated with reduced yield, previous research has not demonstrated a link between compositional traits and plant biomass yield (Miller et al., 1983; Lee and Brewbaker, 1984). When combined with the aforementioned inbred compositional results, this implies that none of these compositional traits are suitable predictors of hybrid yield and/or composition.

The ranges of performance in the hybrid produced from ATx2928 and the RILs from both populations were mostly consistent across years although the magnitudes changed. This shift is the likely cause of the significant genotype x environment interactions. Further, as expected yields increased in the hybrids in comparison to those observed in the inbred lines. The higher yields of the hybrids was expected due to the benefits of heterosis (Packer and Rooney, 2014) and the differences in photoperiod sensitivity between the hybrids and the RIL parents.

In the hybrids from ATx2928 and the R10709/F08331*bmr12* RILs, the ranges in performance averaged 4X which was substantially that the ranges observed among the RIL (Tables 7 and 8). This increase in range was consistent across the two populations. For example, the largest range in both 2017 and 2018 was noted for leaf weight which was also observed in the RILs *per se*. The magnitude of the range shift was greater in 2017 likely due to environment differences affecting leaf growth and drop and a significant genotype x environment effect indicates that the exact response is combination specific. Nevertheless, the response is consistent. Stalk weight was not a trait with detectable variation in the inbred line population; in the hybrid it was year specific. However, the ranges of phenotypes for stalk weight were much wider in the hybrids than in the inbreds. This implies that non-additive gene action is influencing this trait and can only be assessed in hybrid combinations.

Table 7. Phenotypic and composition trait mean, minimum, maximum, standard deviation and standard error of testcross hybrid lines from the ATx2928/(R10709/F08331*bmr12*) RIL population in 2017 College Station.

Trait	Mean	Max	Min	Std Dev	Std Err
Plant height, cm	357.4	424.6	273.8	27.4	2.37
3rd internode diameter, mm	17.7	20.7	14.3	1.31	0.11
3rd internode length, cm	19.5	29.2	6	4.2	0.36
6th internode diameter, mm	16.4	19.8	13.1	1.39	0.12
6th internode length, cm	27.8	35.1	8.9	5.7	0.49
Stalk weight per plant, g	813.2	1277.5	504.1	155.7	13.45
Leaf weight per plant, g	157.6	320	7.5	57.3	4.9
Whole plant weight, g	970.9	1586.6	535.8	193.2	16.6
Moisture, %	70.1	75.6	62.7	2.8	0.24
Yield, Mg ha	14.4	23.4	7.4	3.3	0.29
Leaf length, cm	104.3	118	88.6	5.2	0.45
Leaf width, cm	6.3	8.8	4.1	0.82	0.07
Leaf angle, degrees	30.3	39.8	19.6	4	0.34
Acetyl, %	1.45	1.9	1.1	0.17	0.01
Arabinan, %	2.23	3.2	1.7	0.23	0.02
Ash, %	9.28	12.4	7.1	0.9	0.07
Cellulose, %	28.5	33.3	24.3	1.95	0.16
EtOH, %	3.72	4.6	3.4	0.19	0.01
Galactan, %	1	1.3	0.8	0.07	0.006
Glucan, %	30.38	34.3	26.7	1.6	0.13
Lignin, %	12.85	21.1	10.4	1.35	0.11
NSI, %	2.54	4.1	0.1	0.64	0.05
Protein, %	3.91	5.7	2.5	0.69	0.06
SI, %	6.35	9.8	4.7	0.62	0.05
Solubles, %	16.89	22.9	10.9	2.03	0.17
Structural, %	78.71	83.5	72.7	1.89	0.16
Sucrose, %	3.51	7.2	1.1	1.23	0.1
Starch, %	2.17	6.2	0.3	1.57	0.13
Xylan, %	16.94	18.7	15.4	0.65	0.05

Table 8. Phenotypic and composition trait mean, minimum, maximum, standard deviation and standard error of testcross hybrid lines from the ATx2928(R10709/F08331*bmr12*) RIL population for College Station 2018.

Trait	Mean	Max	Min	Std Dev	Std Err
Plant height, cm	236.4	317.9	151.5	27.5	2.3
3rd internode diameter, mm	19.3	23.8	14	2.1	0.18
3rd internode length, cm	14.9	21.7	5.6	3.3	0.29
6th internode diameter, mm	18.5	22.6	12.6	1.9	0.17
6th internode length, cm	19.2	31.1	9	4.8	0.41
Stalk weight per plant, g	538.8	945.7	278.2	109.4	9.4
Leaf weight per plant, g	129.9	393.1	68.7	35.1	3.03
Whole plant weight, g	668.7	1115.8	356.8	124.4	10.7
Moisture, %	79	82.9	72.6	1.41	0.12
Yield, Mg ha	18.5	23.5	13.8	2.09	0.18
Leaf length, cm	107.1	120.3	90.3	4.84	0.41
Leaf width, cm	6.9	9.1	5.1	0.78	0.06
Leaf angle, degrees	25.8	38.6	19.6	3.4	0.29
Acetyl, %	1.28	1.5	1.1	0.09	0.008
Arabinan, %	2.64	3.1	1.8	0.24	0.02
Ash, %	10.75	12.6	8.4	0.75	0.06
Cellulose, %	26.4	33.3	22.2	2.14	0.18
EtOH, %	3.54	4.0	2.9	0.19	0.01
Galactan, %	1.14	1.2	0.9	0.05	0.004
Glucan, %	28.25	36.0	23.5	2.57	0.22
Lignin, %	11.13	14.7	8.5	1.43	0.12
NSI, %	2.11	3.7	1.2	0.4	0.03
Protein, %	6.77	9	4.2	0.86	0.07
SI, %	8.58	10.2	6.3	0.68	0.05
Solubles, %	17.78	23.7	10.1	3.44	0.29
Structural, %	77.64	84.8	71.8	3.16	0.27
Sucrose, %	2.97	6.7	0.6	1.7	0.14
Starch, %	3.49	7.2	0.3	1.4	0.12
Xylan, %	16.03	18.2	14.6	0.73	0.06

In the hybrids from ATx2928 and the Tx2910/R10712 RILs, the ranges in performance averaged 4X which was substantial given the ranges observed among the RILs *per se* (Tables 9 and 10). This increase in range was consistent across the two populations. For example, the largest range in both 2017 and 2018 was noted for whole plant weight – this was not noted in the RILs *per se*. The magnitude of the range shift was greater in 2017 likely due to environment differences affecting growth and a significant genotype x environment effect indicates that the exact response is combination specific. Nevertheless, the response is consistent. As such ranges observed herein imply that both additive and non-additive effects are manifesting in the observed phenotypes and selection based on hybrid performance should lead to inbred lines that improve hybrid performance and possibly subsequent inbred line performance as well.

Table 9. Phenotypic and composition trait mean, minimum, maximum, standard deviation and standard error of testcross hybrid lines from the ATx2928/(Tx2910/R10712) RIL population for College Station 2017.

Trait	Mean	Max	Min	Std Dev	Std Err
Plant height, cm	346.5	415.2	278.1	31	2.84
3rd internode diameter, mm	16.7	20.6	11	1.6	0.15
3rd internode length, cm	20.8	27.5	15	2.5	0.23
6th internode diameter, mm	15.6	19.1	10.2	1.7	0.15
6th internode length, cm	28.5	36.5	20.2	3.2	0.3
Stalk weight per plant, g	741.6	1149.1	290	172.6	15.82
Leaf weight per plant, g	159.4	355	13.1	69.8	6.4
Whole plant weight, g	901.1	1442.5	323.3	225.8	20.7
Moisture, %	70.7	75.3	63.3	2.48	0.22
Yield, Mg ha	13.8	23.8	6.2	3.83	0.35
Leaf length, cm	101.9	116	83.6	6.3	0.57
Leaf width, cm	6.3	8.3	4.5	0.8	0.07
Leaf angle, degrees	27.8	41.7	16.3	4	0.36
Acetyl, %	1.36	1.6	0.9	0.15	0.01
Arabinan, %	2.18	2.8	1.7	0.21	0.01
Ash, %	9.42	11.9	7.6	0.85	0.07
Cellulose, %	28.54	33.2	24.5	1.97	0.18
EtOH, %	3.71	4.2	3.2	0.17	0.01
Galactan, %	1.01	1.2	0.9	0.06	0.005
Glucan, %	30.69	35	26.6	1.75	0.16
Lignin, %	12.81	15.5	10.9	1.03	0.09
NSI, %	2.73	3.7	0.9	0.45	0.04
Protein, %	4.04	6.7	2.4	0.76	0.06
SI, %	6.45	8.0	5.2	0.54	0.04
Solubles, %	15.54	22.4	9.7	2.45	0.22
Structural, %	79.89	84.9	73.4	2.22	0.2
Sucrose, %	3.04	7.2	0.7	1.19	0.1
Starch, %	2.14	5.1	0.3	1.52	0.13
Xylan, %	17.04	18.7	15.5	0.63	0.05

Table 10. Phenotypic and composition trait mean, minimum, maximum, standard deviation and standard error of testcross hybrid lines from the ATx2928/(Tx2910/R10712) RIL population evaluated in College Station in 2018.

Trait	Mean	Max	Min	Std Dev	Std Err
Plant height, cm	235.2	317	153.6	27.5	2.51
3rd internode diameter, mm	19.3	23.6	13.3	2	0.18
3rd internode length, cm	15.5	24.9	9.8	3.1	0.28
6th internode diameter, mm	18.6	23.5	13.2	2.2	0.2
6th internode length, cm	18.7	31.2	10.3	4.6	0.42
Stalk weight per plant, g	530.1	876.9	241.9	124.5	11.37
Leaf weight per plant, g	127.3	226	65	29.5	2.69
Whole plant weight, g	657.4	1086.3	325	146.7	13.39
Moisture, %	78.5	81	83.2	1.5	0.13
Yield, Mg ha	18.5	22.6	14.1	1.7	0.16
Leaf length, cm	106.3	115.6	88.3	5.2	0.47
Leaf width, cm	6.5	8.3	4.1	0.7	0.06
Leaf angle, degrees	24	32	18	2.5	0.23
Acetyl, %	1.29	1.6	1.0	0.1	0.009
Arabinan, %	2.6	3.1	1.8	0.26	0.02
Ash, %	10.59	12.2	8.1	0.81	0.07
Cellulose, %	26.46	34.1	22.0	2.37	0.21
EtOH, %	3.52	3.9	3.0	0.16	0.01
Galactan, %	1.13	1.2	0.9	0.05	0.005
Glucan, %	28.28	35.7	24.3	2.67	0.24
Lignin, %	11.08	15.2	8.2	1.57	0.14
NSI, %	2.05	3.1	1.2	0.38	0.03
Protein, %	6.74	8.3	4.6	0.86	0.07
SI, %	8.55	10.1	6.5	0.67	0.06
Solubles, %	17.81	25	9.4	3.68	0.33
Structural, %	77.6	85.1	70.8	3.39	0.31
Sucrose, %	3.27	8.1	0.4	1.82	0.16
Starch, %	3.77	8.8	0.3	1.88	0.17
Xylan, %	15.99	18.4	14.2	0.86	0.07

Overall, the data observed herein implies that both additive and non-additive effects are manifesting in the observed phenotypes and selection based on hybrid performance should lead to inbred lines that improve hybrid performance and possibly line *per se* performance as well.

Correlations between inbreds and hybrids

Correlations presented in this analysis are considered both direct and indirect. A direct correlation is a correlation between the same trait in both the inbred and testcross hybrids. It assesses a direct relation between the parent and the hybrid. The indirect correlations assess the relationship between different traits in the inbred and corresponding hybrid. While not necessarily expected to demonstrate an association, any predictive relationships could prove useful in the breeding program.

Direct correlations between inbred and hybrid traits were generally low in both populations for both years (Tables 11-14). Of the 12 possible direct correlations in each year, four and three were statistically significant in the R10709/F08331*bmr12* population in 2017 and 2018, respectively (Tables 11 and 12). Of these associations, only 2 were consistent across both years of hybrid data (6^{th} internode diameter and moisture content). In the Tx2910/R10712 population, 6 and 1 of the 12 possible correlations were statistically significant in 2017 and 2018, respectively (Tables 13 and 14). The single association detected in 2018 was also noted in 2017 (stalk weight/plant). All of these associations were positive correlations. The lower number of associations in 2018 is likely due to the fact that the inbred lines were evaluated only in 2017. Therefore, correlations between inbred and hybrid performance in 2017 use the same environment. However, correlations with 2018 assess the consistency of response across years.

Of the direct correlations detected, none were highly correlated; the highest r value was for moisture content at r = 0.49 (Table 13). Interestingly, moisture content was the only correlation observed across both years and population; this implies that moisture content is controlled by repeatable genetic factors. In fact, numerous genes have been described that influence the juiciness of the stalk and could be segregating in the Tx2910/R10712 population (Shiringani et al., 2010;

Anami et al., 2015). Correlations between standard important agronomic traits (plant height, biomass yield) did not exist in any population or any year. As such given the lack of correlation or low correlations it appears that prediction of the hybrid performance from direct correlation with traits is not possible in bioenergy sorghum.

While the indirect correlations are less obvious, trends here could also be useful in a breeding program if the correlations are consistent and strong enough to be predictive. In the R10709/F08331*bmr12* population, a total of 21 and 42 significant indirect correlations were identified between the inbred lines and 2017 and 2018 hybrids, respectively (Tables 11 and 12). Of these correlations, 13 were detected across both 2017 and 2018 environments (Tables 11 and 12). The traits commonly involved in these consistent associations between inbred and hybrids were 6th internode diameter and leaf weight. While statistically significant, the correlations were generally low and none of them were directly related to biomass yield or plant height.

In the Tx2910/R10712 population, a total of 47 and 14 significant indirect correlations were identified between the inbred lines and 2017 and 2018 hybrids, respectively (Tables 13 and 14). Of these correlations, 7 were detected across both 2017 and 2018 environments (Table 13 and 14). The trait associated with the most correlations was 6th internode length. Given that 6th internode diameter was important indirect correlations in the R10709/F08331*bmr12* population, it appears that the sixth internode, both diameter and length is important and correlated with many structural components of the plant. Like the R10709/F08331*bmr12* population, while statistically significant, the correlations were generally low and none of them were directly related to biomass yield or plant height.

The importance of the sixth internode in these populations maybe associated with leaf angle and productivity. Previous research has suggested that biomass yield can be influenced by leaf

angle due to more erect leaves being better able to capture sunlight, ultimately improving photosynthetic capabilities (Truong et al., 2015). In the present study, inbred leaf angle was only correlated with biomass yield in 2017; in 2018 leaf angle was not statistically significant for genotypes in any hybrid population, thus it is possible that there was not enough variation in hybrid leaf angles in 2018 to elucidate a relationship between inbred leaf angle and hybrid leaf angle and ultimately hybrid yield as the previous study suggested. Therefore, further research is warranted to validate this rationale.

The lack of consistent and repeatable significant correlations suggests that many of the inbred traits are not viable metrics to use as direct prediction of hybrid yield given that they were not stable across multiple years. This finding agrees with those of Venuto and Kindiger (2008), who found that stem traits had low correlations with yield. However, the correlation between inbred 3rd internode diameter and hybrid stalk weight per plant, which was detected in both years and in both populations suggests that thicker stalked inbred parents produce hybrids with higher stalk weights. It could be used as an actionable tool for breeders to use during the selection process.

It is likely that the shift from a PI parent to a PS hybrid affects the correlations in sorghum. In grain sorghum, Jordan et al. (2003) and Habyarimana et al. (2004) did not see a meaningful correlation between inbred traits and hybrid yield. Given that yield is a complex trait, no single metric is likely to possess the ability to predict it. Further, since photoperiod sensitivity differences between inbred and hybrid further reduce the ability to identify correlations, it seems unlikely that inbred phenotypes can be used to predict hybrid performance and testcrosses remain essential.

Table 11. Correlations between agronomic and plant structural traits for a set of RIL from the cross of R10709/F08331*bmr12* evaluated in 2017 (column) and their respective hybrid ATx2928/(R10709/F08331*bmr12*) evaluated in 2017 (top row). Both trials were grown in College Station, Texas. Direct trait correlations between inbred and hybrid performance are highlighted in blue on the diagonal. Indirect correlations between traits in inbred hybrids are above and below the diagonal.

Traits	3rd internode diameter, mm	3rd internode length, cm	6th internode diameter, mm	6th internode length, cm	Leaf angle, degrees	Leaf length, cm	Leaf width, cm	Yield, Mg ha	Moisture %	Plant height, cm	Stalk weight per plant, g	Weight of leaves per plant, g
3rd internode diameter, mm	0.27 ***	-0.07	0.32 ***	0.02	0.02	0.12	0.02	-0.10	0.10	0.02	0.21 *	0.00
3rd internode length, cm	-0.06	-0.08	-0.04	-0.12	0.02	-0.14	-0.17	-0.11	0.15	0.02	0.16	0.06
6th internode diameter, mm	0.25 ***	-0.07	0.36 ***	-0.03	0.02	0.20 *	0.15	-0.03	0.02	-0.03	0.13	0.00
6th internode length, cm	-0.10	0.02	-0.15	0.06	-0.03	-0.18 *	-0.13	-0.09	0.08	0.05	0.03	-0.03
Leaf angle, degrees	-0.01	-0.32 ***	0.03	-0.33 ***	0.00	-0.03	-0.03	-0.28 ***	0.00	-0.44 ***	0.14	0.26 ***
Leaf length, cm	0.01	-0.15	0.08	-0.12	0.06	0.11	-0.03	-0.17 *	0.10	-0.15	0.04	0.09
Leaf width, cm	0.15	0.05	0.30 ***	0.08	-0.03	0.00	0.14	-0.02	-0.18 *	-0.11	0.13	0.04
Yield, Mg ha	0.00	-0.18 *	0.03	-0.19 *	0.01	-0.10	-0.22 *	-0.08	-0.09	-0.23 **	0.18 *	0.36 ***
Moisture %	0.06	-0.04	0.05	0.03	0.07	0.10	0.03	-0.28 ***	0.43 ***	-0.05	0.17 *	-0.10
Plant height, cm	-0.10	-0.08	-0.14	-0.13	0.08	-0.10	-0.16	0.05	0.10	-0.07	-0.03	0.08
Stalk weight per plant, g	0.15	-0.13	0.20 *	-0.06	0.10	0.23 **	0.09	-0.11	0.34 ***	-0.04	0.15	-0.07
Weight of leaves per plant, g	0.23 **	-0.07	0.29 ***	-0.03	0.02	0.09	0.05	-0.14	0.22 *	-0.12	0.21 *	0.11
Weight with pan, g	0.17 *	-0.11	0.24 ***	-0.05	0.07	0.23 **	0.10	-0.08	0.30 ***	-0.05	0.15	-0.04

Table 12. Correlations between agronomic and plant structural traits for a set of RIL from the cross of R10709/F08331*bmr12* evaluated in 2017 (column) and their respective hybrid ATx2928/(R10709/F08331*bmr12*) evaluated in 2018 (top row). Both trials were grown in College Station, Texas. Direct trait correlations between inbred and hybrid performance are highlighted in blue on the diagonal. Indirect correlations between traits in inbred hybrids are above and below the diagonal.

Traits	3rd internode diameter, mm	3rd internode length, cm	6th internode diameter, mm	6th internode length, cm	Leaf angle, degrees	Leaf Length, cm	Leaf width, cm	Yield, Mg ha	Moisture %	Plant height, cm	Stalk weight per plant, g	Weight of leaves per plant, g
3rd internode diameter, mm	0.36	0.05	0.29 ***	0.19 *	0.17	0.00	0.17 *	0.01	0.28 ***	-0.11	0.27 ***	0.08
3rd internode length, cm	-0.08	-0.12	-0.14	-0.18 *	-0.23 **	-0.04	-0.10	0.09	-0.11	-0.02	-0.08	-0.03
6th internode diameter, mm	0.31 ***	0.14	0.26 ***	0.30 ***	0.28 ***	0.03	0.24 ***	0.03	0.25 ***	-0.10	0.23 **	0.00
6th internode length, cm	-0.09	0.00	-0.08	-0.08	-0.24 ***	-0.18 *	-0.23 **	0.13	-0.07	0.18 *	0.08	0.01
Leaf angle, degrees	0.02	-0.14	0.03	-0.13	-0.02	0.02	-0.03	0.00	-0.07	-0.21 *	-0.11	-0.04
Leaf length, cm	0.12	-0.20 *	0.09	-0.15	-0.01	0.09	0.10	-0.14	0.16	-0.20 *	-0.02	-0.03
Leaf width, cm	0.34	0.19 *	0.30 ***	0.39	0.20 *	-0.13	0.24 ***	0.03	0.13	-0.12	0.28 ***	0.13
Yield, Mg ha	0.00	-0.04	-0.01	0.02	-0.05	-0.18 *	-0.06	0.05	-0.02	0.01	-0.03	-0.12
Moisture %	0.05	-0.10	0.04	-0.08	-0.19 *	0.06	0.15	-0.20 *	0.30 ***	-0.24 **	0.05	0.03
Plant height, cm	0.00	-0.15	0.01	-0.19 *	-0.04	0.03	-0.07	0.05	-0.01	0.07	0.04	-0.02
Stalk weight per plant, g	0.19 *	0.00	0.17 *	0.10	0.09	0.12	0.17 *	-0.07	0.38	-0.19 *	0.20 *	0.01
Weight of leaves per plant, g	0.28 ***	0.06	0.26 ***	0.16	0.17 *	0.04	0.24 ***	0.00	0.27 ***	-0.16	0.23 **	0.07
Weight with pan, g	0.21 *	0.04	0.19 *	0.12	0.15	0.10	0.20 *	-0.05	0.36	-0.19 *	0.19 *	0.01

Table 13. Correlations between agronomic and plant structural traits for a set of RILs from the cross of Tx2910/R10712 evaluated in 2017 (column) and their respective hybrid ATx2928/(Tx2910/R10712) evaluated in 2017 (top row). Both trials were grown in College Station, Texas. Direct trait correlations between inbred and hybrid performance are highlighted in blue on the diagonal. Indirect correlations between traits in inbred hybrids are above and below the diagonal.

Traits	3rd internode diameter, mm	3rd internode length, cm	6th internode diameter, mm	6th internode length, cm	Leaf angle, degrees	Leaf length, cm	Leaf width, cm	Yield, Mg ha	Moisture %	Plant height, cm	Stalk weight per plant, g	Weight of leaves per plant, g
3rd internode diameter, mm	0.34 ***	0.12	0.40 ***	0.25 **	-0.11	0.13	0.06	0.12	-0.09	0.09	0.39 ***	0.32 ***
3rd internode length, cm	-0.19 *	0.21 *	-0.22 *	0.33 ***	0.13	0.18 *	-0.02	-0.18 *	-0.09	0.05	-0.23 *	-0.22 *
6th internode diameter, mm	0.33 ***	0.07	0.39 ***	0.20 *	-0.08	0.18	0.12	0.15	-0.16	0.13	0.31 ***	0.19 *
6th internode length, cm	-0.12	0.08	-0.13	0.04	0.13	-0.03	0.04	-0.06	-0.12	-0.09	-0.06	-0.11
Leaf angle, degrees	0.19 *	-0.07	0.18 *	0.10	0.04	-0.02	0.03	-0.19 *	-0.09	-0.24 **	0.28 ***	0.20 *
Leaf length, cm	0.10	-0.02	0.15	0.01	-0.16	0.15	0.02	0.06	0.00	0.02	0.11	0.07
Leaf width, cm	0.23 **	0.03	0.27 ***	0.22 *	-0.06	0.07	0.09	0.02	-0.20 *	0.04	0.21 *	0.14
Yield, Mg ha	0.09	0.01	0.08	0.07	-0.18	0.19 *	0.09	0.10	-0.21 *	0.20 *	0.04	0.07
Moisture %	0.04	-0.23 **	-0.05	-0.19 *	0.12	-0.16	-0.10	0.02	0.49 ***	-0.16	0.13	0.16
Plant height, cm	0.08	0.23 *	0.05	0.35 ***	0.14	0.16	0.10	0.02	-0.10	0.17	0.03	-0.06
Stalk weight per plant, g	0.23 *	0.00	0.23 *	0.16	-0.05	0.08	-0.02	0.05	0.15	-0.01	0.33 ***	0.25 **
Weight of leaves per plant, g	0.27 ***	0.00	0.33 ***	0.05	-0.13	-0.07	0.08	0.02	-0.08	-0.06	0.33 ***	0.38 ***
Weight with pan, g	0.24 **	0.03	0.27 ***	0.19 *	-0.09	0.10	0.02	0.10	0.08	0.01	0.30 ***	0.27 ***

Table 14. Correlations between agronomic and plant structural traits for a set of RILs from the cross of Tx2910/R10712 evaluated in 2017 (column) and their respective hybrid ATx2928/(Tx2910/R10712) evaluated in 2018 (top row). Both trials were grown in College Station, Texas. Direct trait correlations between inbred and hybrid performance are highlighted in blue on the diagonal. Indirect correlations between traits in inbred hybrids are above and below the diagonal.

Traits	3rd internode diameter, mm	3rd internode length, cm	6th internode diameter, mm	6th internode length, cm	Leaf angle, degrees	Leaf length, cm	Leaf width, cm	Yield, Mg ha	Moisture %	Plant height, cm	Stalk weight per plant, g	Weight of leaves per plant, g
3rd internode diameter, mm	0.15	0.18 *	0.18	0.24 **	0.08	0.18	0.16	0.01	0.18	0.06	0.22 *	0.10
3rd internode length, cm	-0.06	0.18	-0.12	0.19 *	0.01	0.03	-0.17	0.04	-0.03	0.13	0.00	-0.11
6th internode diameter, mm	0.12	0.02	0.11	0.13	0.08	0.13	0.12	0.12	0.23 *	0.05	0.16	0.05
6th internode length, cm	-0.06	0.01	-0.06	0.06	-0.05	0.09	-0.15	0.09	-0.20 *	0.10	-0.09	-0.10
Leaf angle, degrees	0.04	0.07	0.06	0.13	0.06	0.03	0.03	-0.08	-0.11	-0.08	0.02	0.08
Leaf length, cm	0.17	-0.07	0.19 *	-0.01	0.05	0.10	0.12	-0.10	0.22 *	-0.08	0.15	0.12
Leaf width, cm	0.06	0.13	0.07	0.21 *	0.03	-0.03	0.01	-0.04	-0.01	0.16	0.15	-0.01
Yield, Mg ha	0.18	-0.07	0.18	0.06	-0.01	0.13	-0.06	0.12	0.15	0.13	0.17	0.06
Moisture %	0.07	-0.19 *	0.09	-0.30 ***	-0.13	-0.04	0.02	-0.11	0.09	-0.17	0.04	0.12
Plant height, cm	0.06	0.13	0.02	0.16	0.03	0.00	-0.10	0.07	-0.04	0.15	0.07	-0.09
Stalk weight per plant, g	0.16	0.05	0.18 *	0.08	-0.02	0.09	0.06	-0.01	0.14	-0.06	0.18 *	0.15
Weight of leaves per plant, g	0.11	0.08	0.10	0.09	-0.01	0.04	0.08	-0.09	0.07	0.00	0.05	0.03
Weight with pan, g	0.17	0.08	0.19 *	0.11	0.02	0.10	0.10	-0.01	0.16	-0.02	0.21 *	0.15

CHAPTER III

HERITABILITY AND HETEROSIS ESTIMATES FOR AGRONOMIC TRAITS IN ENERGY SORGHUM

Introduction

The term heterosis was first used by George Shull and Edward East to describe the observable improvement in performance between a hybrid and its inbred counterparts - after which point, the terms, heterosis and hybrid vigor became synonymous (Berlan, 2018). The concept of heterosis was originally suggested by Charles Darwin who observed that maize hybrids were 15% taller than the inbreds produced by selfing the inbred parents (Darwin, 1876). Ultimately, heterosis or hybrid vigor occurs when the hybrid outperforms either of the inbred parents.

Heterosis can be defined in several different ways. The two most common approaches are high-parent and mid-parent heterosis. High-parent heterosis is the percentage difference between the best parent and the respective hybrid for any trait. Mid-parent heterosis is the percentage difference between the average of the parents and the respective hybrid for any trait. These two methods differ in purpose of use; genetic analyses often use mid-parent heterosis because it focuses on genetic effects while breeding programs focus exclusively on high-parent heterosis because higher performance of the hybrid is required if the hybrid is to be adopted by the commercial producer.

Once methods to produce hybrids were developed, heterosis became the single most important factor in the increased production of many crops including maize, sorghum, and rice (Kaeppler, 2012). The impacts of capturing heterosis in hybrids are immense. In corn, Duvick (2005) demonstrated that maize yields increased by almost 500% from 1930 to 2000 - much of

this is attributed to the discovery and capture of heterosis. Hybrid rice has yield advantages of 20 – 30% over the best inbreds (Cheng et al., 2007). Hybrid sunflowers have heterosis values ranging from 110 – 218% for seed yield (Karasu et al., 2010).

For sorghum, the discovery of cytoplasmic male sterility in 1954 made crossing feasible, allowing the economic production of hybrid seed (Stephens and Holland, 1954). Duvick (1999) estimated that hybrid advantage over inbred performance is 19% annually in grain sorghum. In a more recent study, researchers in Ethiopia reported that high-parent heterosis for hybrid grain yield ranged from 16-52% depending on the environment that it was grown (Mindaye et al., 2016). In a 2014 study on biomass sorghum, high-parent heterosis for biomass yield in hybrids averaged 24.8% across all four environments tested (Packer and Rooney, 2014). However, percent heterosis can be a misleading indicator of combining ability. High yielding parents often produce the highest yielding hybrid, but not the highest percent heterosis; since a poor hybrid can show a high percent heterosis (Liang et al., 1972).

While heterosis has been captured and effectively utilized for many years, there is still no defined cause for the phenomenon. Over the past 100 years, three main hypotheses have developed which are dominance (Davenport, 1908), overdominance (Shull, 1908), and epistasis (Schnell and Cockerham, 1992). The dominance hypothesis states that heterosis is the result of beneficial dominant alleles that mask any unfavorable effect of a recessive allele at any given locus (Davenport, 1908). The overdominance theory states that the heterozygous combination of alleles in the hybrid is better than either of the homozygous states of the parents, which suggests that the two alleles complement each other in the heterozygote and there is an over-expression of those genes in the hybrid. The epistasis hypothesis states that heterosis is due to the non-allelic interactions between the genes at two or more different loci. Based on research, the presence of

both dominant and epistatic theories are the most likely to contribute to heterosis (Meredith and Bridge, 1972; Graham et al., 1997; Shen et al., 2014; Jiang et al., 2017).

The gains from hybrids depend on the amount of heterosis expressed for economically important traits. If heterosis is present, then it is maximized by utilizing the genetic variability and complementary genes found between heterotic breeding populations. In grain crops, heterosis is most commonly defined as high-parent heterosis because if the hybrid is not better than the best parent, producers would simply plant the best parent and not use a hybrid. Mindaye et al. (2016) reported that the magnitude of heterosis observed in Ethiopian sorghum was directly related to the genetic distance between the parental lines that were used to make the hybrids. Blum (1970) found that hybrids benefitted from heterosis in almost all yield measures, with number of grains per panicle averaging 132% and weight of grain per panicle averaging 127% of their best parents. In a study looking at seedling growth under cold temperatures, researchers found that heterosis had favorable effects on all seedling traits that were measured (Yu and Tuinstra, 2001). Jordan et al. (2003) suggested that valid potential exists to develop a prediction system for hybrid grain sorghum yield using molecular markers, as their research group was able to account for 71% of variation in yield using RFLP markers and major effect QTL.

Most heterosis studies focus on traits of economic importance; usually this is grain/fruit or some specific portion of the plant which is only a portion of the total biomass produced by the crop. In forage and energy crops, heterosis levels are typically lower because it is not possible to partition improvement to a specific portion of the crop. Consequently, heterosis differs between energy and grain sorghum. Heterosis in forage bromegrass was identified to be around 14% (Casler et al., 2005). Biomass Arabidopsis heterosis was observed to be 50% (Meyer et al., 2004). Previous studies wherein maturity varied have reported different levels of heterosis in biomass sorghums.

In most cases, heterosis for biomass yield is relatively low. Knoll and Anderson (2016) saw reduced heterosis for biomass yield when examining a panel of sweet sorghum lines; Packer and Rooney (2014) saw high-parent heterosis values of 24.8% for biomass yield in photoperiod sensitive energy sorghum hybrids; and Corn (2011) observed high-parent heterosis values for biomass yield ranging from 40 – 190% in sweet sorghum hybrids.

Further complicating the measurement of heterosis in energy sorghum is the effect of complementation between parents and their resulting hybrid. As described previously, PI parents and their PS hybrids differ greatly for maturity and height traits. This difference in phenotypes between parent and progeny, inflates heterosis values (Pedersen et al., 2013; Bunphan et al., 2015). These heterosis values are primarily a function of the complementary *Ma* and *Dw* genes, which are not yield genes *per se*.

Once these alleles are fixed, further improvement based solely on their presence is not possible. Once *Ma* and *Dw* are fixed in a breeding population, future improvement must rely on the identification of other factors influencing yield in elite photoperiod sensitive hybrids. In Chapter II, predicting yield of a hybrid based on inbred performance was not effective. Given that there has not been any study to assess the genetic variation observed within a set of testcross hybrids that are genetically uniform for the photoperiod sensitive response. Likewise, while genomic selection methods have been effective in several grain crops, they have not yet been applied in forage or energy sorghum (Schrag et al., 2010).

Within this context, the objectives of this chapter are i) to assess the heritability of phenotypic traits between the inbred parents and biomass hybrids, and ii) assess the heterosis estimates of phenotypic traits in the hybrids to attempt to identify a trend that could be used for breeding purposes. The underlying hypothesis is that the narrow-sense heritability of traits is

decreased in hybrids compared to inbred parents and that heterosis values are skewed due to the effects of the *Ma* genes. If this hypothesis is true, it follows that selection and improvement of bioenergy sorghum is more difficult than improvements in a grain sorghum.

Materials and Methods

Heritability and Heterosis Estimates

The parental RIL populations and the resulting hybrid progeny used for this study were the same as those detailed in Chapter II for correlation of inbred phenotypic traits with hybrid biomass yield. From that data set, a subset of traits was selected for analysis. The selected traits are plant height, 3rd internode length, 3rd internode diameter, 6th internode length, 6th internode diameter, percent moisture, leaf weight per plant, stalk weight per plant, yield, leaf length, leaf angle, and leaf width.

Data was analyzed using JMP 14.3 PRO software using a combined model. All dependent variables were analyzed by year for both inbreds and hybrids in each population using an all random model of $Y = \alpha i + \beta(\alpha)ij + yk + \alpha yik + \varepsilon$ where α= year (i = 1, 2), β = repetitions (j = 1, 2), y = genotypes (k = 1… 86), and ε = error.

Heterosis estimates were calculated for high-parent heterosis (HPH) as (F_1 value - (high parent)) / (high parent). Variance components were estimated from this analysis using the formula $\sigma^2_{Trait} = \sigma^2_G + \sigma^2_R + \sigma^2$error where σ^2_G represents the variance due to genotype, σ^2_E is the variance due to the environment (Year), σ^2_R is the variance due to replications, and σ^2_{error} is the variance due to experimental error. Variance estimates of yield across years were calculated using the formula $\sigma^2_{Trait} = \sigma^2_G + \sigma^2_E + \sigma^2_R + \sigma^2_{GxE} + \sigma^2_{error}$ with all terms the same and where σ^2_{GxE} is the

variance due to the genotype by environment interactions. These variance components were used to calculate broad-sense Heritability (H^2) on an entry mean basis using the formula $H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gxe}^2}{e} + \frac{\sigma_e^2}{re}}$. Correlations between the heterosis of phenotypic traits and biomass yield were calculated. Significance of heterosis and correlations were tested by least significant differences after an F test of the experiment was conducted.

Results

Range of Heterosis

High-parent heterosis varied widely depending on the trait but HPH was consistent for a trait across the populations (Tables 15 and 16). As expected, biomass yield expressed the highest HPH averaging between 200 - 400 percent (Tables 15 and 16). As predicted, much of this effect is due to the epistatic interaction of the *Ma1* and *Ma5* loci; when combined in the proper orientation, they become *de facto* yield genes manifested in hybrid offspring that are photoperiod sensitive and do not flower and far out yield either of the parents.

Most traits had moderate HPH (20 to 70% for plant height, leaf length and leaf width), whereas a few traits expressed little to no HPH (-30 to 20% for internode length and moisture content). Leaf angle had a consistently negative average HPH, inferring that across both populations, the hybrids' leaves from the 6th internode were more erect compared to their inbred parents. Furthermore, both 3rd and 6th internode length likely resulted in a low HPH value as the internodes measured were located close to the ground. Previous research has demonstrated the longest internodes in biomass sorghum are internodes 17 and 21, respectively, and those contribute the largest height differences between biomass sorghum and photoperiod insensitive sorghum (Fujii et al., 2014). If true, higher HPH values would likely have been observed in this study if

internodes were sampled higher up on the hybrids and the same internode on their respective inbred parents. Moisture content had low HPH which is not surprising, given that the moisture content of a sorghum plant is highly influenced by specific temporal environmental factors such as available moisture at time of harvest.

Besides average HPH, the range between the minimum and maximum HPH varied widely. While there is minimal differences in the average heterosis of the 3rd internode length, 3rd internode diameter, 6th internode length and 6th internode diameter across populations or environments, the range of heterosis values for the 6th internode diameter are much higher than the range for the 3rd internode diameter. This can be observed in both populations and across years, which might be more of an indication of growing conditions rather than heterosis values.

The estimate of HPH indirectly predicts the hybrid potential of energy sorghum and the relative importance of non-additive effects to their productivity. For biomass yield the R10709/F08331*bmr12* population, HPH averaged 250% and 344% in 2017 and 2018, respectively. The biomass yield HPH averaged in the Tx2910/R10712 population was 262% and 387% in 2017 and 2018, respectively (Tables 15 and 16). These high numbers demonstrate the value of introducing photoperiod sensitivity to the system and confirms its massive effect on productivity. Given that this significant jump is the result of qualitative genes that are now uniformly homozygous, further exploitation of improvement will rely on other traits influencing biomass yield in a photoperiod sensitive background.

For plant height, heterosis is higher for both hybrid populations in 2017 but that is again most likely due to the combination of environmental effects and the fact that the inbred parents were only grown in 2017. Since the environment has such a large effect on heterosis values, the estimates of these should be used in a general sense (Shang et al., 2016; Li et al., 2018; Knoll et

al., 2018). The wide range of heterosis values here and the wide range of phenotypic traits discussed in Chapter II highlight how the differences are most likely due to a combination of environmental effects and the effects of the height and maturity genes, which skew and mask the various phenotypic traits that would normally be used as criteria for selection. This ultimately makes selection based on those traits difficult and less impactful.

Table 15. The minimum, average, maximum, and range of high-parent heterosis values for both 2017 and 2018 ATx2928/(R10709/F08331*bmr12*) hybrid populations. High-parent heterosis was calculated by using inbred line data from the respective RIL parent compared with testcross yield data of that line in 2017 and 2018.

	2017				2018			
Trait	Min	Avg	Max	Range	Min	Avg	Max	Range
Plant height (cm)	22.7	70.4	174.2	151.5	-31.2	12.6	79	111.2
3rd internode length (cm)	-73.5	-6.7	132.6	206.2	-70.7	-28.7	46	116.9
3rd internode diameter (mm)	2.6	68	126.8	124.1	16.5	83.1	168	152.1
6th internode diameter (mm)	-66.9	7.3	71.7	138.7	-67.5	-25.1	35	102.5
6th internode length (cm)	22.7	74.1	212.4	189.6	28.3	97	266	238.4
% Moisture	-17.3	-6.6	21.4	38.7	-6.2	5.3	31	37.4
Biomass yield (Mg ha)	26.5	250.3	720.5	694	99.4	344.6	1156	1057.5
Leaf length (cm)	3.2	29.3	83.3	80.1	3.4	32.8	80	76.7
Leaf width (cm)	-27.2	20.3	89.2	116.5	-16.2	31.1	81	96.9
Leaf angle (degrees)	-55	-17.3	33	88	-57.6	-29.6	24	81.3

Table 16. The minimum, average, maximum, and range of high-parent heterosis values for both 2017 and 2018 ATx2928(Tx2910/R10712) hybrid populations. High-parent heterosis was calculated by using inbred line data from the respective RIL parent compared with testcross yield data of that line in 2017 and 2018.

	2017				2018			
Trait	Min	Avg	Max	Range	Min	Avg	Max	Range
Plant height (cm)	18	65.8	115.1	97.1	-22	12.5	50.9	72.9
3rd internode length (cm)	-40.7	0	73.9	114.7	-67.7	-25.7	21.7	89.4
3rd internode diameter (mm)	4.5	70.9	160.1	155.6	28	97.7	187.5	159.4
6th internode diameter (mm)	-45	13.7	64.8	109.9	-68	-25.3	57.5	125.6
6th internode length (cm)	-23.1	75.2	179.4	202.6	-29.6	109.4	261.9	291.6
% Moisture	-18	-5.4	12.3	30.4	-7	5	27.6	34.7
Biomass yield (Mg ha)	13.7	262	1019.3	1005.6	88	387.1	1195.8	1107.7
Leaf length (cm)	-7	30.9	102.4	109.5	-2.5	36.6	102.4	104.9
Leaf width (cm)	-23.6	25.8	123.5	147.2	-30.5	31	144.4	175
Leaf angle (degrees)	-53.1	17.7	50.6	103.8	-51.8	-28.7	23.4	75.3

Heterosis Correlations

Correlations between biomass yield and HPH for phenotypic traits revealed that repeated significant correlations exist between hybrid biomass yield and HPH plant height, HPH 3rd internode length, and biomass yield in both populations (Table 17). In the R10709/F08331*bmr12* hybrids, the correlations between biomass yield and heterosis were 0.49 and 0.33 in 2017 and 2018, respectively. In the Tx2910/R10712 hybrids the correlations between biomass yield and heterosis were 0.54 and 0.23 in 2017 and 2018, respectively. In both populations the correlations between heterosis and biomass yield are much higher in 2017 than in 2018. Again, this is most likely because the inbred population was only grown in 2017, so their HPH values better represent the 2017 growing environment.

These relationships indicate the importance of HPH in this system to overall productivity. Packer and Rooney (2014) evaluated a closed photoperiod sensitivity system and reported HPH values that average 25%. Given the drastic differences between that system and the one tested herein, it is surmised that most of the heterosis herein is epistatic and associated with *Ma1* and *Ma5*. Further, the numerical values for the correlations are significant, but they are probably not sufficient for breeding purposes else a stronger correlation between inbred and hybrid performance would have been detected in the first study (Lorencetti et al., 2006). Further, given that the only way to obtain heterosis values is by making test crosses and growing the hybrids, which is the very step breeders would like to bypass. Moreover, of all of the heterosis values only biomass heterosis is significantly correlated because it is a measurement of the biggest difference between the hybrid and the inbred parents.

Table 17. Correlation between trait heterosis values and hybrid biomass yield for ATx2928(R10709/F08331*bmr12*) and ATx2928(Tx2910/R10712) populations in 2017 and 2018.

	ATx2928(R10709/F08331*bmr12*)		ATx2928(Tx2910/R10712)	
Population	2017	2018	2017	2018
Trait	Yield (Mg ha)	Yield (Mg ha)	Yield (Mg ha)	Yield (Mg ha)
Plant height (cm)	0.15	0.33 ***	0.40 ***	0.27 **
3rd internode diameter (mm)	0.09	-0.05	-0.02	-0.07
3rd internode length (cm)	0.21 *	0.20 *	0.25 **	0.15
6th internode diameter (mm)	0.00	-0.03	-0.11	-0.14
6th internode length (cm)	0.17 *	0.04	0.15	0.09
Stalk weight per plant (g)	-0.06	0.21 *	-0.12	0.14
Weight of leaves per plant (g)	-0.10	0.01	-0.18	0.10
% Moisture	0.11	0.16	-0.20 *	0.02
Yield (Mg ha)	0.49 ***	0.33 ***	0.54 ***	0.23 *
Leaf length (cm)	0.24 **	0.07	0.10	0.13
Leaf width (cm)	0.18 *	-0.13	0.11	-0.09
Leaf angle (degrees)	0.19 *	0.10	0.04	0.12

Heritability

Heritability estimates ranged from 0.49 to 0.92 across all traits, populations and years (Tables 18 and 19). While heritability estimates for hybrids were generally similar over years, for a few traits, heritability estimates differed greatly between the two years. For example, 6th internode diameter had heritability estimates of 0.66 and 0.80 for 2017 and 2018, respectively (Table 18).

The average heritability over all traits for the inbred population R10709/F08331*bmr12* was 0.79, while the corresponding hybrid populations average heritabilities were 0.67 and 0.69 in 2017 and 2018, respectively. The average heritability over all traits for the inbred population Tx2910/R10712 was 0.78, while the corresponding hybrid populations average heritabilities were 0.70 and 0.65 in 2017 and 2018, respectively. Higher heritability in the inbreds allows for the potential of a greater selection compared to hybrids, corroborating that breeders generally select inbreds rather than hybrids.

For most traits and both populations, heritability estimates were higher in the inbred parents than the hybrids. First, biomass yield heritability in the inbred was near 0.80 while in the hybrids it dropped to approximately 0.60. While all of these are higher than originally expected, the drop in heritability in hybrids is due to the uniformity of response for photosensitivity. When that variability is lost it offset any potential gains from dominant variation that may be exposed in the hybrid. This general reduction in phenotypic variation reflects that the variation remaining has a relatively higher error variation associated because of the loss of the genetic variation underlying the phenotypic trait.

The heritability of traits within the hybrid populations and between the hybrid populations are consistent, specifically stalk weight per plant and leaf weight per plant (Tables 18 and 19). Heritability of the stalk weight per plant was consistently high in both inbred populations but substantially lower across years in each of the hybrid populations. Stalk weight makes up the majority of biomass yield and as such is an important trait to examine when normally breeding for biomass yield. However, in this case the heritability of stalk weight per plant drops off significantly and the highest stalk weight parents did not consistently result in the highest stalk weight hybrids.

When comparing only 2017 data, the inbred heritability for plant height in the R10709/F08331*bmr12* RIL population is equivalent to the hybrids. For all other traits the inbreds have higher heritability than the hybrids and thus will better respond to selection. In the Tx2910/R10712 RIL population inbred heritability is equal to or lower than the hybrids for 3rd internode diameter, 6th internode length, and 6th internode diameter, all other trait heritabilities are higher in the inbreds (Table 19).

Table 18. Broad sense heritability (H^2) for phenotypic traits measured in 2017 and 2018 in College Station for the inbred parents and hybrid offspring for the R10709/F08331*bmr12* population.

Trait	2017 inbreds	2017 hybrids	2018 hybrids
Plant height, cm	0.75 ± 0.038	0.78 ± 0.027	0.72 ± 0.034
3rd internode diameter, mm	0.77 ± 0.027	0.68 ± 0.039	0.71 ± 0.035
3rd internode length, cm	0.76 ± 0.028	0.65 ± 0.043	0.72 ± 0.033
6th internode diameter, mm	0.74 ± 0.030	0.66 ± 0.041	0.80 ± 0.024
6th internode length, cm	0.87 ± 0.015	0.70 ± 0.037	0.71 ± 0.036
Stalk weight per plant, g	0.78 ± 0.025	0.74 ± 0.030	0.62 ± 0.042
Leaf weight per plant, g	0.92 ± 0.009	0.66 ± 0.042	0.68 ± 0.038
Whole plant weight, g	0.77 ± 0.027	0.65 ± 0.039	0.59 ± 0.050
Moisture, %	0.80 ± 0.023	0.65 ± 0.041	0.63 ± 0.045
Yield, Mg ha	0.82 ± 0.021	0.58 ± 0.051	0.58 ± 0.051
Leaf length, cm	0.70 ± 0.035	0.63 ± 0.045	0.62 ± 0.045
Leaf width, cm	0.87 ± 0.015	0.76 ± 0.029	0.79 ± 0.025
Leaf angle, degrees	0.70 ± 0.035	0.63 ± 0.045	0.62 ± 0.045
Biomass moisture, %	0.87 ± 0.015	0.76 ± 0.029	0.79 ± 0.025
Lignin	0.75 ± 0.029	0.64 ± 0.044	0.85 ± 0.017

Table 19. Broad sense heritability (H^2) for phenotypic traits measured in 2017 and 2018 in College Station for the inbred parents and hybrid offspring for the Tx2910/R10712 population.

Trait	2017 inbreds	2017 hybrids	2018 hybrids
Plant height, cm	0.85 ± 0.019	0.77 ± 0.029	0.68 ± 0.040
3rd internode diameter, mm	0.72 ± 0.035	0.80 ± 0.025	0.69 ± 0.040
3rd internode length, cm	0.74 ± 0.035	0.67 ± 0.042	0.63 ± 0.048
6^{th} internode diameter, mm	0.72 ± 0.043	0.83 ± 0.021	0.75 ± 0.032
6^{th} internode length, cm	0.66 ± 0.035	0.72 ± 0.036	0.66 ± 0.043
Stalk weight per plant, g	0.71 ± 0.035	0.74 ± 0.032	0.55 ± 0.057
Leaf weight per plant, g	0.89 ± 0.014	0.69 ± 0.039	0.68 ± 0.041
Whole plant weight, g	0.72 ± 0.035	0.65 ± 0.044	0.64 ± 0.047
Moisture, %	0.92 ± 0.010	0.72 ± 0.036	0.52 ± 0.061
Yield, Mg ha	0.84 ± 0.020	0.59 ± 0.052	0.62 ± 0.049
Leaf length, cm	0.74 ± 0.033	0.61 ± 0.050	0.49 ± 0.066
Leaf width, cm	0.88 ± 0.015	0.76 ± 0.030	0.75 ± 0.032
Leaf angle, degrees	0.74 ± 0.033	0.61 ± 0.050	0.49 ± 0.066
Biomass moisture, %	0.88 ± 0.015	0.76 ± 0.030	0.75 ± 0.032
Lignin	0.73 ± 0.034	0.54 ± 0.059	0.84 ± 0.019

Some dominance effects were expected in the hybrids, but given the structure of this study it is impossible to definitively estimate the relative effects. Most studies evaluating sorghum for dominance variation have had limited success in its detection (Murty et al., 1988; Mohammed et al., 2015), however, at some level dominance effects are manifested in the heterosis values that are produced. Broad sense heritability (H^2) estimates were calculated given that the inbred populations were expected to be completely fixed at all loci, consequently there should be only additive effects and no dominance effects. However, dominance effects will still be present in the hybrids since they were crossed to a common tester. Therefore, the only fair way to compare inbreds vs. hybrids is to use broad sense heritability rather than narrow sense. Differences in heritability estimates between the inbreds and hybrids, while still numerically different, were not of the expected magnitude.

Heritability is one of the most important selection tools for plant breeders, given that its main use is for calculating the response to selection for a given trait. Hence, when heritability is reduced it ultimately makes evaluation of the hybrids difficult and less impactful. Liang et al. (2018) found in pearl millet that genomic prediction accuracy of hybrid yield using only hybrid heritabilities was low (0.48). Furthermore, they found that indiscriminately using inbred heritabilties decreased the prediction accuracy even further (0.30). Rather, the combination of both inbred and hybrid heritiabilities resulted in the highest prediction accuracy (0.52) for hybrid yield.

It is apparent that the effects of the environment, and the maturity and height genes that cause photoperiod sensitivity reduce the relevance of these heterosis and heritability values. Furthermore, specifically the dominance effect in both the *Ma* and *Dw* genes that can only be observed in this situation cause hybrid test crosses to be necessary. It is this dominance effect that masks heterosis and makes heterosis a poor metric for biomass sorghum evaluation. Hence, if there is a dominant allele at all three loci in the parents, seed production is impossible outside of tropical and subtropical environments – which is ultimately the crux of the situation, a method of hybrid prediction needs to be identified in the same breeding system that hybrid seed can be realistically produced (PI x PI = PS). It can again be seen that there is no substitute for growing out hybrid testcrosses, specifically when it comes to predicting the performance of inbred parent combinations to produce biomass hybrids.

CHAPTER IV

IDENTIFICATION OF GENOMIC REGIONS AND QTL ASSOCIATED WITH BIOMASS PRODUCTION

Introduction

Crop improvement programs continually strive to combine methods, technology and approaches to make the breeding process as efficient and effective as possible. This means that programs are continually assessing the existing approaches and how new technology could effectively be deployed within the limits and biological constraints of the specific crop.

One such technology that has been integrated in breeding programs have been molecular markers. From their humble beginnings in the 1980s, molecular markers have become more numerous, easier to use, faster to complete, better quality, and ultimately more effective to deploy within a breeding program (Paterson et al., 2009; Sonah et al., 2013).

Molecular technology has many applications, more suited for plant breeding application than others. Marker-assisted selection (MAS) has be applied in plant breeding to improve selection efficiency, to identify genomic regions under selection, to assess cultivar purity, and to evaluate breeding material (Collard and Mackill, 2008). More recently, genomic selection can be used for improving complex traits and highly quantitative traits, and for developing new marker-based models for genetic evaluation (Bhat et al., 2016).

In sorghum, molecular markers have been used to generate many high-density genetic linkage maps which are useful for the study of QTL (Menz et al., 2002; Nelson et al., 2011; Zou et al., 2012). These QTL studies have covered a wide variety of traits with varied levels of success. Several studies have identified potential QTL that are related to biomass yield and yield-related

traits (Murray et al., 2008; Salas Fernandez et al., 2009). Felderhoff et al. (2012) identified QTL for maturity and height that co-localized with biomass yield as had been observed in several previous studies (Pereira and Lee, 1995; Ritter et al., 2008; Mathur et al., 2017). Many studies have identified QTL for traits related to grain yield, specifically seed size and flowering time (Lin et al., 1995; Natoli et al., 2002; Shiringani and Friedt, 2011; Olson et al., 2012). Most of these QTL explain a significant amount of the variation but it is likely that the effect of these QTL are over-estimated because yield is a highly quantitative trait commonly subjected to overestimation in QTL analysis (Lande and Thompson, 1990; Melchinger et al., 1998; Utz et al., 2000; Carlborg and Haley, 2004). Carlborg and Haley (2004) conclude that it is unlikely that yield is controlled by a single gene, but is rather controlled by hundreds of genes and involves multiple gene-gene epistatic interactions. In addition, most of these studies used highly variable mapping populations related to the number of individuals, generation examined, and parental cross phenotypes (short early flowering x tall late flowering, or sweet sorghum x grain sorghum). This results in often inflated heritability estimates, and inconsistent identification of QTL.

In bioenergy sorghum, studies to detect important QTL for bioenergy traits have focused on photoperiod sensitive cultivars in GWAS analyses and RIL populations. Disasa et al. (2018) identified two QTL for brix content to make more energy dense bioenergy sorghum but was unable to correlate brix and total plant mass. Zou et al. (2012) identified a total of 57 QTL across eight traits in a sorghum RIL population, however only one location and one replication was used in the study. While these studies are useful for the detection of loci underlying important agronomic traits, they are not likely to direct application in the breeding of hybrid bioenergy sorghums.

Therefore, it is important to assess the QTL underlying traits in bioenergy breeding populations. To date, no QTL studies involving populations that are fixed with the allelic

composition of *Ma*$_1$ *ma*$_5$ *Ma*$_6$ have been completed. It is important to assess these populations because they are the basis of the pollinators in bioenergy breeding; when they are hybridized to a seed parent (*ma*$_1$ *Ma*$_5$ *ma*$_6$), they produce uniformly photoperiod sensitive hybrids. In the absence of the effect of the *Ma*$_1$, *Ma*$_5$ and *Ma*$_6$ loci (because of their homozygosity), variation in additional, albeit smaller, effect loci should be manifested. The identification of such QTL could further assist breeders in accelerating the breeding process by allowing them to use marker-assisted selection.

The objectives of this chapter are i) develop a molecular map for each of the RIL populations, ii) identify QTL related to biomass yield in the inbreds and hybrids that are consistent across environments, and iii) identify overlapping genomic regions in both the inbreds and hybrids that are related to yield and can be used for future breeding purposes. Inherent in this assumption is that the complex and quantitative nature of yield combined with the consistent photoperiod sensitivity in the hybrids will make the detection of QTL more challenging.

Materials and Methods

Plant Material and Experimental Design

The parental RIL populations and the resulting hybrid progeny used for this study were the same as those detailed in Chapter II for correlation of inbred phenotypic traits with hybrid biomass yield.

Linkage Map Construction

A total of 86 RILs from the Tx2910/R10712 population and 84 RILs from the R10709/F08331*bmr12* population were used for the construction of two maps. A minimum of 10 seeds from a single panicle of each RIL entry was grown in the greenhouse until sufficient leaf tissue had accumulated. Leaf tissue was harvested, ground and homogenized followed by total genomic DNA extraction with the use of the Quick-DNA™ Plant/Seed Miniprep Kit (Zymo Research, Irvine, CA) according to the manufacturer's protocol.

Digital Genotyping (DG) was used for genotyping the population (Morishige et al., 2013). The DG template was generated using the restriction enzyme *Fse*I and single-end sequencing was conducted using a HiSeq2500v4 (Illumina, San Diego, CA). Sequencing data obtained consisted of 150 bp reads initiating from a barcode and the partial restriction enzyme site. Data was processed using custom Perl and Python scripts, which sorted sequences to individual progenies using a unique barcode as well as trim them for downstream analysis. Parental lines were aligned to the BTx623 reference sorghum genome (Sbicolor_454 v3.0.1) using BLASTN and scored through the progeny using methods described by Morishige et al. (2013). A genetic map was constructed for both populations using JoinMap V5.0 (Van Ooijen, 2018) and a custom R script.

To calculate map distances from recombination frequencies, Kosambi's mapping function was used.

The molecular maps generated from each population was used with the phenotypic information for the inbred population to identify potential QTL in the parental RIL population. That same molecular map was used with the phenotypic information from the corresponding hybrid population to identify possible regions in the parental RIL that were related to biomass traits in the hybrids.

All collected phenotypic traits and NIR estimated traits were used for QTL analysis by single-marker analysis, interval mapping, and composite interval mapping with 1000 permutations using the software WinQTL Cartographer V2.5 (Wang et al., 2012) and the R/qtl package. These traits were plant height, stalk weight per plant, 3rd internode length and diameter, 6th internode length and diameter, percent moisture, leaf length, leaf width, and leaf angle, yield values (metric tons per hectare), and the NIRS estimated traits: ash content, protein, sucrose, ethanol extractives, lignin, whole starch, cellulose, glucan, xylan, galactan, arabinan, and acetyl.

Results and Discussion

Genetic Mapping

For genetic mapping in the Tx2910/R10712 population, 801 markers were scored through 55 $F_{4:5}$ individuals. The resulting map consisted of 10 linkage groups spanning 956 cM with an average of 1.19 cM between markers (Figure 1). A total of 9.3% of the loci in the final map were heterozygous, lower than the theoretical 12.5% heterozygosity that would be expected for an F_4 population. The distribution of alleles from each parent was similar (43.3% for Tx2910 and 47.3% for R10712).

While the original population had 86 individuals, 31 of them were removed from the study because the resulting biomass hybrids were shown to be segregating for early flowering time. Due do the specific nature of the present experiment it was determined important that the hybrids that were evaluated be completely photoperiod sensitive. Moreover, approximately 6.7 percent of all marker data was reported as missing and not included in map construction.

For genetic mapping in the R10709/F08331*bmr12* population, 459 markers were scored through 67 $F_{4:5}$ individuals. The resulting map consisted of 11 linkage groups spanning 1,952.2 cM with an average of 4.25 cM between markers (Figure 2). A total of 6.3% of the loci in the final map were heterozygous which is lower than the theoretical 12.5% heterozygosity that would be expected for an F_4 population. The distribution of alleles from each parent was similar (45.5% for R10709 and 48.1% for F08331).

While the original population had 84 individuals, 17 of them were removed from the study because the resulting biomass hybrids were shown to be segregating for early flowering time. Approximately 2.6 percent of all marker data was reported as missing and not included in map construction.

Figure 1. Genetic map for the R10709/F08331*bmr12* population.

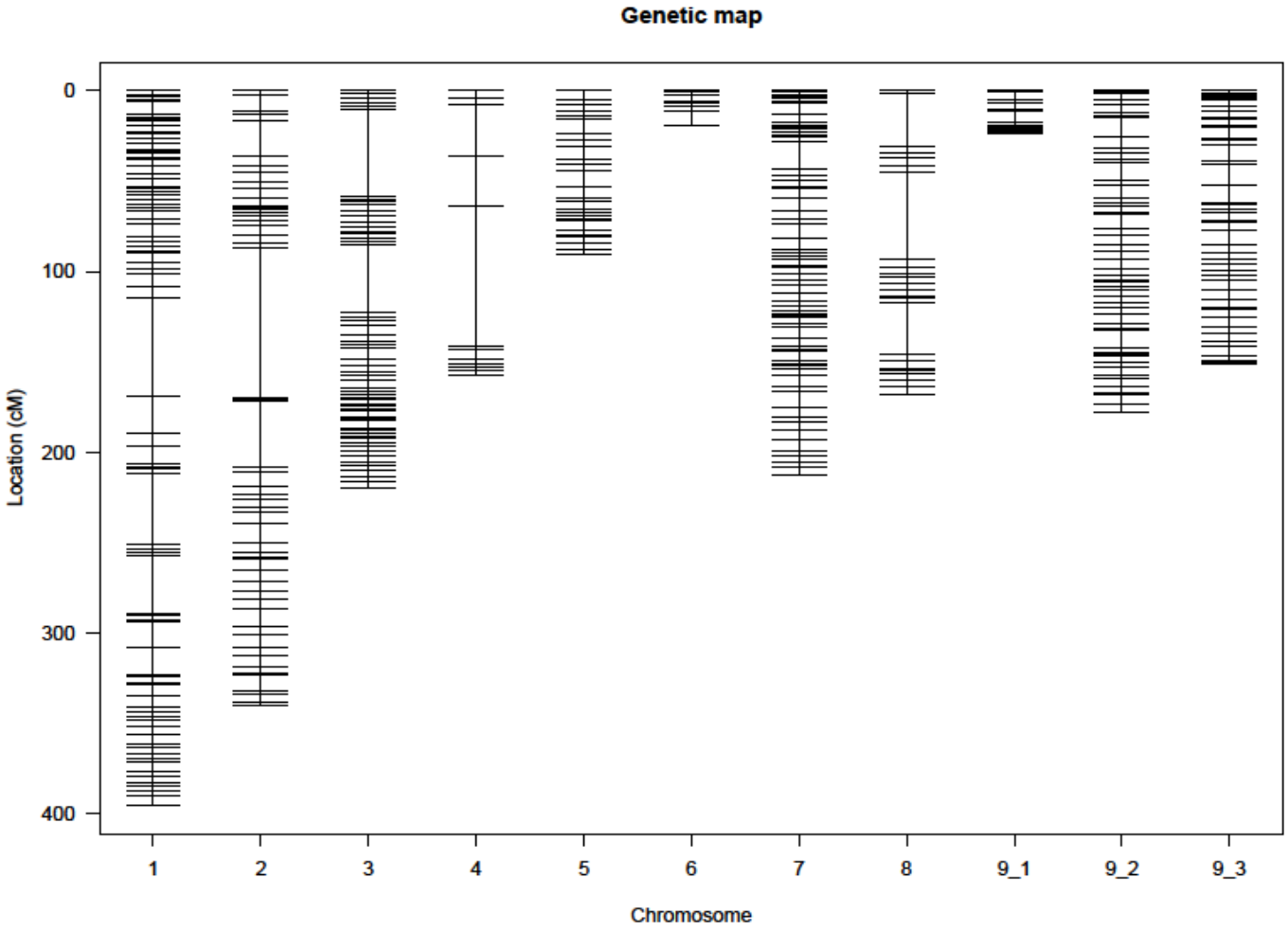
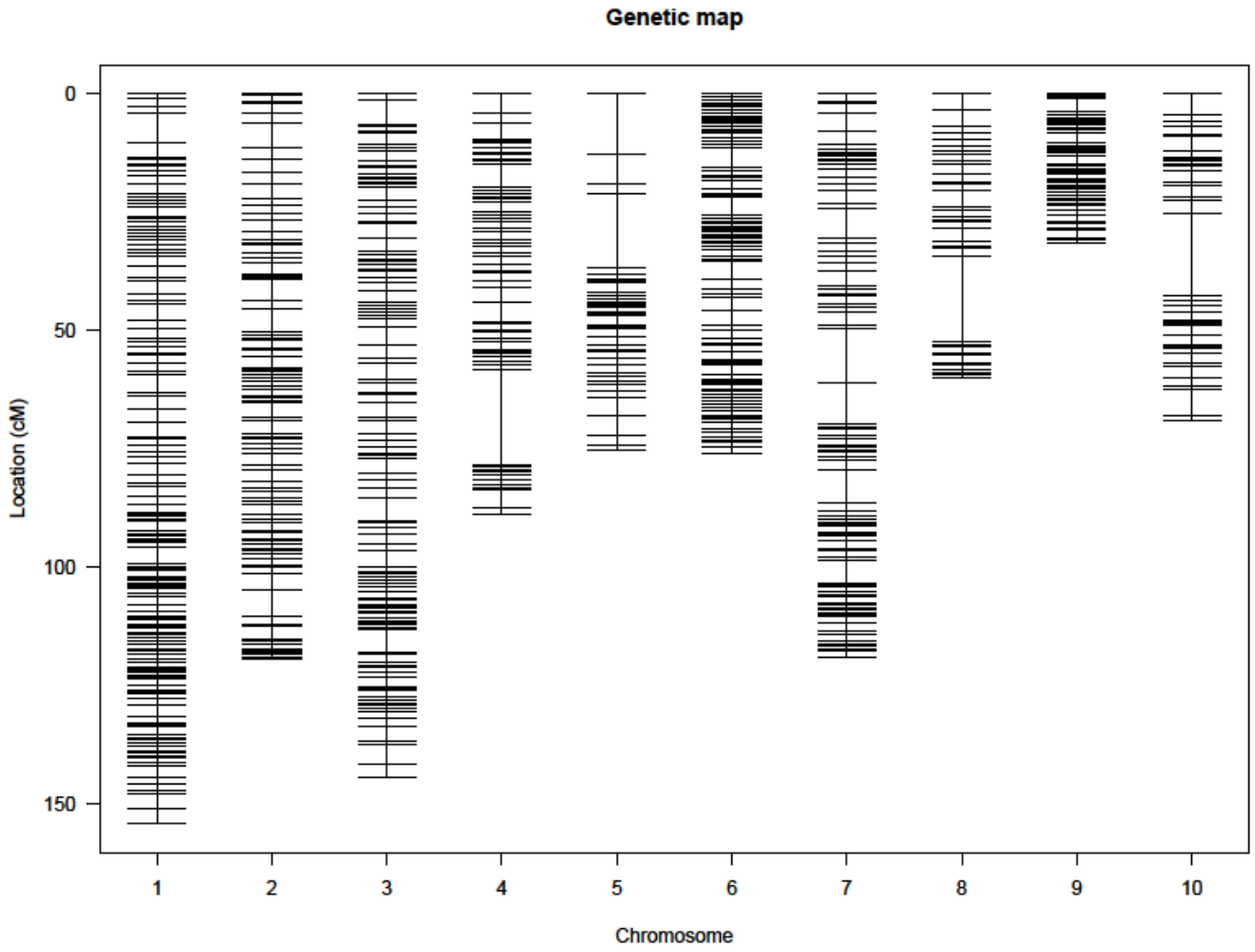


Figure 2. Genetic map for the Tx2910/R10712 population.



QTL Mapping and Analysis

A total of 20 QTL were identified across both populations, their inbreds and hybrids, and environments. Of these 20, ten of them were located on chromosome 7 and were related to an internode measurement (Tables 20 and 21). Chromosome 7 is a major contributor to internode characteristics, which are stable across years, which has been confirmed in several previous studies (Higgins et al., 2014; Hilley et al., 2016, 2017), hence this result was expected. The gene *Dw3*, a major height gene, is also located on chromosome 7 and related to stalk traits, which further confirms the identification of QTL in this study related to internode traits. However, neither internode length or internode diameter have been found to be predictive of overall plant height or biomass yield (Nakamura et al., 2011).

In the Tx2910/R10712 population a total of eight phenotypic QTL were identified in the inbreds and the hybrids across both years; three in the inbred parents and five in the hybrids (Tables 20 and 21). Of these eight, none of the QTL were identified in both the inbreds and the hybrids in the same position. However, several of the QTL did appear on the same chromosome between years in the hybrids for the same traits.

In the Tx2910/R10712 RIL population, two QTL on chromosome 6, one for percent moisture at harvest and the other for biomass yield, explained 61% and 29% of the variation for those traits, respectively (Table 20). A QTL identified for leaf length was located on chromosome 3; it accounted for 46% of the variation for leaf length in that environment.

Table 20. QTL identified in the Tx2910/R10712 inbred population grown in 2017 College Station, Texas.

Chromosome number	Trait name	LOD score	% Variance	Peak		Peak between markers		Additive Effect	Parental Allele Contribution
				From (cM)	To (cM)				
6	Percent moisture	11.3	61.18	21.18	21.62	chr06_50567049	chr06_50656486	2.39	R10712
6	Yield	4.24	29.88	32.28	34.33	chr06_52145218	chr06_52566426	0.4	Tx2910
3	Leaf length	7.53	46.77	107.01	110.54	chr03_61509197	chr03_61933663	8.29	Tx2910

Moreover, in the hybrids of the ATx2928(Tx2910/R10712) population, five QTL were identified and all were related to some type of internode measurement (Table 21). Two QTL for third internode length was identified in 2017 and 2018, one on chromosome 1 and the other on chromosome 7 which explained 25% and 29% of the variation, respectively. A QTL region for sixth internode length was identified in 2017 and 2018. In both years the region was similar but the two QTL did not overlap. The QTL in 2017 explained 31% of the variation for sixth internode length and in 2018 it accounted for 39% of the variation. Due to their close proximity, it is possible that these are the same QTL but larger population sizes would be required to delimit the region. A single QTL was identified in 2017 for the sixth internode diameter, and it explained 25% of the variation in that trait.

Table 21. QTL identified in the 2017 and 2018 ATx2928(Tx2910/R10712) hybrid population grown in College Station, Texas.

Chromosome number	Trait name	LOD score	% Variance	Peak		Peak between markers		Additive Effect	Parental Allele Contribution
				From (cM)	To (cM)				
7	2018 3rd internode length	4.23	29.83	74	79.37	chr07_57056070	chr07_57077785	1.4	Tx2910
7	2018 6th internode length	5.92	39.08	75.25	76.57	chr07_57229867	chr07_57288688	2.81	Tx2910
1	2017 3rd internode length	3.52	25.53	137.75	139.05	chr01_77250987	chr01_77366305	0.98	R10712
7	2017 6th internode diameter	3.54	25.65	70.92	72.21	chr07_56520815	chr07_56525921	1.62	Tx2910
7	2017 6th internode length	4.49	31.34	79.37	86.26	chr07_57837375	chr07_59462562	1.02	Tx2910

In the R10709/F08331*bmr12* population a total of 12 QTL were identified in the inbreds and the hybrids across both years; 7 in the inbred parents and 5 in the biomass hybrids (Tables 22 and 23). Of these 12, no QTL were identified in both the inbreds and the hybrids in the same position, however, several of the QTL did show similar genetic locations between years for similar traits. For example, QTL detected for third internode length and sixth internode length appeared on chromosome 7 in close proximity to each other (Table 22).

In the R10709/F08331*bmr12* RIL population, seven QTL were identified (Table 22) and all the QTL were located on chromosomes 3 and 7 and most were for internode traits. Two QTL for third internode diameter were identified on chromosomes 3 and 7, which explained 25% and 23% of the variation, respectively. For sixth internode diameter, two QTL were also identified on chromosomes 3 and 7, which explained 35% and 30% of the variation in sixth internode diameter, respectively. Several studies have also identified QTL related to plant height and internode traits on chromosome 3 (Hilley et al., 2017; Kong et al., 2018). Two QTL for leaf width were also identified on chromosomes 3 and 7, which explained 27% and 39% of the variation in leaf width, respectively. Finally, a single QTL for leaf length was identified in chromosome 3 which explained 43% of the variation in leaf length.

Table 22. QTL identified in the R10709/F08331*bmr12* inbred population grown in 2017 College Station, Texas.

Chromosome number	Trait name	LOD score	% Variance	Peak		Peak between markers		Additive Effect	Parental Allele Contribution
				From (cM)	To (cM)				
3	3rd internode diameter	4.31	25.64	127.42	130.14	chr03_63093082	chr03_63093987	0.72	F08331bmr12
7	3rd internode diameter	3.96	23.83	180.19	187.51	chr07_56520815	chr07_56626110	1.06	R10709
3	6th internode diameter	6.32	35.23	127.42	130.14	chr03_63093082	chr03_63093987	0.89	F08331bmr12
7	6th internode diameter	5.33	30.67	180.19	193.36	chr07_56520815	chr07_56633892	0.67	F08331bmr12
3	Leaf length	8.32	43.55	122.71	125.07	chr03_63065473	chr03_63079607	6.52	F08331bmr12
3	Leaf width	4.59	27.06	125.07	130.14	chr03_63079607	chr03_63093987	0.33	F08331bmr12

A total of five QTL were detected in the hybrids of the ATx2928(R10709/F08331*bmr12*) population, and these were associated primarily with internode length (Table 23). A QTL for sixth internode length was identified on chromosome 7 in both 2017 and 2018 explaining 27% and 55% of the variation, respectively. These two QTL were between the same flanking markers in both years so they are at least within the same QTL region. A single QTL for sixth internode diameter was identified on chromosome 7 in 2017, explaining 25% of the variation in sixth internode diameter. For leaf angle, a single QTL was identified in 2018 on chromosome 7 that explained 34% of the variation in leaf angle. For third internode length a single QTL was identified on chromosome 7 in 2018 that explained 39% of the variation in third internode length.

Table 23. QTL identified in the 2017 and 2018 ATx2928(R10709/F08331*bmr12*) hybrid population grown in College Station, Texas.

Chromosome number	Trait name	LOD score	% Variance	Peak		Peak between markers		Additive Effect	Parental Allele Contribution
				From (cM)	To (cM)				
7	2018 3rd internode length	7.28	39.37	187.51	193.37	chr07_56626110	chr07_56633892	1.67	F08331*bmr12*
7	2018 6th internode length	11.8	55.56	166.11	175.59	chr07_56081328	chr07_56482132	3.31	F08331*bmr12*
7	2018 Leaf angle	6.24	34.88	193.36	198.79	chr07_56633892	chr07_57065460	0.1	R10709
7	2017 6th internode diameter	4.36	25.89	93.13	125.69	chr07_13794922	chr07_26576944	0.77	F08331*bmr12*
7	2017 6th internode length	4.71	27.66	166.11	187.52	chr07_56081328	chr07_56626110	2.45	R10709

The gene *Dw3*, located on chromosome 7, controls plant height which is highly related to internode traits. Previous research has reported the identification of QTL for internode traits on chromosome 7 (Higgins et al., 2014; Hilley et al., 2016, 2017). Therefore, the identification of QTL related to internode traits on chromosome 7 in the present study are in close proximity to the location of a major height gene, *Dw3*, also located on chromosome 7 (Li et al., 2015). Plant height and internode traits are associated; thus, the identification of these QTL highlight the potential of chromosome 7 being a major contributor to stalk characteristics.

Biomass yield and plant height are highly quantitative traits; no QTL for either were identified in the present study. Due to the quantitative nature of these traits it is unlikely that major effect QTL would be identified in any scenario. Thus, these traits are best examined in genomic selection and prediction experiments, where all markers can be evaluated simultaneously to develop estimated breeding values and selection criteria is no longer placed on a single marker or marker interval (Wang et al., 2018).

As expected, this population did not segregate for the major loci controlling height, specifically *Dw3* and major *Ma* genes. Thus, the lack of segregation at these loci limited and reduced our ability to identify major effect QTL for height, photoperiod sensitivity and ultimately biomass yield. It is interesting to note that while biomass composition was not the focus of this study, many of the biomass composition traits were measured and obtained through NIR. There were no QTL identified in either population – inbreds or hybrids, in either year that related to any of the biomass composition traits as obtained by NIR.

Overall, it is likely that QTL detection was limited by the small population sizes which reduced the power of detection. The population sizes of 55 and 67 individuals utilized herein likely reduced the ability to identify QTL, given that most studies recommend at least 200

individuals for optimal QTL identification (Charmet, 2000; Ferreira et al., 2006; Li et al., 2010; Xu et al., 2017). Statistical variation exists within populations for most phenotypic traits, although it is possible this variation was not sufficient for QTL identification.

In each instance of an identified QTL, the explained variation was high - unexpectedly so, especially since most of the traits are highly quantitative in nature. Due to the low population size it is likely that the explained variation is inflated. Beavis (1994) demonstrated that as population size decreases, estimates of the phenotypic variances associated with identified QTL were greatly overestimated. This phenomenon aptly named "The Beavis Effect" is especially severe when population sizes are smaller than 100 individuals, which is the case for both populations used in this study. Therefore, it is likely that larger populations than what were used would have resulted in identified QTL with explained phenotypic variation closer to the true magnitude.

Future research regarding the applications of genomic prediction on increasing biomass yield are warranted, specifically when photoperiod insensitive parents are used to develop photoperiod sensitive biomass hybrids.

CHAPTER V

CONCLUSIONS

In order to speed up the breeding process, reduce the number of test crosses required, and conserve resources, there has been motivation to examine the relationship between inbred parent performance and hybrid performance. Eliminating the need to make test crosses has the potential to speed up the breeding cycle by at least one year, ultimately allowing growers access to superior genetics more rapidly. Although, previous research has resulted in conflicting reports on the relationship between selections based solely on inbred performance in order to develop superior hybrids and becomes more distorted once differences in photoperiod sensitivity come in to play. This photoperiod sensitivity causes the inbreds and resulting hybrids to look nothing alike phenotypically, making prediction difficult.

In the present study, the examination of both photoperiod insensitive inbred parents and their resulting biomass hybrids revealed few trends between the two populations, regarding both phenotypic and NIR trait means. Statistically different trait variation existed in all populations for phenotypic traits. Despite this, correlations of phenotypic and NIR traits for PI inbred parents between their PS hybrid offspring were inconsistent across years and populations apart from inbred 3rd internode diameter and hybrid stalk weight per plant. Moreover, the vastly different phenotypes of the inbreds and hybrids make meaningful comparisons between the two of them difficult.

Furthermore, the range of HPH for the hybrid population varied widely depending on the trait. Specifically, biomass yield HPH had a range of 1000% while percent moisture HPH had a range of 30%. The difference in biomass yield heterosis range can be mainly attributed to the presence of maturity genes. In the inbred parents these maturity genes are in the homozygous

allelic configuration of *Ma*$_1$ *ma*$_5$ *Ma*$_6$. The common tester parent, ATx2928 is homozygous for the *Ma* genes in the configuration *ma*$_1$ *Ma*$_5$ *ma*$_6$. As such, the resulting testcross hybrids are photoperiod sensitive because they are heterozygous at *Ma*$_1$, *Ma*$_5$ and *Ma*$_6$. This photoperiod sensitivity causes a significant delay in flowering, consequently all of the hybrids' energy is focused on biomass accumulation. It is this key photoperiod sensitivity that results in large heterosis values for biomass yield. The narrow range of percent moisture heterosis values can be attributed primarily to the length of growing season and available moisture at time of harvest.

Heritability is one of the most important selection tools for plant breeders, given that its main use is for calculating the response to selection for a given trait. In the present study, when examining inbreds and hybrids grown in the same year, we report that inbred heritabilties were generally higher for phenotypic traits than their corresponding hybrids. It is speculated that the effects of the maturity genes and heterosis are not only confounded with each other, but that their biomass increasing effects reduced the heritability for most of the phenotypic traits in the hybrids. Hence, when heritability is reduced it ultimately makes evaluation of the hybrids difficult and less impactful.

Finally, a genetic map was developed for both populations and both inbred and hybrid phenotypic and NIR trait data was analyzed using these genetic maps. Mapping of QTL was conducted, and several QTL were identified in both the inbreds and hybrids of both populations. Overall, most of the QTL identified were related to stalk characteristics and found on chromosome 7, the chromosome where *Dw3*, a major height gene, is located. These results suggest chromosome 7 plays a large part in both stalk traits and height. No QTL were identified that explain any traits being correlated with biomass yield. While other studies found QTL for internode traits in the same chromosomal regions, none of these traits have been found to be related to overall biomass

yield. The small population size utilized herein made identification of QLT difficult as it is recommended that populations consist of at least 200 individuals for reliable QLT mapping. No QTL were identified for biomass yield, which was expected due to the quantitative nature of biomass yield – it is unlikely that such a quantitative trait is controlled by a single gene(s). In turn, the presence and expression of the maturity genes are confounded with heterosis and skew the heritabilties and trait correlations, making dependence on them for selection criteria unreliable.

The advancements in genomic prediction, the unique composition of this crossing scheme used to make biomass hybrids, and the highly quantitative nature of biomass yield make it a prime candidate for the application of genomic prediction. Based on the results of this study, and corresponding literature, there is no substitute for making test crosses when predicting the best inbred combinations, specifically when it comes to developing biomass hybrids.

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APPENDIX A

Table A1. Test of homogeneity for the ATx2928(R10709/F08331*bmr12*) 2017 and 2018 hybrid populations.

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	35.8568	1	237	<.0001*
Brown-Forsythe	39.9900	1	237	<.0001*
Levene	41.2080	1	237	<.0001*
Bartlett	63.7243	1	.	<.0001*
F Test 2-sided	4.6418	118	119	<.0001*

Table A2. Test of homogeneity for the ATx2928(Tx2910/R10712) 2017 and 2018 hybrid populations.

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	35.8568	1	237	<.0001*
Brown-Forsythe	39.9900	1	237	<.0001*
Levene	41.2080	1	237	<.0001*
Bartlett	63.7243	1	.	<.0001*
F Test 2-sided	4.6418	118	119	<.0001*