HIGH-BIOMASS SORGHUMS FOR BIOMASS BIOFUEL PRODUCTION

A Dissertation

by

DANIEL J. PACKER

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

DOCTOR OF PHILOSOPHY

May 2011

Major Subject: Plant Breeding

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Approved by:

Chair of Committee,	William L. Rooney
Committee Members,	Patricia Klein
	Brent Bean
	Steve Hague
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ABSTRACT

High-Biomass Sorghums for Biomass Biofuel Production. (May 2011)

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Chair of Advisory Committee: Dr. William Rooney

High-biomass sorghums provide structural carbohydrates for bioenergy production. Sorghum improvement is well established, but development of high-biomass sorghums for biofuels is not. Thus the objectives of this research were to develop information on sorghum improvement of high-biomass sorghums including marker-assisted selection, use of exotic germplasm, heterosis, and GxE variability of biomass composition.

Marker-assisted selection was compared to testcross selection for identifying photoperiod-insensitive (PI) experimental lines that yield photoperiod-sensitive (PS) hybrids within the *Ma1*/*Ma5*/*Ma6* hybrid production system. Four hundred eighty-three sorghum lines were genotyped at the *Ma1* and *Ma5* loci to predict their hybrid photoperiod reactions and testcrossed to establish actual hybrid photoperiod reactions. *Ma1*/*Ma5* marker selections for lines producing PI hybrids were reliable. *Ma1*/*Ma5* marker selections for lines producing PS hybrids were not reliable and identification of such lines will require testcrossing or genotyping at *Ma6* or other additional loci.

An attempt was made to determine whether relationships exist between the passport data (geographic origin) of sorghum accessions and high-biomass desirability.

Such a relationship could prioritize sorghum accessions for breeding evaluations. Seventeen hundred ninety two exotic sorghum accessions from 7 different geographic origins were evaluated for high-biomass desirability in 3 environments. Significant relationships between passport data and high-biomass desirability were identified within environments, but not across environments because of large GxE interactions. A larger sampling of environments will be needed to establish reliable passport data and high-biomass desirability GxE patterns.

Hybrid entries derived from high-biomass sorghum pollinators and grain sorghum females were evaluated for biomass heterosis. Moderate levels of biomass high-parent heterosis were widely available in the hybrids. Heterosis and biomass yields were maximized in specific hybrid combinations and were subject to GxE interactions.

Biomass composition (% cellulose, hemicellulose, etc.) affects the conversion efficiency of biomass to fuel and may be subject to GxE interactions. The biomass composition of 12 sorghums grown across 5 environments was estimated using Near-Infrared Spectroscopy to identify GxE patterns. Significant GxE interactions for composition were identified. Differences between genotypes for compositional traits were small (1-3 %), but may prove important with large-scale biomass processing.

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I. INTRODUCTION

Increasing global energy demands and concerns regarding fossil fuel consumption are increasing the need to develop alternative and renewable energy sources. Global petroleum consumption is projected to increase 28.4% (86.1 million barrels/day to 110.6 million barrels/day) and global electricity consumption is expected to increase 87% (18.8 trillion kWh to 35.2 trillion kWh) between 2007 and 2035. (USEIA, 2010). It is worth noting that both of these increases include improvements in energy use efficiency (Greene, 2007).

In addition to projected energy demand, climate change issues also favor the use of carbon neutral and renewable fuels. Fossil fuels are associated with climate change; their replacement with alternative energy is proposed to mitigate detrimental effects (Jacobson, 2009). Potential alternative energy sources include biomass, wind energy, solar-photovoltaic and others. Among alternative energy options, biomass is the most readily suited to produce liquid fuels (Agrawal et al., 2006).

Biofuels refer to energy derived from some type of biomass produced via the photosynthetic activity of plants or algae. Depending on the plant species, biomass type will vary; all will produce structural carbohydrates (lignin, hemicellulose, and cellulose), but some are effective at producing non-structural carbohydrates (sugar, starch), proteins or oils.

This dissertation follows the style of Crop Science.

Carbohydrates (structural or non-structural) can be fermented into alcohol fuels. In the U.S., over 4.5 B bushels of maize grain were used from Sep-Aug 2009/2010 to produce starch-based ethanol (USDA ERS, 2010a). The fatty acids in plant oils can also be readily converted to liquid fuels and from Sep-Aug 2009/2010, over 940 thousand Mg of soybeans were used for biodiesel production (USDA ERS, 2010b).

In the U.S., the Energy Independence Act of 2007 established a renewable fuel standard stipulating that renewable fuels must supply 36.0 billion gallons of liquid transportation fuels by 2022 (Jessup, 2009). That amount would replace approximately 30% of current U.S. petroleum consumption (Perlack et al., 2005). Currently, most biofuels (mostly ethanol) produced in the U.S. are derived from maize starch. However, maize supplies are finite; maize alone cannot meet legislated goals. For example, Hill et al. (2006) reported that if the entire 2005 U.S. maize grain and soybean crops were converted to biofuels, only 12% of U.S. gasoline consumption and 6% of diesel consumption would be replaced. Additionally, maize is an important feed/food crop, and its large-scale use for biofuels may have negative impacts on food supply. If biofuels are to make a significant contribution to energy supplies, other feedstocks must be developed.

It is has been hypothesized that biofuels derived from plant structural biomass have the potential to meet needed volumes (Schubert, 2006). Perlack et al. (2005) estimated that the U.S. has the capacity to reasonably produce 1 billion dry tons of biomass annually. Such a supply of biomass would be sufficient to replace 30% of U.S. petroleum consumption if converted into alcohol fuels (Perlack et al., 2005). If biomass biofuels are to meet these goals and be economically viable, abundant and low-cost

biomass sources are needed. To provide this, dedicated bioenergy crops are necessary (Epplin et al., 2007).

For a variety of reasons, the C4 grass sorghum (*Sorghum bicolor* L. Moench) is among the plant species being developed as a bioenergy crop (Rooney et al., 2007). First, sorghum has extensive diversity available with types that can be tailored for starch, sugar, or biomass production. Second, it has superior adaptation to hot and/or semi-arid environments relative to most crops, which reduces seasonal productivity fluctuations due to the environment. Third, it is well established as a grain, fodder, and syrup crop with a mature seed industry and agronomic practices that can facilitate its adoption. Finally, it is generally managed as an annual, thus providing management flexibility relative to other proposed energy crops, most of which are perennials.

To date, sorghum has remained relatively unexploited for biofuel production. The exception is grain sorghum, where many of the existing elite hybrids are readily suited for starch based ethanol production and have been rapidly adopted (USCP 2009; Wang et al., 2008). For other bioenergy applications, energy sorghums specifically tailored to those applications are needed.

While there are several types of energy sorghums, one group classified as high-biomass sorghums is the focus of projected future U.S. production. High-biomass sorghums produce large amounts of structural carbohydrates (lignocellulose), are tall (3.5-5 meters) and have pithy and dry stems that do not accumulate large amounts of soluble sugar. A specific feature of these sorghums is delayed flowering through photoperiod sensitivity, meaning that in temperate latitudes they remain in vegetative growth phase until very late in the growing season; some never flower.

High-biomass sorghums are specifically photoperiod sensitive (PS) because delaying maturity has particular advantages in the production of biomass. PS sorghums generally out-yield comparable photoperiod-insensitive (PI) sorghums, particularly when water is limiting and the growing season is long (McCollum et al., 2005). With a long growing season, non-flowering plants continue to accumulate biomass in vegetative structures while biomass accumulation in flowering plants has slowed or ceased. When water is limiting, vegetative PS sorghums are able to become dormant and will resume growth when sufficient water is available. This is in contrast to a plant which has initiated reproductive growth; once flowering is initiated, they must continue through reproductive development regardless of water supply.

In temperate latitudes, there is not enough growing season for the production of PS sorghum seed. To enable seed production of PS sorghums, the *Ma1*/*Ma5*/*Ma6* seed production system uses complementary dominant interactions to produce seed of PS hybrids with PI parents (Rooney and Aydin, 1999). This system manipulates the sorghum maturity loci *Ma1*, *Ma5* and *Ma6*. With a dominant allele at each locus, flower initiation is delayed until day length is ≤ 12h 20 min. By crossing a PI parental line that is homozygous recessive at one of these two loci and homozygous dominant at the other with another PI parent having the opposite allelic configuration (e.g. *ma1ma1Ma5Ma5ma6ma6* / *Ma1Ma1ma5ma5Ma6Ma6*), PS hybrid progeny with a dominant allele at both loci (e.g. *Ma1ma1* / *Ma5ma5* / *Ma6ma6*) are obtained.

Using existing data, the reported biomass yields of current sorghums with high-biomass potential (sweet, forage, and limited bioenergy) range from 15.6 Mg ha^{-1} to 40.3 Mg ha^{-1} (dry weight basis) across many environments (Habyarimana et al., 2004,

McCollum et al., 2005, Rooney, 2007, Venuto and Kindiger, 2008). For comparison, recent U.S. maize grain yields averaged approximately 9.3 Mg ha^{-1} (Egli, 2008) and Burns et al. (2008) released a switchgrass cultivar with superior dry matter yields of 15.7 Mg ha^{-1} per year across two locations in North Carolina after one establishment year. Thus, sorghum competes favorably with these species in yield and most sorghums in these studies were bred for forage use; their biomass yield is constrained by animal palatability and nutrition parameters. To date, there has been limited breeding specifically for total biomass yield and bioenergy production. Lifting animal palatability and nutrition requirements offers real potential to further increase high-biomass sorghum yields.

Breeding high-biomass sorghums for bioenergy has the advantage that sorghum improvement methods and resources are well established. But high-biomass sorghum improvement efforts are recent, thus many criteria such as the development of marker-assisted selection, establishment of new breeding pools, identifying heterosis availability, and determining the variability of bioenergy traits must be addressed. With these criteria in mind, this research has the following objectives:

1. **A comparison of marker-assisted selection vs. testcross selection for identifying the photoperiod reactions of high-biomass sorghum experimental lines in hybrid combination.** As new high-biomass sorghum experimental lines are developed, those that produce PS hybrids in the *Ma1*/*Ma5*/*Ma6* seed production system must be identified. Two methods exist that must be compared for efficacy; genotyping at the *Ma1*/*Ma5* loci

with molecular markers and testcrossing. Marker-assisted selection would be substantially more efficient than testcrossing.

2. **Determining the potential of passport information (geographic origin) for predicting the high-biomass potential of exotic sorghum accessions.** Exotic sorghum accessions are a source of germplasm for building new high-biomass sorghum breeding pools. A predictive relationship between passport data and high-biomass desirability may exist. Such a relationship could be used to prioritize accessions from specific geographic origins for high-biomass evaluations to select new germplasm for breeding pools.
3. **Measuring the availability of high-parent heterosis for biomass yield in sorghum hybrids derived from high-biomass pollinators and grain sorghum females.** The availability of high-parent heterosis would increase biomass yields. High-biomass sorghum hybrids may or may not capture biomass high-parent heterosis with the use of grain sorghum females (required for large-scale seed production).
4. **Measure and describe genotype x environment interactions for biomass compositional traits (cellulose, xylan, and lignin content) in high-biomass sorghums.** Biomass composition affects the conversion efficiency of biomass to liquid fuels and could be altered to improve conversion efficiencies. Cellulose, xylan (hemicellulose proxy), and lignin are the primary components of biomass composition and their individual proportions may be subject to GxE interactions. Breeding and genotype deployment of

altered compositional profiles in high-biomass sorghums may be complicated by GxE interactions.

II. MARKER-ASSISTED SELECTION VS. TESTCROSS SELECTION FOR IDENTIFYING PHOTOPERIOD EFFECTS OF HIGH-BIOMASS SORGHUM EXPERIMENTAL LINES IN HYBRID COMBINATION

Introduction

A specific feature of high-biomass sorghums being developed for biofuels is delayed flowering through photoperiod-sensitivity. Sorghum is a quantitative short-day plant, and photoperiod-sensitive (PS) genotypes delay flowering until a minimum day length is reached. The specific day lengths vary, but are highly heritable. This photoperiod regulation of flowering evolved as an adaptation to sorghum's tropical African origins that coordinates flowering with optimal conditions (Morgan and Finlayson, 2000) At temperate latitudes, many PS sorghums flower near the end of the growing season or not at all.

For biomass production, delaying flowering provides several advantages. Photoperiod-sensitive plants can continue to accumulate biomass in vegetative structures after biomass accumulation in flowering plants has slowed or ceased. When water is limiting, vegetative PS sorghums can become dormant and resume growth when sufficient water is available. This is in contrast to a plant that has initiated reproductive growth and must continue with reproductive development regardless of water supply. Because they flower earlier, photoperiod-insensitive (PI) sorghums are more susceptible to early and mid-season water stress and are unable to utilize any late-season moisture, unless through a ratoon crop. Thus PS sorghums generally out-yield comparable PI

sorghums, particularly when water is limiting and the growing season is long (McCollum et al., 2005).

Six maturity loci (*Ma1-Ma6*) have been discovered and described in sorghum that mediate photoperiod-sensitivity and its control of flowering (Quinby, 1974; Rooney and Aydin, 1999). Photoperiod-sensitivity in *Sorghum* actively represses FLOWERING LOCUS T (FT) and APETAL1 (AP1) which mediate the transition of the shoot apex from vegetative to reproductive (Colasanti and Coneva, 2009; Klein et al., 2008). Thus in these loci, photoperiod-sensitivity and delayed flowering is dominant to photoperiod-insensitivity and early flowering. But depending on the allelic combinations of these loci, flowering can be delayed by as little as two weeks to eighteen weeks or more. Of the maturity loci that have been described, *Ma1* has the largest effect and is the primary locus separating early and late flowering genotypes (Quinby, 1974; Klein et al., 2008).

For sorghum grain and seed production in temperate latitudes, early-flowering genotypes are required. Thus farmers in the US selected early-flowering mutants from PS sorghum introductions early in the 20th century (Smith and Frederiksen, 2000). The development of early-flowering temeprate sorghums was formalized with the creation of the USDA Sorghum Conversion Program in 1963. This program crossed PI temperate varieties to PS exotic lines to create new PI lines from the exotic PS lines. The conversion of these PS lines to PI was mostly accomplished by converting *Ma1* to *ma1* and most temperate sorghums are *ma1* (Klein et al. 2008).

Reproductive phase induction in most PS sorghums occurs when day lengths are $\leq$ 12 h 20 min (Rooney and Aydin, 1999). After reproductive phase initiation, flowering does not occur for another 30-35 days and mature seed requires another 30-35 days. In

most all temperate climates, there is not enough growing season for the production of mature seed. To alleviate this problem, a unique three-gene system with complementary dominant epistasis is used for hybrid seed production of PS hybrids in temperate climates (Rooney and Aydin, 1999). The genes involved are the sorghum maturity loci, *Ma1*, *Ma5* and *Ma6*. With a dominant allele at each locus, flower initiation is delayed until day length is ≤ 12h 20min (Rooney and Aydin, 1999). By crossing a parental line that is homozygous recessive at one of these loci and homozygous dominant at the other with another parent having the opposite allelic configuration (e.g. *ma1ma1Ma5Ma5ma6ma6* / *Ma1Ma1ma5ma5Ma6Ma6*), PS hybrid progeny with dominant alleles at all loci (e.g. *Ma1ma1Ma5ma5Ma6ma6*) are obtained. In this manner, PS hybrid sorghum seed can be produced in temperate latitudes.

For higher seed yields and mechanical seed harvest, the *Ma1*/*Ma5*/*Ma6* system for producing PS hybrids uses short, grain-type sorghums as female parents and high-biomass sorghums as male parents. Most U.S. grain sorghums have the *ma1ma1Ma5Ma5ma6ma6* allelic configuration, implying that high-biomass males must be *Ma1Ma1ma5ma5Ma6Ma6*. Currently there are very limited sources of *Ma6* alleles (Rooney and Aydin, 1999). Further diversification of germplasm with the *Ma1Ma1ma5ma5Ma6Ma6* allelic composition is clearly needed. Hybridization of promising new breeding lines with a source of the *Ma1ma5Ma6* alleles is the initial step, but significant testing and selection will be required to ultimately develop high-biomass sorghum populations fixed for the *Ma1Ma1ma5ma5Ma6Ma6* allelic configuration.

There are two approaches to identify progeny with the desired *Ma1*/*Ma5*/*Ma6* alleles. The first entails testcrossing the progeny to a tester with a known

ma1ma1Ma5Ma5ma6ma6 genotype and proven performance in the *Ma1*/*Ma5*/*Ma6* system followed by evaluation of testcross photoperiod responses. The second method uses molecular markers to genotype the *Ma1*/*Ma5*/*Ma6* loci and predict hybrid photoperiod reactions. Molecular markers have been developed for several traits in sorghum (Ejeta and Knoll, 2007) and SSR and INDEL markers for *Ma1* and *Ma5* alleles are available within the Texas A&M sorghum improvement program. The genetic map location of *Ma6* is currently unknown, thus markers for *Ma6* are unavailable.

Despite the unavailability of *Ma6* markers, markers at *Ma1* and *Ma5* could eliminate a portion of new lines that produce PI hybrids based on their genotypes at *Ma1* and *Ma5*. Remaining lines would then be testcrossed to account for *Ma6* and identify those producing PS hybrids. Such a marker-assisted selection scenario would reduce the amount of testcrossing required when breeding PI high-biomass sorghum males that produce PS hybrids in the *Ma1*/*Ma5*/*Ma6* hybrid production system.

To determine the viability of such a system, the selection efficiency of *Ma1* and *Ma5* markers for identifying lines producing PI hybrids must be established and compared to testcrossing. Thus the objective of this research is to compare marker-assisted selection to phenotypic testcross selection across multiple populations using Texas A&M SSR and INDEL markers for *Ma1* and *Ma5* alleles to identify PI sorghum males that produce very late flowering PS hybrids in the *Ma1*/*Ma5*/*Ma6* seed production system.

Materials and Methods

Population Development

Eleven breeding populations were created by crossing 11 male-fertile sorghums with high-biomass potential and the *ma1ma1Ma5Ma5ma6ma6* allelic configuration to R07007, a *Ma1Ma1ma5ma5Ma6Ma6* allele donor (Figure 2.1). These parental lines included tropical plant introductions converted to temperate adaptation by the USDA Sorghum Conversion Program (Quinby, 1974), and forage sorghum cultivars. Crosses were made in College Station, TX in 2007 using plastic bag emasculations as described in Rooney (2004) to sterilize the high-biomass males and use R07007 as the pollinator. The resulting PS F_1 was grown and selfed in Puerto Rico during the winter of 2007-2008 to recover F_2 seed. Progeny of the individual crosses were managed as separate populations. The F_2 population of each cross was grown in College Station in 2008 to recover $F_{2:3}$ seed. As expected, these populations segregated for photoperiod sensitivity; F_2 plants that were PS (presumably *Ma1__Ma5__Ma6__*) were vegetative in growth and were not advanced. Photoperiod insensitive plants were self-pollinated and harvested as single heads to produce $F_{2:3}$ families. $F_{2:3}$ seed from the selected F_2 plants were planted in head to row plots at a fall nursery in Weslaco, TX in 2008. In Weslaco, pollen from 483 F_3 lines was bulked within head plots to reconstitute the F_2 and make testcrosses to one of four *ma1ma1Ma5Ma5ma6ma6* male-sterile sorghum testers (Table 2.1). These testers are standard grain sorghums routinely used in producing sorghum hybrids, including *Ma1*/*Ma5*/*Ma6* hybrids.

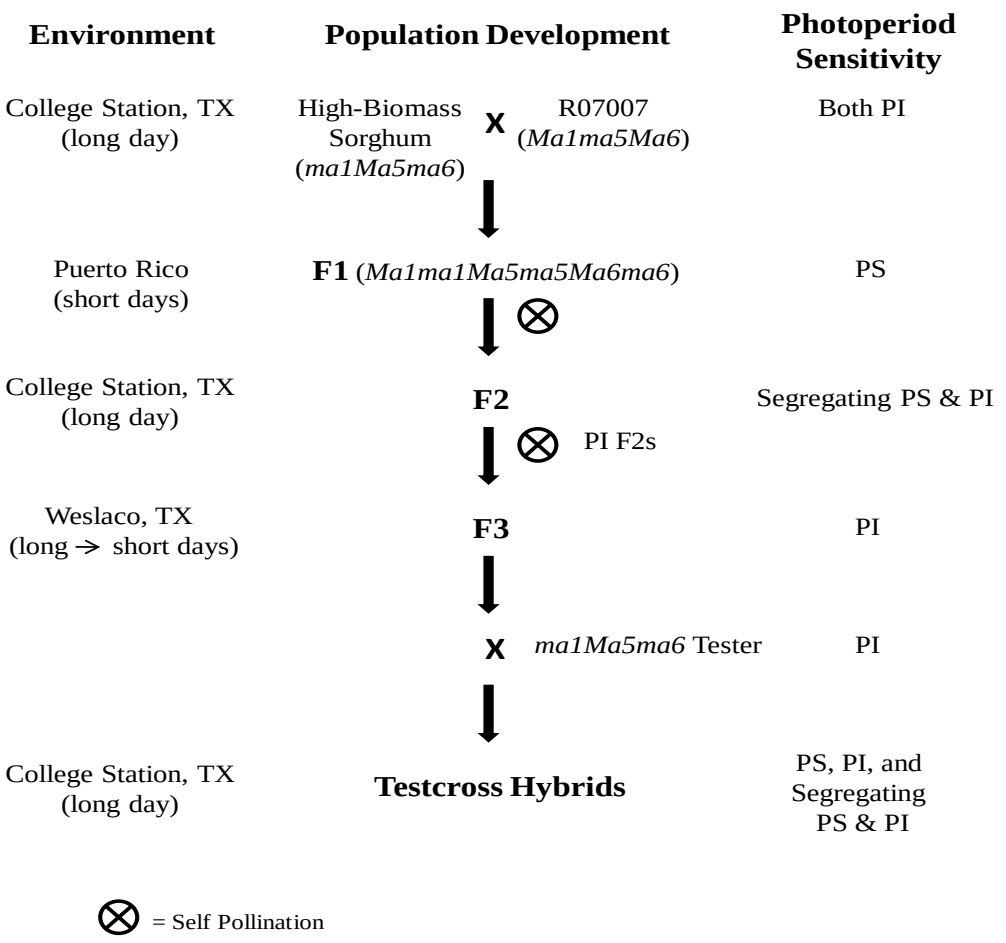

Figure 2.1 Schematic for the development of F_3 sorghum breeding populations to identify photoperiod-insensitive (PI) lines with the *Ma1ma5Ma6* genotype for use in the *Ma1/Ma5/Ma6* photoperiod-sensitive (PS) hybrid production system. Sorghum lines were originally crossed to a *Ma1ma5Ma6* donor and progeny managed in environments with differing day lengths for seed production or differentiation of PS and PI individuals. *ma1Ma5ma6* testers were used to identify lines with the *Ma1ma5Ma6* genotype

Table 2.1 11 populations derived from crosses of sorghums with high-biomass potential to a *Ma1*/*ma5*/*Ma6* (R07007) allele donor to identify photoperiod-insensitive (PI) lines that produce photoperiod-sensitive (PS) hybrids in the *Ma1*/*Ma5*/*Ma6* hybrid production system. The parental lines represent temperately adapted plant introductions and forage cultivars. PI F_3 lines were crossed to grain sorghum testers to identify those that produce PS progeny.

Population	# of F_3 Lines	Testers
1	30	ATx623
2	46	ATx645
3	36	ATx2928/BTx2752
4	39	ATx645/BTX2752
5	53	
6	50	
7	40	
8	55	
9	17	
10	80	
11	37	
Total	483	

The PI F_3 lines used in making testcrosses were expected to be minimally homozygous recessive at one of the three loci. The other loci could be either homozygous recessive or dominant, or heterozygous. Therefore they were expected to produce one of three types of testcross phenotypes; PS progeny that remain vegetative until daylengths are < 12h 20min (*Ma1Ma1ma5ma5Ma6Ma6* F_3), progeny segregating for PS (F_3 heterozygous at *Ma1*, *Ma6*, or both), and PI progeny with early to mid-season flowering (F_3 homozygous recessive at *Ma1*, *Ma6*, or both).

Phenotypic Evaluation for Genotype

Four hundred eighty-three testcrosses were planted in late March 2009 in College Station, TX for phenotypic evaluation. Testcrosses were planted grouped by population in single replications in one-row plots and managed with standard agronomic practices.

At 2-4 week intervals throughout the growing season, the testcrosses were phenotyped for their growth stage to determine when they became reproductive and thus their level of photoperiod sensitivity. A total of seven phenotypic evaluations occurred from June 12th to October 19th. At each evaluation, testcrosses were categorized as either being vegetative, reproductive, or segregating for these growth stages. The absence or presence of a flag leaf or reproductive organs was used to differentiate vegetative vs. reproductive.

To separate the F_3 lines producing the expected Ma_6 PS response, the lines were placed in 1 of 3 photoperiod reaction categories based on their phenotype in early September (Sept. 7th) when day lengths were approaching 12h 20min:

1. Photoperiod Sensitive
2. Photoperiod Insensitive
3. Segregating for photoperiod sensitivity

Uniformly vegetative testcrosses were categorized as PS and represent the expected *Ma1Ma1ma5ma5Ma6Ma6* response. Uniformly reproductive lines were categorized as PI and represent the expected *ma1ma1*, *Ma5__* and *ma6ma6* responses. Those segregating for growth phase were categorized as segregating for photoperiod sensitivity and represent the expected *Ma1ma1* and *Ma6ma6* response. Because the goal was to identify lines producing the expected *Ma1Ma1ma5ma5Ma6Ma6* extreme PS response,

testcrosses with delayed flowering but that were nonetheless reproductive in early September were classified as PI.

Genotype Analysis

The pollinator lines (F_3 lines used for testcrossing) were also genotyped using molecular markers at the *Ma1* and *Ma5* loci. From the seed used to establish the $F_{2:3}$ plots, 15 seedlings were germinated in a greenhouse to collect seedling tissue for DNA extraction and marker analysis. *Ma1* and *Ma5* have been genetically mapped on the Texas A&M sorghum genetic map and multiple flanking SSR and INDEL markers polymorphic within the individual populations were available and used (Table 2.2). *Ma1* maps to linkage group 6 at ~11-21 cM and *Ma5* maps to linkage group 2 at ~145-148 cM (Mullet et al., 2008).

Table 2.2 Texas A&M sorghum SSR and INDEL markers used to genotype F_3 sorghum lines from 11 populations for alleles at the *Ma1* and *Ma5* sorghum maturity loci. SSR markers are designated with the *txp* prefix whereas INDEL markers are designated with the *txi* prefix.

Ma1 Molecular Markers		*Ma5* Molecular Markers
*txp*640	*txp*598	*txp*348
*txp*604	*txp*597	*txp*566
*txp*694	*txp*434	*txp*428
*txp*695	*txp*547	*txp*431
*txi*48	*txp*568	
*txp*658	*txp*535	
*txp*696	*txp*559	
*txi*49		

Because *Ma1* is minimal to elicit the *Ma1*/*Ma5/Ma6* PS response, genotyping was performed first for *Ma1*. Lines that contained *Ma1* donor marker alleles were then genotyped at *Ma5* to confirm that they also contained the *Ma5* marker alleles from the *Ma1ma5Ma6* donor and had the expected *ma5ma5* marker genotype. Genotyping was performed using an Applied Biosystems 3130 DNA analyzer for sequencing and Applied Biosystems GeneMapper for allele calling. Based on the *Ma1* marker genotypes, each F_3 line was placed into one of three genotypic categories:

1. Homozygous for the *Ma1* donor marker alleles
2. Heterozygous for the *Ma1* donor marker alleles
3. Homozygous for the *ma1* parent marker alleles

Based on the marker genotypes, the photoperiod reactions of the F_3 testcrosses were predicted to be PI, PS, or segregating for PS as described in the phenotype evaluations. The predicted photoperiod reactions were then compared to the actual photoperiod reactions of the testcrosses in the phenotypic evaluations. Chi-square analyses using the PROC FREQ procedure of SAS 9.2 were performed to determine if the predicted photoperiod reactions were significantly different from the observed phenotypes.

Results and Discussion

Based on the *Ma1* and *Ma5* marker genotypes, it was predicted that 388 of the 483 testcrosses would be PI and flower before September. Per the marker genotypes, these testcrosses were derived from *ma1ma1Ma5__* or *ma1ma1ma5ma5* F_3 lines that would not produce PS testcrosses (*Ma1ma1Ma5ma5Ma6ma6*) when crossed to a

ma1ma1Ma5Ma5ma6ma6 tester. Of the 322 predicted to be PI by the markers, 321 were PI and had reproductive phenotypes by early September (Table 2.3). In fact, 95% of these testcrosses already had reproductive phenotypes by the first evaluation on June 12th, demonstrating the major effect of *Ma1* for producing photoperiod sensitivity and delaying flowering (Klein et al., 2008). The difference between the predicted PI count and the observed PI count were not significantly different in a chi-square analysis. Overall the markers were 99.5% correct in identifying F_3 lines that would produce PI testcrosses across the eleven populations.

Based on the *Ma1* and *Ma5* marker genotypes, it was predicted that 39 of the 483 testcrosses would be PS and remain vegetative until daylengths were ≤ 12h and 20min when testcrossed to a *ma1ma1Ma5Ma5ma6ma6* tester. These testcrosses were derived from F_3 lines with a *Ma1Ma1ma5ma5* marker genotype. Alleles at *Ma6* are unaccounted for and segregating in these populations. Because of the importance of *Ma6* in producing the desired PS response, these marker predictions of PS testcrosses were not expected to be reliable.

And indeed they were not, of the 39 testcrosses predicted to be PS by the markers, 7 were in fact PS in the September field evaluations (Table 2.4). The difference between the observed results and the predicted results was highly significant in a chi-square analysis. Overall, the markers were 18.0% correct in identifying F_3 lines that would produce PS testcrosses. Their predictive ability differed between populations (Table 2.4) but was never greater than 50% correct.

Based on the *Ma1and Ma5* marker genotypes, it was predicted that 56 of the 483 testcrosses would segregate for PS until daylengths were ≤ 12h and 20min. These

Table 2.3 Comparison between *Ma1*/*Ma5* molecular marker predicted photoperiod-insensitive (PI) photoperiod reactions of sorghum testcrosses from 11 populations to their actual phenotype across six dates.

Population	Predicted # of PI Testcrosses	12-Jun Actual	26-Jun Actual	10-Jul Actual	24-Jul Actual	10-Aug Actual	7-Sep Actual	7-Sep % Correct by Pop.
1	28	28	28	28	28	28	28	100.0
2	37	37	37	37	37	37	37	100.0
3	25	25	25	25	25	25	25	100.0
4	27	25	26	27	27	27	27	100.0
5	35	34	34	34	35	35	35	100.0
6	44	40	40	44	44	44	44	100.0
7	36	36	36	36	36	36	36	100.0
8	50	50	50	50	50	50	50	100.0
9	12	10	11	11	11	11	11	92.0
10	66	58	60	62	66	66	66	100.0
11	28	28	28	28	28	28	28	100.0
Overall	388	371[ns]	375[ns]	382[ns]	387[ns]	387[ns]	387[ns]	
% Correct		95.6	96.6	98.4	99.7	99.7	99.7	

[ns] No significant difference between the observed count and the expected count at $\alpha = 0.05$

Table 2.4 Comparison between *Ma1*/*Ma5* molecular marker predicted photoperiod-sensitive (PS) photoperiod reactions of sorghum testcrosses from 11 populations to their actual phenotype across six dates.

Population	Predicted # of PS Testcrosses	12-Jun Actual	26-Jun Actual	10-Jul Actual	24-Jul Actual	10-Aug Actual	7-Sep Actual	7-Sep % Correct by Pop.
1	2	2	2	1	0	0	0	0.0
2	9	9	7	4	4	4	4	44.4
3	2	2	1	1	1	1	1	50.0
4	6	6	2	2	2	2	2	33.3
5	6	3	1	1	1	0	0	0.0
6	1	1	0	0	0	0	0	0.0
7	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
8	1	1	0	0	0	0	0	0.0
9	5	3	0	0	0	0	0	0.0
10	6	6	0	0	0	0	0	0.0
11	1	1	1	0	0	0	0	0.0
SUM	39	34^{ns}	14*	9**	8**	7**	7**	
% CORRECT		87.2	36.0	23.1	20.5	18.0	18.0	

* Statistically significant deviation of the observed count from the expected count at $\alpha = 0.05$
** Statistically significant deviation of the observed count from the expected count at $\alpha = 0.01$
ns No significant difference between the observed count and the expected count at $\alpha = 0.05$

testcrosses were derived from F_3 lines with a *Ma1ma1ma5ma5* marker genotype and were expected to produce PS and PI testcrosses when crossed to a ma1ma1*Ma5Ma5ma6ma6* tester. But again, alleles at *Ma6* were segregating and unaccounted for. Thus marker predictions of testcrosses segregating for PS were not expected to be reliable. Of the 56 predicted to segregate for PS by the markers, 6 were actually segregating for PS in the September field evaluations (Table 2.5). The difference between the observed results and the predicted results was highly significant. Overall, the markers were 10.7% correct.

Based on the results, the *Ma1* and *Ma5* markers were very effective at identifying a large portion of the F_3 lines that produce PI hybrids across the 11 populations used. Given the high heritability of the *Ma1*/*Ma5*/*Ma6* PS response and the few loci involved, these results are consistent with previous research determining that markers are most effective and easiest to implement for highly heritable traits controlled by a few large effect loci (Holland, 2004).

In a high-biomass breeding program, markers at *Ma1* and *Ma5* could very

effectively be used to eliminate new PI lines producing PI hybrids before testcrossing. The reduced number of remaining lines would then be testcrossed to establish their hybrid photoperiod reactions and identify lines producing PS hybrids. In the populations used here, over 80% of the lines were predicted to produce PI hybrids and would thus be discarded. Such an ability to reliably discard undesirable lines before phenotyping is a substantial improvement in efficiency and would allow resources to be focused on material with greater potential.

Stepwise selection methods based on marker-assisted selection before phenotyping have been proposed to improve the population means of many crops and traits and thus genetic gain (Bertrand and Mackill, 2008; Langridge and Chalmers, 2005). In the population used here, elimination of the lines producing PI hybrids based on their *Ma1* and *Ma5* marker alleles would increase the proportion of lines producing PS hybrids from 1.4% to 7.4%. Further improvements would be seen with the development of markers for the *Ma6* locus. Published research of marker-assisted stepwise selection of photoperiod sensitivity is

Table 2.5 Comparison between the *Ma1*/*Ma5* marker predicted segregation of testcrosses for photoperiod reactions to their actual photoperiod phenotypes across six dates

Population	Predicted # of Segregating Testcrosses	12-Jun Actual	26-Jun Actual	10-Jul Actual	24-Jul Actual	10-Aug Actual	7-Sep Actual	7-Sep % Correct by Pop.
1	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	9	2	4	2	2	2	2	22.2
4	6	0	0	0	0	0	0	0.0
5	12	5	5	3	1	0	0	0.0
6	5	2	2	1	0	0	0	0.0
7	4	0	3	0	0	0	0	0.0
8	4	1	1	0	0	0	0	0.0
9	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
10	8	3	4	1	0	0	0	0.0
11	8	7	7	4	4	4	4	50.0
SUM	56	20**	26**	11**	7**	6**	6**	
% CORRECT		35.7	46.4	19.6	12.5	10.7	10.7	

[*] Statistically significant deviation of the observed count from the expected count at $\alpha = 0.05$
[**] Statistically significant deviation of the observed count from the expected count at $\alpha = 0.01$
[ns] No significant difference between the observed count and the expected count at $\alpha = 0.05$

currently unavailable. But it is routinely used for many other qualitative and quantitative traits in a host of crops (Bertrand and Mackill, 2008; Eathington et al., 2007).

As high-biomass sorghum breeding pools expand and incorporate new germplasm, additional maturity loci besides those described here may be identified. US sorghums themselves are a minor representation of sorghum diversity (Rosenow & Dahlberg, 2000). Rooney and Aydin (1999) discovered that crosses made between a source of the dominant *Ma6* allele and the sudangrass-type sorghum 'Lahoma' did produce a PS F_1. However, the F_2 of this cross did not segregate in the 9:7 ratio of a two-gene system with complementary dominant interaction that they were expecting. Mullet et al. (2010) recently described a sorghum maturity locus, *Ma7*, that potentially has *Ma5*-like activity. Additionally, there are sorghum genotypes requiring substantially shorter day lengths for flowering than those mediated by *Ma1*, *Ma5*, and *Ma6*. If high-biomass sorghum breeding begins to utilize germplasm with PS responses controlled by novel maturity loci, then the use of markers at *Ma1* and *Ma5* as described here may require modification or have reduced utility.

Conclusions

Molecular markers for alleles at the *Ma1* and *Ma5* maturity loci were used to predict the photoperiod reaction of sorghum testcrosses made with 483 PI F_3 lines from 11 populations crossed to PI testers in the *Ma1*/*Ma5*/*Ma6* hybrid production system.

Marker-predicted photoperiod reactions were compared to the actual testcross phenotypes.

The markers studied identified F_3 lines that would produce PI hybrids with an accuracy of 99.5% across the 11 populations. The markers studied identified F_3 lines that would produce PS hybrids with an accuracy of 18.0% across the 11 populations. The markers studied identified F_3 lines that would produce hybrids segregating for photoperiod-sensitivity with an accuracy of 10.7% across the 11 populations. Alleles at *Ma6* were unaccounted for and the development of *Ma6* markers would improve the ability to predict hybrid photoperiod reactions.

Markers for *Ma1* and *Ma5* could be used to reliably identify and discard a large proportion of new lines that produce PI hybrids before testcrossing. The remaining material would then be testcrossed and phenotyped to identify lines producing PS hybrids.

III. PREDICTING THE POTENTIAL OF EXOTIC SORGHUM ACCESSIONS FOR HIGH BIOMASS PRODUCTION BASED ON PASSPORT INFORMATION

Introduction

Many different plant species ranging from switchgrass to *Miscanthus* are being developed as energy crops. Among these, sorghum (*Sorghum bicolor*) has the advantage of already being a well established crop with equally well established improvement methods and resources with a history of achieving substantial changes in sorghum. Sorghum breeders have developed elite sorghums for grain, fodder, and syrup applications (Rooney, et al., 2007). These resources can now be marshaled for biofuel breeding goals. A particularly useful resource in sorghum breeding has been the availability of exotic plant introductions. In 1963, the Sorghum Conversion Program was established to convert tropical sorghum plant introductions to temperate adaptation for US breeding (Quinby, 1974). From this program, 840 new lines were developed, many of which have made important contributions to sorghum improvement and have provided much needed genetic variability to the narrow germplasm base of US sorghums (Duncan et al., 1991; Klein, et al., 2008). However, these lines were developed for grain or fodder applications, and their applicability to biofuels is limited.

For future biofuel sorghum breeding, a vast quantity of exotic germplasm is still available in germplasm banks. As of June 2009, the USDA National Plant Germplasm System had 43,957 *Sorghum* genus accessions, 43,533 of which were *Sorghum bicolor*

from 112 different countries (NPGS, 2009). The germplasm collection of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has ~37,000 *Sorghum bicolor* accessions from 90 countries (Grenier et al., 2001).

Sorghum breeding for biofuels will include the development of high-biomass sorghums for lignocellulosic biofuels. Currently, most sorghums with high-biomass potential were developed for fodder applications and their yield is constrained by animal nutrition and palatability parameters. Also, biofuel applications will likely require selection for new or modified traits. Thus high-biomass sorghum breeding will require new breeding pools incorporating new germplasm. An important source of new high biomass germplasm will be the exotic accessions contained in germplasm banks. As previously mentioned, the large number of accessions available in germplasm banks highlights their potential utility. However, this abundance presents a challenge as the number of accessions available greatly exceeds the evaluation capacities of any breeding program. Additionally, most accessions have limited utility for applied breeding applications; including high-biomass breeding. Thus from the vast quantity of accessions available, breeders must prioritize which accessions are evaluated for potential use.

Germplasm banks strive to provide relevant descriptor information for their accessions to aid researchers in selecting material. Sorghum accession descriptors can include passport data (geographic origin), race, photoperiod sensitivity, growth habit, resistance to biotic and abiotic stresses and other information. However, the availability of descriptor information for most accessions is limited; only passport data is widely

available. Additionally, many descriptors are subject to genotype x environment interactions that limit their reliability across environments. Thus researchers are often limited to passport data when selecting germplasm bank accessions for evaluation. This combination of size with a dearth of descriptor information contributes to the under-utilization of most germplasm collections by breeders (Glaszmann et al., 2010).

To manage the large size of germplasm collections and facilitate their use, the development and evaluation of representative subsamples has been proposed (Brown, 1989). Towards this, core and mini-core collections that represent the diversity of a larger collection with a subsample based on passport data, genomic data, morphology, or other available descriptors have been developed (Brown, 1989; Upadhaya and Ortiz, 2001; Glaszmann et al., 2010; Upadhaya et al., 2009, Vaughn 1991). Researchers can identify germplasm-phenotype associations in these smaller core collections and extrapolate the results to the larger collection to inform its future use.

In sorghum, Grenier et al. (2001a) sampled accessions from the ~37,000 accession ICRISAT sorghum germplasm collection based on their photoperiod sensitivity to create a 2247 accession core collection. While this core collection provides researchers with a smaller subsample organized into photoperiod sensitivity categories, it is still excessively large for extensive evaluations. To remedy this, Upadhyaya et al. (2009) developed a 242 accession mini-core collection from the ICRISAT core collection. A 3011 accession core collection has also been developed for the USDA National Plant Germplasm System sorghum collection based on a 10%

accession subsample of each country of origin in the larger collection (Dahlberg et al., 2004).

By evaluating a core collection or another germplasm subsample for a trait of interest, links between descriptors and traits can be established to prioritize accessions from the larger collection for evaluation. In the USDA NPGS sorghum collection, passport data (geographic origin) is a widely available descriptor for sorghum and is not subject to GxE interactions as other available descriptors may be. In general, geographic origin and phenotypes are often related because of evolutionary history (Loveless and Hamrick, 1984). Thus passport data may be an indicator of various traits, including high-biomass potential. Grenier et al. (2001b) identified a significant relationship between the latitudinal origin and race of sorghum accessions with photoperiod sensitivity. Others have identified mixed results in associating geography with specific traits across several crop species (Reid et al., 1990; Holbrook et al., 2000; Jarosz and Bordon, 1991; Westaman et al., 1990).

With these considerations in mind, the objective of this research is to determine whether significant relationships exist between the passport data of sorghum accessions from the USDA NPGS sorghum collection and high-biomass desirability. Passport data for the USDA NPGS sorghum collection is typically limited to an accession's country of origin rather than more detailed information such as originating elevation or soil type. Also, racial classification data is one of the more widely available descriptors for USDA NPGS sorghum accessions and identification of significant relationships between sorghum race and high biomass desirability will also be attempted. A large portion of

sorghum accessions in the USDA NPGS have been categorized into one of fifteen sorghum races based on panicle traits and differences may exist between races for high biomass desirability. If meaningful relationships between passport data alone or passport data with race and high biomass desirability exist, then sorghum germplasm bank accessions can be prioritized for high biomass breeding based on such information.

Materials and Methods

Selection of Accessions and Plot Establishment

Seven hundred ninety one sorghum accessions from the USDA National Plant Germplasm System sorghum collection were obtained for evaluation in 2007 and 1001 different accessions in 2009. Most accessions in this collection are landraces with passport data (country of origin) as the most widely available descriptor. Accessions described as late flowering (75-90 days) per the NPGS were randomly sampled from 7 different countries; Burundi, Zimbabwe, Sudan, Yemen, Nigeria, and Kenya (Table 3.1). These countries represent a diversity of sorghum growing environments and have sufficient accessions available in the NPGS collection for large scale evaluation. A large number of accessions for evaluation were needed because most accessions were expected to have undesirable phenotypes, thus large numbers are needed to identify desirable phenotypes and provide sufficient variability for comparisons. Also, a large number of accessions aids in mitigating the effects of duplications in the collection.

Table 3.1 Number of unique USDA National Plant Germplasm System sorghum accessions from 7 origins phenotyped for high-biomass desirability. 2009 accessions were planted twice (March and June)

Origin	2007	2009	Total
Burundi	96	143	239
India	95	143	238
Kenya	49	143	192
Nigeria	87	143	230
Sudan	62	143	205
Yemen	245	143	388
Zimbabwe	157	143	300
Total	791	1001	1792

In 2007, the accessions for this study were part of a larger observation nursery designed to identify high biomass germplasm for breeding. In 2007 the accessions were not placed in an experimental design and were planted in the order received from the NPGS, which generally placed the accessions in blocks by country of origin. In 2009, the accessions were not part of a larger nursery and were grouped into 13 blocks; each block had 11 accessions from each origin for a total of 77 accessions per block. Accessions were randomized within blocks. In 2007 accessions were planted in College Station, TX in late March and in 2009, this test was planted twice in College Station, TX; an early planting in March and a late planting in June. Each plot was planted with 100 seed.

Based on soil fertility tests, plots in 2007 received 143.7 kg/ha of 10-34-0-4 (Zn) pre-plant fertilizer and were side-dressed with 67.3 kg/ha of N_2 in early May. Both 2009 plantings received 168 kg/ha of 32-0-0 pre-plant fertilizer and were side dressed with 350 kg/ha of 32-0-0 six weeks after planting. All plots received standard sorghum

agronomic practices for weed and insect control and supplemental irrigation was not provided.

High-Biomass Evaluation and Racial Classification

In August and October of 2007, plots were visually scored for high-biomass desirability by 3 individuals with the Texas A&M sorghum breeding program. High-biomass desirability is largely a function of height, stem diameter, delayed flowering and lodging. Plots that maximize height and stem diameter, delay flowering until late in the growing season or do not flower, and with little to no lodging represent the desired phenotype. Accessions were rated with a + or – scale, with a + signifying desirability for high-biomass breeding. Accessions rated with a + by all three individuals had high biomass desirability.

In August of 2009, plots in the early planting were visually scored for high-biomass desirability using the method defined in the 2007 evaluation. Scoring of the 2009 late planting for high-biomass desirability occurred in October. When available, accession racial classification data from the NPGS was added to the high biomass phenotyping results. The NPGS sorghum collection classifies sorghum into 15 races based primarily on panicle traits as described by Harlan and de Wet (1972). When multiple racial classifications were available for an accession, the most recent classification was used. The availability of racial classification was somewhat inconsistent across origins as was the distribution of races within origins (Table 3.2).

For example, the Guinea race was overly associated with Nigerian accessions and the Caudatum race with Kenyan accessions.

Table 3.2 Racial classification data for USDA National Plant Germplasm System sorghum accessions from 7 different geographic origins. Data includes the percentage of accessions from each origin classified for race, the number of races represented in each origin, and the two most widely represented races (Race 1 & 2) in each origin with their level of representation (%).

Origin	Accessions with Race (%)	# of Races	Race 1	Race 2
Burundi	97.1	13	Kafir-Durra (18.2 %)	Kafir (15.7 %)
India	87.4	14	Durra (32.2 %)	Caudatum (16.2 %)
Kenya	80.7	14	Caudatum (45.6 %)	Guinea-Caudatum (20.9 %)
Nigeria	70.0	13	Guinea (49.4 %)	Caudatum (12.4 %)
Sudan	52.7	13	Caudatum-Bicolor (26.5 %)	Caudatum (22.0 %)
Yemen	94.3	11	Durra-Caudatum (31.3 %)	Durra (21.8 %)
Zimbabwe	35.7	10	Kafir (35.4 %)	Caudatum (24.7 %)
Total	74.6			

Analysis

Analyses were performed individually for the 3 environments and with the environments combined. Accessions were analyzed first by their passport data (country of origin) alone. Then the subset of accessions with both passport data and racial classification data were analyzed using both factors. For comparisons of race rank and performance, only the racial categories represented by at least 30 accessions were used (11 of the 15 races).

Analyses of variance were performed using the PROC MIXED procedure of SAS 9.1. Accession origin and race were treated as fixed effects and all other factors as

random effects. A two-component AMMI analysis was performed for the origin x environment, data using IRRISTAT 5.0 (IRRI, 2005).

Results and Discussion

In 2007, 15.6% of the accessions visually scored for high biomass desirability (primarily superior height and stem diameter, late flowering and low lodging) were selected as desirable (Table 3.3). Nigeria and Zimbabwe were the geographic origins with the highest proportion of selected accessions (41.3% and 26.1%, respectively) while Kenya and Yemen had the lowest proportion (8.1% and 0.80%, respectively). In the 2009 early planting, 10% of the accessions had high biomass desirability with Yemen and India having the highest proportion of selected accessions (26.6 % and 18.1%, respectively) and Nigeria and Burundi with the lowest proportion (4.2% and 0.70%, respectively). The 2009 late planting environment had the lowest proportion of accessions selected for high-biomass desirability, 3.4%, with Zimbabwe and Nigeria having the largest proportion of selected accessions (8.4% and 5.6%, respectively) and Sudan and Kenya with the lowest proportion (1.4% and 0.70%, respectively). In all of the environments, lodging was the most common criteria separating accessions for high biomass desirability, which was typically severe in most accessions. Large rank shifts between environments for the accession high biomass desirability of each geographic origin were readily observed (Table 3.3).

Table 3.3 Percentage of sorghum accessions from 7 different geographic origins selected for high biomass desirability in 3 College Station, TX environments; one in 2007, and an early (March) and late (June) planting in 2009.

	2007		2009 Early		2009 Late	
Origin	% Selected	Rank	% Selected	Rank	% Selected	Rank
Burundi	14.6	4	0.7	7	2.8	3
India	12.6	5	18.9	2	2.1	4
Kenya	8.1	6	5.6	5	0.7	6
Nigeria	41.3	1	4.2	6	5.6	2
Sudan	24.2	3	6.3	4	1.4	5
Yemen	0.8	7	26.6	1	2.8	3
Zimbabwe	26.1	2	7.7	3	8.4	1
Total	15.6		10.0		3.4	

Table 3.4 Analysis of variance results for sorghum accessions from 7 different geographic origins evaluated for high biomass desirability across 3 College Station, TX environments. Environments included a 2007 planting, and an early (March) and late (June) planting in 2009.

Variance	Across Env		2007		2009 Early		2009 Late	
Source	DF	MS	DF	MS	DF	MS	DF	MS
Origin	6	0.61	6	2.28**	6	1.15**	6	0.02
Env	2	4.25	.	.	.	.	.	.
Block	.	.	.	.	12	0.44**	12	0.07**
Origin*Env	12	1.52**	.	.	.	.	.	.
Error	2772	0.07	784	0.11	982	0.08	982	0.03

** Statistically significant at $\alpha = 0.01$

In ANOVA analyses of the individual environments, geographic origin was a highly significant variance source for high biomass desirability in the 2007 and 2009 early planting environments but not in the 2009 late planting environment (Table 3.4). The 2009 late planting environment had less variability than the other environments that may have reduced the differences between geographic origins.

In an ANOVA analysis with the environments combined, the geographic origin of the accessions was no longer a significant variance source for high biomass desirability (Table 3.4). However, the interaction between the origins and the environments was highly significant. This result combined with the large rank shifts indicate that the relationships between high biomass desirability and geographic origin varied greatly by environment. Some of these origin x environment interactions can be visualized in an AMMI-2 biplot of the combined data (Figure 3.1) accounting for 99.1% of the interaction variance. In this biplot, specific adaptations of Nigerian accessions to the 2007 environment and India and Yemen to the 2008 early planting environment can be identified. Also, Zimbabwean accessions appear to have a more general adaptation. These observations are reflected in the ranks, including the rank stability of Zimbabwe; which was consistently ranked in the top 3 geographic origins.

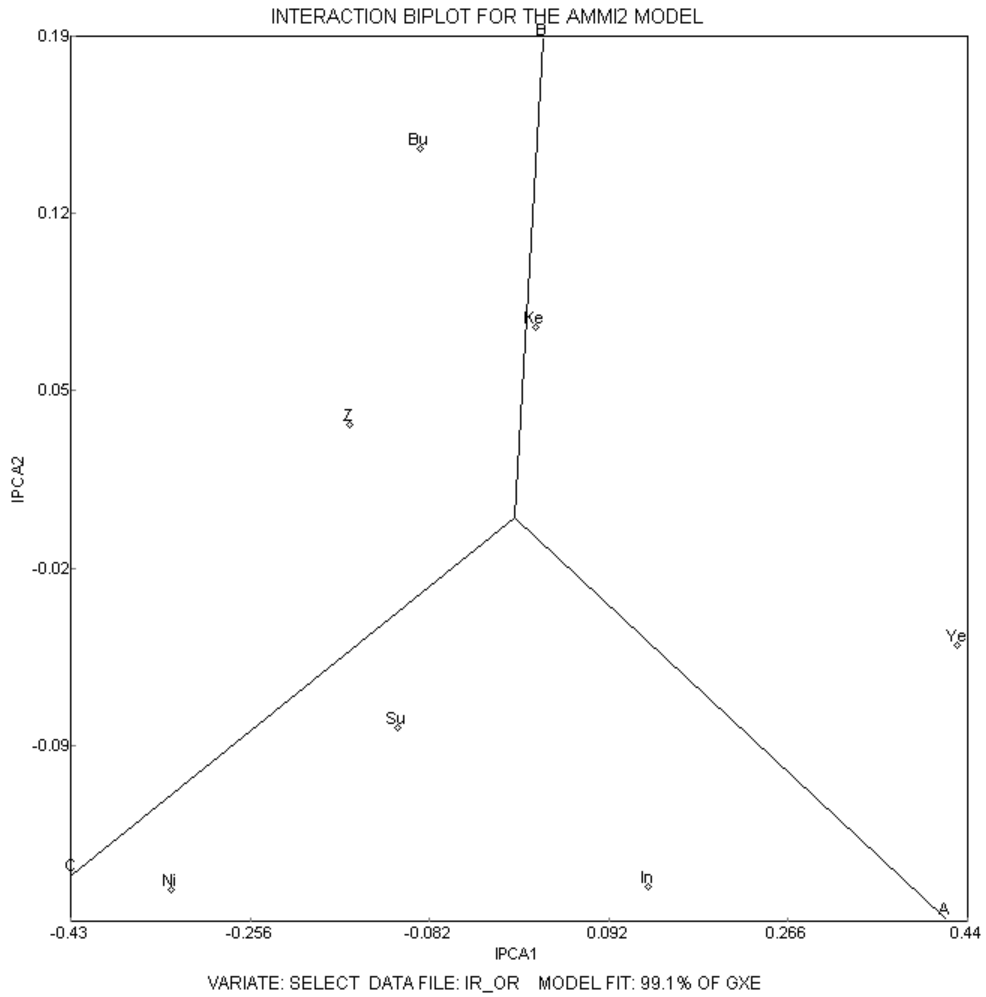

Figure 3.1 AMMI-2 plot (biplot of IPCA1 x IPCA2 scores) of sorghum accessions from 7 different geographic origins evaluated across 3 environments for high biomass desirability. Geographic origins are represented by their abbreviations and environments by the letters A, B, and C (A = 2009 early planting, B = 2009 late planting, C = 2007 environment).

Thus significant relationships between geographic origins and high biomass desirability can be identified within environments. Evidence that the phenotypes were related to the evolutionary environments of the accessions (Loveless and Hamrick, 1984) can be seen in the divergent performances of Nigerian and Yemeni accessions in the

2007 and 2009 early planting environments. The 2007 and 2009 early planting environments differed greatly for rainfall particularly during the summer months (36.3cm May-Aug. 2007; 11.3cm May-Aug. 2009) (NOAA, 2010). The 2009 late planting environment had significant rainfall the latter half of its growing season. Nigeria is a high rainfall, high humidity environment, and Nigerian accessions performed best in the moister 2007 environment. Yemen is a low rainfall low humidity environment, and Yemeni accessions did best in the drier 2009 early planting environment. Obtaining additional information regarding the environmental origins of germplasm accessions would further improve phenotypic predictions based on geography. Street et al. (2008) proposed their focused identification of germplasm strategy (FIGS) that couples accession collection site coordinates with geographic information system (GIS) databases to estimate environmental variables to further predict phenotypes and inform germplasm collection use. FIGS has successfully been used in several examples to improve the identification of novel traits and alleles in germplasm collections (Endreson, 2010; Street et al., 2008). But use of FIGS requires coordinates for accession collection sites, which are unavailable for most USDA NPGS sorghum accessions. Additionally, others have cautioned that phenotypes and alleles are not always related to geography and recommend evaluating whole core collections (Spooner et al., 2009).

In the limited data presented here, there is evidence that geographic origin is related to high-biomass desirability. However, these relationships are subject to genotype x environment interactions that limit their applicability across environments.

Given the quantitative nature of high biomass desirability and the diversity of their origins, their diverse environmental reactions are not surprising.

Because of these interactions, identifying robust relationships between accession passport data and high biomass desirability will require more environments than those used here. With data from more environments, G x E interaction patterns and reliable relationships established. Evidence that meaningful relationships may still be identified is the rank stability of the Zimbabwean accessions in the limited environments used here.

Accession racial classification data was also analyzed to determine whether it could aid in predicting high biomass desirability, particularly when combined with passport data. However, the effects of race and geographic origin are difficult to separate as sorghum races are often associated with specific geographic origins such as the Guinea race with West Africa and the Durra race with the Horn of Africa and India (Brown et al., 2011). Individual races were not evenly distributed across origins and most of the origins were overly represented by a limited set of races that were less prominent in other origins. For example, Guineas in the 2007 Nigerian accessions (53%) vs. Guineas in the 2007 Yemeni accessions (0%) (data not shown). Thus the effect of Guineas is difficult to separate from possible differences between Nigeria and Yemen and the detection of an origin x race interaction for Guineas in Yemen is not possible. Additionally, the availability of racial data was limited for some origins. Only 35.7% of Zimbabwean accessions were classified for race (Table 3.2). Zimbabwe was a consistently superior origin for high biomass desirability, but the lack of racial data

limits the ability to establish the role of race in Zimbabwean accessions for high biomass desirability.

Race was a highly significant variance source for high biomass desirability in the 2007 environment but was non-significant in the remaining environments and across environments. The limited significance for race may be related to the reduced variance in the 2009 environments that may have precluded the detection of a significant racial effect. Or conversely, sorghum races are differentiated by panicle traits that may not translate into prominent differences for high biomass desirability. In the 2007 environment, the Guinea and Bicolor races had the highest proportion of their accessions selected (44.2% and 32.0%, respectively) while the Durra-Caudatum and Kafir-Durra had the lowest proportion (2.7% and 3.2%, respectively) (Table 3.5). Across environments, Guinea and Bicolor had the highest proportion of their accessions selected (15.9% and 14.3%, respectively) while the Caudatum-Bicolor and Kafir-Durra had the lowest proportion of their accessions selected (4.1%, and 6.0%, respectively). The superior performance of Guineas and Bicolors across environments may be skewed by the disproportionately larger percentage of accessions with high biomass desirability in the 2007 environment vs. the other environments. Additionally, a highly significant race x environment interaction was detected that was also reflected in the ranks (Table 3.5 and Table 3.6). The superior performance of Guineas in the wetter 2007 environment is likely related to their West African origins as was discussed for the Nigerian accessions, most of which were Guinea. As with the origins, data across more environments would be needed to establish robust relationships regarding race and high biomass desirability.

Table 3.5 Percentage of sorghum accessions from 11 different sorghum races selected for high biomass desirability in 3 College Station, TX environments. Environments included a 2007 planting, and an early (March) and late (June) planting in 2009.

	2007		2009 Early		2009 Late		Across Environments	
Race	% Selected	Rank	% Selected	Rank	% Selected	Rank	% Selected	Rank
Bicolor	32.0	2	7.7	7	3.9	3	14.3	2
Caudatum	12.0	6	7.9	6	0.7	9	6.4	8
Caudatum-Bicolor	25.0	3	0.0	9	1.6	8	4.1	11
Durra	9.6	8	23.5	1	4.1	2	12.6	3
Durra-Bicolor	10.0	7	7.7	7	3.9	3	6.9	7
Durra-Caudatum	25.0	10	21.0	2	0	10	7.5	6
Guinea	44.2	1	0.0	9	8.1	1	15.9	1
Guinea-Caudatum	.	.	11.1	4	2.2	6	8.0	5
Kafir	14.6	4	10.8	5	3.1	5	8.9	4
Kafir-Caudatum	12.8	5	3.6	8	3.6	4	6.0	10
Kafir-Durra	3.2	9	14.3	3	1.8	7	6.3	9
Average	18.8		9.8		3.0		8.8	

Table 3.6 Analysis of variance results for sorghum accessions from 7 different geographic origins and 15 sorghum races evaluated for high biomass desirability in 3 College Station, TX environments. Environments included a planting in 2007, and an early (March) planting and late (June) planting in 2009.

Variance	Across Env		2007		2009 Early		2009 Late	
Source	DF	MS	DF	MS	DF	MS	DF	MS
Origin	6	0.09	6	0.59**	6	0.17*	6	0.02
Race	14	0.07	14	0.30**	14	0.12	14	0.01
Env	2	0.98*	.	.	.	.	.	.
Block	.	.	.	.	12	0.35*	12	0.05*
Origin*Race	66	0.08	66	0.15**	66	0.07	66	0.02
Origin*Env	12	0.49**	.	.	.	.	.	.
Race*Env	28	0.15**	.	.	.	.	.	.
Error	1951	0.07	.	0.09	.	0.08	.	0.02

* Statistically significant at $\alpha = 0.05$

** Statistically significant at $\alpha = 0.01$

In the 2007 environment, a significant origin x race interaction was detected, indicating that potential relationships between race and high biomass desirability may vary by origin. If so, combining passport data with racial data would improve the predictability of accession high biomass desirability. Grenier et al. (2001b) successfully stratified a subset of the ICRISAT sorghum collection for photoperiod sensitivity by combining both passport data and racial classifications. However, they had a more balanced and complete dataset than the one available here.

Given the data available here, the ability of race to predict high biomass desirability and how it varies by origin cannot be reliably determined. In many ways, the use of NPGS sorghum accession racial data described here highlights some of the challenges in using available descriptor data to prioritize germplasm bank accessions for use, even for a comparably widely available descriptor such as race.

Conclusions

An attempt was made to determine whether meaningful relationships exist between the geographic origin (passport data) of exotic sorghum accessions and high biomass desirability. Identification of such relationships could aid researchers in prioritizing exotic sorghum accessions for evaluation to develop new high biomass sorghums.

Within environments, geographic origin was a significant variance source for high biomass desirability and geographic origins could be differentiated for performance. However, relationships between geographic origins and high biomass

desirability were subject to large G x E interactions. Thus relationships established within environments had little applicability across environments. To develop robust and meaningful relationships, data from a larger number of environments than those used here will be needed to understand and account for G x E interactions.

Racial classification data was also analyzed to determine if it could contribute to predicting high biomass desirability. Using the data available from the USDA National Plant Germplasm System, a relationship between accession race and high biomass desirability was identified in only one of the three environments and not across environments. The availability of racial data was inconsistent across origins as was the distribution of races within origins. These factors combined with the limited number of environments used hindered the identification of reliable relationships between race and high biomass desirability.

Overall, the results failed to meet the desired objective of establishing reliable relationships between USDA NPGS sorghum accession passport and racial data with high biomass desirability to prioritize accessions for high biomass evaluations. However, these results are based on limited data and future research may yet obtain this objective.

IV. HIGH-PARENT HETEROSIS FOR BIOMASS YIELD IN HIGH-BIOMASS SORGHUM HYBRIDS DERIVED FROM HIGH-BIOMASS POLLINATORS AND GRAIN SORGHUM FEMALES

Introduction

Photoperiod-sensitive (PS) high-biomass sorghums for biofuels will be produced as hybrids to enable commercial seed production. In temperate latitudes, there is insufficient growing season for the production of PS sorghum seed. To circumvent this, a seed production system (*Ma1*/*Ma5*/*Ma6* seed production system) that uses two photoperiod-insensitive (PI) parents to yield PS hybrid seed is used (Rooney and Aydin, 1999). This system manipulates the sorghum maturity loci *Ma1*, *Ma5* and *Ma6*. With a dominant allele at each locus, flower initiation is delayed until day length is ≤ 12h 20 min. By crossing a PI parental line that is homozygous recessive at one of these two loci and homozygous dominant at the other with another PI parent having the opposite allelic configuration (e.g. *ma1ma1Ma5Ma5ma6ma6* / *Ma1Ma1ma5ma5Ma6Ma6*), PS hybrid progeny with a dominant allele at all loci (e.g. *Ma1ma1Ma5ma5Ma6ma6*) are obtained.

In addition to facilitating seed production, this hybrid production system allows for the capture of heterosis for biomass yield. Heterosis is the superior performance of a hybrid compared to its parents. Two measures of heterosis exist, mid-parent heterosis

and high-parent heterosis. Mid-parent heterosis is the superior performance of the hybrid relative to the mean parental performance and is calculated as:

$$\text{MPH (\%)} = \frac{F1 - ((P1 + P2)/2))}{(P1 + P2)/2} * 100$$

$F1$ = hybrid performance, $P1$ & $P2$ = performance of parents

High-parent heterosis is the improved performance of the hybrid relative to the performance of the superior parent and is calculated as:

$$\text{HPH (\%)} = \frac{F1 - HP}{HP} * 100$$

$F1$ = hybrid performance, HP = performance of the superior parent

For applied breeding applications, high-parent heterosis is the only measure of practical importance because the goal of a breeding program is to improve the crop beyond what already exists in the parents and/or germplasm.

High-parent heterosis is regularly exploited for increasing sorghum grain yield. For dry biomass yield, high-parent heterosis values ranging from 38% to 199% have been identified in sweet sorghums (Corn, 2008; Meshram et al., 2005). High-parent heterosis values for biomass yield ranging from -18% to 38% have been identified in the

potential bioenergy crops switchgrass and alfalfa (Vogel and Mitchell, 2008; Riday and Brummer, 2005).

High-parent biomass heterosis likely exists in high-biomass PS hybrid sorghums and could be exploited to increase yield. Regardless of the crop, increasing biomass feedstock yields is among the most productive breeding goals for improving biomass biofuels viability (Epplin et al., 2007; Towler et al., 2004) because increased yields reduce fixed costs per unit of biofuel, particularly land and transportation costs (Epplin et al., 2007). Using existing data, the reported biomass yields of current sorghums with high-biomass potential (sweet, forage, and limited bioenergy) range from 15.6 Mg ha^{-1} to 40.3 Mg ha^{-1} (dry weight basis) across many environments (Habyarimana et al., 2004, McCollum et al., 2005, Rooney, 2007, Venuto and Kindiger, 2008). For comparison, recent U.S. maize grain yields averaged approximately 9.3 Mg ha^{-1} (Egli, 2008) and Burns et al. (2008) released a switchgrass cultivar with superior dry matter yields of 15.7 Mg ha^{-1} per year across two locations in North Carolina after one establishment year.

For higher seed yields and mechanical seed harvest, high-biomass hybrid sorghums will be produced using traditional grain sorghum seed parents and newly developed biomass sorghums as pollinator parents. It is possible that grain sorghum seed parents may preclude the availability of high-parent heterosis in the hybrids. Published research documenting the availability of biomass high-parent heterosis in high-biomass sorghums is currently unavailable. Therefore, the objective of this research is to measure the availability of high-parent heterosis for biomass yield in PS sorghum hybrids created with grain sorghum females and high-biomass pollinators.

Materials and Methods

Fifty-two sorghum entries were evaluated for biomass yield in four environments (Table 4.1). The entries were composed of PS high-biomass sorghum hybrids and their respective high-biomass pollinator lines and grain sorghum female lines. The pollinators represent a sample of current high-biomass pollinator diversity (PI and PS) available in the Texas A&M sorghum breeding program and the grain sorghum females are routinely used for hybrid sorghum production.

Table 4.1 High-biomass sorghum pollinators and grain sorghum females used to create hybrids evaluated for biomass yield in four environments. Hybrid representation of a genotype within an environment is indicated with an X.

Pollinator Genotype	College Station 2007	Halfway 2008	College Station 2008 - 1	College Station 2008 - 2
R07007		X	X	X
R07008	X	X	X	X
R07009	X			
R07010	X			
R07011	X			
R07012				X
R07014				X
R07015	X			
R07018	X	X	X	X
R07019				X
R07020	X			X
R07021	X			
R07024	X			X
R07025	X			
Female Genotype				
BTx645	X		X	
BTx623	X		X	
BTx378		X	X	X
ATx623/BTx2752			X	X
ATx623/BTx2928		X		
A3Tx436/RTx437		X	X	X

Hybrids were created via one of two methods. Hybrids with PI pollinators adapted to the *Ma1*/*Ma5*/*Ma6* seed production system were made in College Station, TX via *Ma1*/*Ma5*/*Ma6* seed production. The PI pollinators had the *Ma1ma5Ma6* allelic configuration that when crossed to the *ma1Ma5ma6* grain sorghum females yielded *Ma1ma1Ma5ma5Ma6ma6* PS hybrids. Hybrids with PS pollinators were made in a Puerto Rico winter nursery. Puerto Rico's tropical latitude permitted the simultaneous flowering of the PS pollinators with the grain sorghum females for hybrid seed production.

Entries were evaluated in four environments; College Station, TX in 2007 and twice in 2008 (CS-1 and CS-2), and in Halfway, TX in 2008. In each environment, entries were arranged in a RCB design with 3 replications in the CS 2007 and CS-2 environments and 2 replications in the CS-1 and Halfway 2008 environments. Plots were 6.7 meters long with 75 cm row spacing in all environments and were either one-row (CS 2007 and CS-1) or two-row (CS-2 and Halfway) and were all planted with 3g of seed per row. Seed weight varied per entry, and thus plot plant populations also varied. Plots were planted in mid-March for all of the environments except Halfway, which was planted in late May. Plots were managed with standard sorghum agronomic practices including fertilization, cultivation, herbicide, and pesticide applications. Based on soil fertility tests, CS 2007 plots received 177 kg/ha of 10-34-0-4 (Zn) pre-plant and 112 kg/ha of N_2 sidedressed one month after planting. CS-1 and CS-2 plots received 168 kg/ha of 10-34-0-4 (Zn) pre-plant and 135 kg/ha of N_2 sidedressed one month after

planting. Halfway plots received 112 kg/ha of N_2 approximately one month after planting. Supplemental irrigation was only provided in Halfway (52 cm).

Plots in the College Station environments were harvested in mid-October while Halfway was harvested in early November. CS 2007 plots were manually harvested by cutting 1.5 meters from the center of each plot and weighing it. The full lengths of both rows of CS-2 plots were harvested by a self propelled forage harvester with a row crop head. Harvested material was collected in a silage wagon modified with an Avery Weigh-Tronix system with weigh spindles to collect plot weights. CS-1 and Halfway plots were harvested by a tractor mounted PTO-driven forage harvester with a row crop head. Harvested material was collected in a suspended bucket mounted to the forage harvester modified with an Avery Weigh-Tronix system with a weigh bar underneath the bucket to collect plot weights. In Halfway, the full length of only one of the two plot rows was harvested.

An approximately 500g sample of harvested biomass was collected for each plot at the time of harvest. In the manually harvested CS 2007 plots, these were collected by sampling three whole plants from each plot and processing them through a wood chipper. The biomass samples were weighed, placed in paper bags, oven dried for a week at 57° C, then re-weighed to estimate dry matter content and yields for each plot.

Yield means were calculated with the PROC MEANS procedure of SAS 9.1. Mean (protected LSD) separation tests were performed with the MEANS statement of PROC GLM in SAS 9.1. High parent heterosis was calculated with the following formula:

$$\text{HPH (\%)} = \frac{F1 - HP}{HP} * 100$$

$F1$ = hybrid dry biomass yield, HP = superior parent dry biomass yield

For every hybrid entry, the pollinator was the high parent.

Analyses of variance for heterosis within environments and across environments were performed using PROC MIXED of SAS 9.1 with all random effects. For analyses performed across environments, a reduced dataset containing only the entries represented across all of the environments was used.

Correlation analyses relating hybrid biomass yield to pollinator yield and high-parent heterosis were performed with PROC CORR of SAS 9.1. A regression analysis with hybrid yield as the dependant variable and pollinator yield and high-parent heterosis as independent variables was performed with PROC REG of SAS 9.1.

Results and Discussion

The dry biomass yields of the high-biomass pollinators and their hybrids were not statistically different when measured across environments (Table 4.2). Within the individual environments, they were statistically different in two of the four environments (CS-1 and CS-2 from 2008) and the hybrid yields were numerically superior in all environments. It should be noted that yields of the College Station 2007 environment were exceptionally high. This may be due to the differences in harvest methodology between this environment and the others (manual subsamples vs. mechanical whole plots) combined with the exceptionally wet summer College Station had in 2007. While

environmental differences in harvest methods may hinder direct yield comparisons, they should not affect comparisons of heterosis values.

Table 4.2 Average dry biomass yields (Mg ha^{-1}) of high-biomass sorghum hybrids, high-biomass pollinators, and grain sorghum females evaluated in four environments and across environments. Averages sharing the same letter within a column are not statistically different at $\alpha = 0.5$

Entry Type	College Station 2007	Halfway 2008	College Station 2008 - 1	College Station 2008 - 2	Across Environments
Hybrid	41.4 a	24.2 a	26.4 a	23.3 a	31.6 a
Pollinator	36.3 a	19.9 a	21.3 b	14.8 b	27.6 a
Female	3.6 b	12.8 b	8.1 c	8.9 b	8.3 b

High-parent heterosis averaged 40.9% across the environments and ranged from a low of 29.4% in College Station 2007 to a high of 52.9% in Halfway (Table 4.3). These values are comparable to the high-parent heterosis values seen for grain yield in sorghum, and biomass production in switchgrass swards, *Arabidopsis thaliana*, and alfalfa (Barth et al., 2003; Duvick, 1997; Vogel and Mitchell, 2008; Riday and Brummer, 2005). But higher high-parent heterosis values have been observed in sweet sorghum for several traits, including dry biomass yield (Corn, 2009; Meshram, 2005). Additionally, a majority of the pollinators produced positive high parent heterosis values in their hybrids (Table 4.4).

Table 4.3 Average high-parent heterosis values and their ranges for high-biomass sorghum hybrids evaluated for biomass yield in four environments.

	Heterosis		
Environment	Average	High	Low
College Station 2007	29.4	208.6	-49.8
Halfway	52.9	95.7	-27.4
College Station 2008-1	38.6	154.0	-15.8
College station 2008-2	49.8	111.1	12.5
Across Locations	40.9	208.6	-49.8

The observed yields and heterosis availability demonstrate that in general, pollinator yield was maintained or improved in the hybrids. Given that increased height, a very important factor in biomass yield, is dominant in sorghum, this is not surprising (Morgan and Finlayson, 2000). The top 2 yielding hybrids consistently out-yielded the top 2 yielding pollinators by an average of 11.8 Mg ha^{-1} across locations with a range from 7.1 Mg ha^{-1} in CS-1 to 22.9 Mg ha^{-1} in College Station 2007 (Table 4.5). Based on theoretical ethanol yields for corn stover, the extra yield in the superior hybrids across locations represents an additional 4572.5 liters of ethanol per ha compared to the top pollinators (USDOE, 2009). For comparison, theoretical ethanol yields for maize grain yields of 10.05 Mg ha-1 are 10176.9 liters of ethanol per ha (USDOE, 2009). Thus producing high biomass sorghum hybrids with grain sorghum females permits temperate seed production and captures heterosis for improved yields.

Table 4.4 Line biomass yield, average hybrid biomass yield, and average high-parent heterosis values and range for high-biomass pollinators evaluated in four environments. Ranks are given for each value represented. Yields are dry biomass yields in Mg ha^{-1}.

Environment	Pollinator	Line Yield	Rank	Avg. Hybrid Yield	Rank	Heterosis	Rank	Heterosis Range High	Heterosis Range Low
2007	R07008	51.3	2	38.7	6	-24.4	9	-21.2	-27.7
College Station	R07009	39.4	4	19.7	10	-49.8	10	.	.
	R07010	28.9	8	31.8	4	9.3	6	-1.5	20.1
	R07011	31.8	6	32.0	7	1.9	7	17.1	-15.2
	R07015	32.2	5	51.9	5	60.8	3	86.5	35.1
	R07018	52.2	1	69.0	2	32.2	4	.	.
	R07020	40.5	3	67.2	3	65.8	2	.	.
	R07021	29.8	7	22.8	8	-22.8	8	.	.
	R07024	26.0	9	80.4	1	189.9	1	208.6	152.5
	R07025	21.7	10	21.7	9	30.8	5	.	.
	Average	35.4		43.7		29.4			
Halfway	R07007	15.9	2	26.4	1	60.8	2	80.7	49.5
	R07008	14.5	3	26.4	1	81.4	1	95.7	67.1
	R07018	26.0	1	19.0	2	-27.4	3	.	.
	Average	18.8		23.9		38.3			
2008	R07007	12.7	8	25.5	3	99.9	1	154.0	80.7
College Station-1	R07008	23.5	4	31.1	1	32.4	4	52.4	9.5
	R07012	23.9	3	24.2	5	1.5	6	18.7	-15.8
	R07018	20.8	5	24.4	4	17.2	5	35.5	-1.1
	R07020	19.2	6	31.1	1	61.6	2	62.8	60.5
	R07024	29.8	1	27.7	2	-6.7	7	.	.
	R07014	25.3	2	19.5	6	-23.0	8	.	.
	R07019	15.9	7	24.2	5	52.1	3	.	.
	Average	21.4		26.0		29.4			
2008	R07007	10.1	3	17.5	3	75.5	1	111.1	40.0
College Station-2	R07008	18.6	1	23.9	2	29.5	3	33.7	25.3
	R07018	17.9	2	25.7	1	44.3	2	76.2	12.5
	Average	15.5		22.4		49.8			

Table 4.5 Dry biomass yield and high-parent heterosis values of high-biomass sorghum hybrids in four environments. The dry biomass yields of their respective pollinator and female parents are also presented. Yields are in Mg ha^{-1}.

Environment	Hybrid	Hybrid Yield	Pollinator	Pollinator Yield	Female	Female Yield	High-Parent Heterosis (%)
2007	ATx645/R07021	19.8	R07021	29.8	ATx645	3.5	-33.3
College	ATx645/R07024	80.2	R07024	26.0	ATx645	3.5	208.6
Station	ATx645/R07018	69.2	R07018	52.3	ATx645	3.5	32.2
	ATx645/R07015	43.6	R07015	32.3	ATx645	3.5	35.1
	ATx645/R07008	37.1	R07008	51.4	ATx645	3.5	-27.7
	ATx645/R07010	28.6	R07010	29.0	ATx645	3.5	-1.5
	ATx645/R07009	19.8	R07009	39.5	ATx645	3.5	-49.8
	ATx645/R07011	37.2	R07011	31.8	ATx645	3.5	17.1
	ATx623/R07021	26.1	R07021	29.8	ATx623	3.8	-12.4
	ATx623/R07025	28.4	R07025	21.7	ATx623	3.8	30.8
	ATx623/R07020	67.3	R07020	40.6	ATx623	3.8	65.8
	ATx623/R07015	60.2	R07015	32.3	ATx623	3.8	86.5
	ATx623/R07008	40.5	R07008	51.4	ATx623	3.8	-21.2
	ATx623/R07010	34.9	R07010	29.0	ATx623	3.8	20.1
	ATx623/R07011	26.9	R07011	31.8	ATx623	3.8	-15.2
	Average	41.3		35.2		3.6	22.3
Halfway	ATx378/R07007	28.60	R07007	15.8	ATx378	12.1	80.7
	ATx623/BTx2752//R07007	24.10	R07007	15.8	ATx623/BTx2752	12.9	52.2
	ATx623/BTx2752//R07008	24.30	R07008	14.5	ATx623/BTx2752	12.9	67.1
	A3Tx436/RTx437//R07007	23.70	R07007	15.8	A3Tx436/RTx437	13.2	49.5
	A3Tx436/RTx437//R07008	28.50	R07008	14.5	A3Tx436/RTx437	13.2	95.7
	A3Tx436/RTx437//R07018	18.90	R07018	26.0	A3Tx436/RTx437	13.2	-27.4
	Average	24.7		17.1		12.9	52.9

Table 4.5 Continued

Environment	Hybrid	Hybrid Yield	Pollinator	Pollinator Yield	Female	Female Yield	High-Parent Heterosis (%)
	ATx623/BTx2752//R07012	20.2	R07012	23.9	ATx623/BTx2752	11.6	-15.8
	ATx623/BTx2752//R07018	28.2	R07018	20.8	ATx623/BTx2752	11.6	35.5
	ATx623/BTx2752//R07020	31.4	R07020	19.2	ATx623/BTx2752	11.6	62.8
	ATx623/BTx2752//R07024	27.8	R07024	29.8	ATx623/BTx2752	11.6	-6.7
	ATx623/BTx2752//R07014	19.5	R07014	25.3	ATx623/BTx2752	11.6	-23.0
	ATx623/BTx2752//R07019	24.2	R07019	15.9	ATx623/BTx2752	11.6	52.1
	A3Tx436/RTx437//R07007	23.1	R07007	12.7	A3Tx436/RTx437	6.7	80.7
	A3Tx436/RTx437//R07008	31.8	R07008	23.5	A3Tx436/RTx437	6.7	35.2
	A3Tx436/RTx437//R07012	28.5	R07012	23.9	A3Tx436/RTx437	6.7	18.7
	A3Tx436/RTx437//R07018	20.6	R07018	20.8	A3Tx436/RTx437	6.7	-1.1
	ATx623/R07020	30.9	R07020	19.2	ATx623	9.6	60.5
	ATx645/R07008	25.8	R07008	23.5	ATx645	6.5	9.5
	Average	26.8		20.5		9.5	38.7
2008	ATx378/R07007	14.1	R07007	10.1	ATx378	10.7	40.0
College	ATx623/BTx2752//R07007	20.6	R07007	10.1	ATx623/BTx2752	8.3	111.1
Station-2	ATx623/BTx2752//R07008	24.8	R07008	18.6	ATx623/BTx2752	8.3	33.7
	ATx623/BTx2752//R07018	31.5	R07018	17.9	ATx623/BTx2752	8.3	76.2
	A3Tx436/RTx437//R07008	23.3	R07008	18.6	A3Tx436/RTx437	7.8	25.3
	A3Tx436/RTx437//R07018	20.1	R07018	17.9	A3Tx436/RTx437	7.8	12.5
	Average	22.4		15.5		8.5	49.8

In ANOVA analyses for heterosis, pollinators were a significant effect only in the 2007 College Station environment (Table 4.6). However, prominent differences between the pollinators were readily observed in each environment. For example, in the CS-1 environment, hybrids of the R07007 pollinator had an average heterosis value of 99.9% while those made with R07012 averaged 1.5% (Table 4.4). Within environments, the maximum differences between pollinators for heterosis production ranged from a high of 239.7% in College Station 2007 to a low of 46.0% in Halfway (Table 4.4) and in mean separation tests, multiple differences between pollinators for heterosis production were detected. Thus while the average heterosis values seen here were comparable to those of other biomass crops, the range of heterosis values observed was much greater (Vogel and Mitchell, 2008; Riday and Brummer, 2005)

Some of these differences for heterosis production are related to the yield of the pollinators themselves. There was a modest, but highly significant, negative correlation of -0.38 between pollinator yield and heterosis production (Table 4.7). This relationship can be seen in the heterosis production of the top yielding pollinators. The average dry

Table 4.6 Analysis of variance results for high-parent heterosis of high-biomass sorghum hybrids evaluated for biomass yield in four environments. All effects are random.

Environment	Variance Source	DF	MS
Across Locations	Pollinator	14	8224.2
	Female	4	2987.1
	Env	3	683.6
	Rep(Env)	6	2170.4
	Pollinator*Female	11	2222.7
	Pollinator*Env	5	3497.9
	Error	61	1551.8
2007 College Station	Pollinator	11	11241.0**
	Female	1	388.0
	Rep	2	3460.3
	Pollinator*Female	6	1137.8
	Error	20	1342.1
Halfway	Pollinator	2	7543.3
	Female	2	873.9
	Rep	1	42.2
	Pollinator*Female	1	564.5
	Error	5	578.0
2008 College Station-1	Pollinator	7	9968.2
	Female	4	5034.4
	Rep	2	2291.6
	Pollinator*Female	4	3377.4
	Error	26	1957.8
2008 College Station-2	Pollinator	2	1771.4
	Female	2	2122.9
	Rep	1	1366.6
	Pollinator*Female	1	1476.9
	Error	4	842.3

** Statistically significant at $\alpha = 0.01$

biomass yield of the top yielding pollinators in each environment was 31.4 Mg ha^{-1} compared to an average pollinator yield of 22.6 Mg ha^{-1}. These same pollinators produced an average heterosis value of 10.6% compared to the average of 40.9%. The top heterosis producing pollinators in each environment produced an average heterosis value of 111.6% but only yielded an average of 15.7 Mg ha^{-1} as pollinator lines. Thus in general, as pollinator yield increased, heterosis decreased.

Table 4.7 Correlation coefficients between the biomass yield of high-biomass sorghum hybrids, the biomass yield of high-biomass sorghum pollinators, and the expression of high-parent heterosis in the hybrids.

	Hybrid Yield	Pollinator Yield	Heterosis
Hybrid Yield		0.44**	0.58**
Pollinator Yield			-0.38**
Heterosis			

** Statistically significant at $\alpha = 0.01$

This negative relationship between pollinator yield and heterosis is partially explained by the relationship between photoperiod sensitivity and biomass yield. Increasing photoperiod sensitivity allows a pollinator or hybrid to remain vegetative longer and thereby increase biomass yields given a long growing season and sufficient water. PS pollinators and PS hybrids sharing this photoperiod sensitivity based yield advantage will express less yield differences and thus, heterosis. Indeed the previously mentioned top yielding pollinators that produced below average heterosis were PS. PS hybrids created with a PI pollinator using the *Ma5*/*Ma6* seed production system will

generally express substantially more heterosis because of the biomass yield advantage of the hybrids based on photoperiod sensitivity. Such was the case here, as R07007; the only PI pollinator used; had consistently low biomass yields but exceptionally high heterosis values that were sufficient to produce hybrid yields comparable to the other pollinators. Thus as PS pollinators are converted to PI for temperate seed production and lose a portion of their biomass yield, those yield losses can be recovered via heterosis in their PS hybrids.

A significant pollinator x environment interaction was not detected, but the establishment of reliable conclusions regarding pollinator by environment interactions is difficult because of the unbalanced nature of pollinator representation across environments. Those pollinators that were more widely represented allow some insight into heterosis reactions across environments. For example, R07007 hybrids were evaluated in 3 of the 4 environments and consistently had high heterosis levels. R07008 and R07018 hybrids were evaluated in all 4 environments and each pollinator produced consistently moderate heterosis levels, but each with an exception. R07008 hybrids had a large negative heterosis value in College Station 2007 while R07018 produced a large negative value in Halfway. Another interesting example is R07024 which produced the highest heterosis value in College Station 2007 (189.9%) but also the 2nd lowest value in CS-1 (-6.7%). Thus examples of both relative stability and environmental interactions for heterosis existed. Additionally, heterosis varied substantially for some pollinators depending on hybrid combination. Several pollinators produced heterosis value ranges of over 50% depending on their hybrid combinations (Table 4.6). Despite this, a

significant pollinator x female interaction was not identified (Table 4.5), but again, this may be related to the unbalanced nature of the data. Thus high-parent heterosis is widely available in high-biomass sorghums, but can vary based on hybrid combination and G x E interactions. These same results have been seen for multiple traits in other sorghums as well (Corn, 2009). More balanced and extensive future datasets will be required to make reliable interpretations of these factors.

Correlation analyses were used to measure the relationship between biomass yield with high-parent heterosis and pollinator yield. The correlation coefficient for hybrid biomass yield with heterosis was 0.51 and 0.47 for hybrid biomass yield with pollinator biomass yield. Both values were highly significant. These highly significant, but moderate values indicate a modest relationship between high-parent heterosis and pollinator yield with hybrid biomass yield. Use of a high-yielding pollinator or large heterosis values improve the likelihood of producing a high yielding hybrid, but do not guarantee it. High-yielding hybrids likely involve both components. A regression analysis with hybrid yield as the dependent variable and pollinator yield and heterosis as the independent variables had an R^2 value of 0.86, evidence that pollinator yield and heterosis combined are important factors in hybrid biomass yield. However, very high yielding pollinators can be excessively tall for effective pollinations of seed parents. Thus a balance between pollinator yield and seed production must be achieved.

Conclusions

High-biomass sorghum hybrids created from high-biomass pollinators and grain sorghum females were evaluated for biomass yield high-parent heterosis in four environments. Moderate levels of heterosis (40.9% across environments) were widely available and hybrid biomass yields were consistently similar or superior to the pollinators. Also, the top yielding hybrids consistently out-yielded the top yielding pollinators. Thus creating high-biomass sorghum hybrids with grain sorghum females enables commercial seed production and captures heterosis for improved yields. However, heterosis availability did vary by pollinator, hybrid combination, and environment.

In general, heterosis increased with decreasing pollinator yield. This is partially attributable to differences in photoperiod sensitivity, with PI pollinators yielding less than PS pollinators, but the PS hybrids of both had similar yields. So as PS pollinators are converted to PI for temperate seed production, the associated yield losses can be recovered in their PS hybrids.

V. GENOTYPE X ENVIRONMENT INTERACTIONS OF SORGHUM COMPOSITIONAL TRAITS

Introduction

High-biomass sorghums specifically targeted for biofuels applications are being developed. Yield has been established as the most important breeding goal, but other traits will also be important in high-biomass sorghums for biofuels. In addition to yield, the conversion efficiency of biomass to fuel is important in developing biofuel feedstocks. Biomass conversion efficiency is the ease with which the energy in biomass can be converted into a convenient fuel and is one of the most important considerations for producing economically viable biofuels, especially alcohol fuels (Banarjee et al., 2010; Han et al., 2007).

Biomass conversion efficiency is largely determined by the composition and quantity of structural carbohydrates in plant cell walls. The cell walls of vascular plants are composed of cellulose, hemicellulose, and lignin that together form lignocellulose. Lignocellulose provides cell wall structure, strength, and rigidity and is the primary reservoir of stored energy in biomass.

Cellulose is a high molecular weight glucose polymer that accounts for ~25-40% of grass cell wall composition. It can exhibit varying degrees of structural organization from amorphous to highly crystalline. Hemicellulose is a shorter polymer of pentose sugars. In grass cells, hemicellulose is primarily composed of a xylose backbone with

arabinose and glucoronic acid side chains. Hemicellulose provides additional structure to cellulose and cell walls and accounts for ~20-50% of grass cell wall composition (Vogel, 2008). Lignin is a complex polymer of phenylpropanoids (diverse type of organic compounds derived from phenylalanine) that is very heterogeneous in structure and subunit composition. Lignin surrounds cellulose and hemicellulose in secondary cell walls for additional strength, rigidity, and hydrophobicity; and constitutes ~20% of grass secondary cell walls. The remaining proportion of lignocellulose weight is a diverse mixture of proteins, phenols, chlorophyll, and other molecules (Suiter et al., 2008; Vogel, 2008). The individual proportion of these cell wall components varies by species, genotype, structure, and environment (Pauly and Keegstra, 2008).

The sugar monomers of cellulose and hemicellulose provide the needed substrates for lignocellulosic biomass to be fermented into ethyl alcohol. These sugars are made available via the enzymatic hydrolysis of cellulose and hemicellulose. However, extracting cellulose and hemicellulose is difficult; lignin surrounds both components, inhibits enzyme access and thus reduces the biomass conversion efficiency and alcohol yield. Furthermore, lignin can adsorb cellulolytic enzymes (Saballos, 2008). Lignin content is among the most important factors in determining biomass conversion efficiency and alcohol yield (Chang and Holtzapple, 2000). Other compositional traits such as the acetylation of xylose, cellulose crystallinity, and lignin cross-linking also have importance (Chang and Holtzapple, 2000; Corredor et al., 2009; Grabber, 2005), but most research on biomass degradability and composition has focused on lignin.

Thermochemical pretreatments of biomass are used to remove or degrade lignin and this improves enzyme access to cellulose and hemicellulose. Pretreatments increase the costs and timing of biomass conversion into liquid fuels and are a significant barrier to their economic viability (Wyman, 2007). Multiple studies have demonstrated in sorghum and other crops that reducing biomass lignin contents improves degradability and glucose yields both with and without pretreatments (Chang and Holtzappple, 2000; Dien, et al., 2009; Grabber, 2005; Saballos et al., 2008; Sarath et al., 2008). Research to determine the role of lignin composition on biomass degradability has been inconclusive. Some have found important effects (positive and negative) while others have not (Chang and Holtzapple, 2000; Corredor, et al., 2009; Dien et al., 2009; Grabber, 2005; Saballos et al., 2008).

With these factors in mind, breeding biomass crops with altered compositional profiles to improve conversion efficiencies and alcohol yield has been proposed (Dhugga, 2007; Pauly and Keegstra, 2008; Wyman, 2007). Burns et al. (2009) released a switchgrass cultivar selected for improved conversion efficiency. In sorghum, breeding for compositional traits has been successful, but with animal palatability and nutrition in mind (Pederson and Fritz, 2000). An example would be the development of low lignin brown-midrib (*bmr*) sorghums (Cherney et al., 1991).

Significant variability for compositional traits has been identified in several crops, indicating their potential alteration via breeding selections (Lorenz et al., 2009; Cassida et al., 2005; Dien et al., 2006). In sweet sorghums, Corn (2009) reported glucan concentrations (cellulose proxy) ranging from 24.7% to 38.5%, xylans (hemicellulose

proxy) from 8.5% to 13.9% and lignin from 9.3% to 13.0%. In a grain sorghum x sweet sorghum RIL population, Murray et al. (2008) reported cellulose content ranging from 29% to 46%. Additionally, several brown midrib (*bmr*) mutants with unique modes of action and effects on lignin content are known in sorghum (Cherney et al., 2001; Saballos et al., 2008).

Despite the many proposals to alter biomass composition, a consensus on compositional ideotypes for biofuels is currently unavailable. The most interest focuses on reducing lignin content (Li et al., 2008; Sattler et al., 2010; Chapple et al., 2007; Chen et al., 2007; Lewis et al., 2010). However, lignin is an important component of plant fitness with roles in water transport, defense, and structural integrity. Lignin is also very energy dense and can itself provide energy or co-products. Reductions in lignin have been associated with reduced forage and grain yield, increased animal digestibility and weight gain, and increased lodging (Pederson et al., 2005; Pauly and Keegstra, 2008). However, the negative effects of reduced lignin may be ameliorated through the use of specific genes or genetic backgrounds (Sattler et al., 2010; Pederson et al., 2005; Oliver et al., 2005a, b). Modifying other compositional traits such as cellulose crystallinity or phenolic cross-linking may also improve biofuels production. However, such changes will likely elicit the same concerns as lignin modification. A consensus on composition will likely remain unavailable until large-scale biomass biofuels production provides practical experience.

Regardless of the composition breeding goals established, the traits selected for improvement will almost certainly be subject to genotype x environment interactions. A

GxE interaction is when the relative performance of genotypes differs across environments (Bernardo, 2002). An example would be the differential response of genotypes in lignin content across environments. The type and magnitude of GxE interactions influences criteria such as breeding and evaluation methods, genotype recommendations, processing optimization, and the ability to forecast energy yields. A trait with a smaller GxE interaction will be easier to modify by requiring less testing, facilitating the interpretation of genotypic effects, and producing more stable and adaptable results. Also, a smaller GxE interaction would facilitate optimization of biofuel production by providing a more consistent feedstock.

Prior research has shown that variation for compositional traits and their GxE interactions are significant, but smaller than variation for yield or environmental effects (Buxton and Cassler, 1993, Murray et al., 2008; Templeton et al., 2009). However, small effects are critical when large-scale biomass processing occurs. For example, an increase of sorghum whole-plant glucan content from 34% to 35% increases theoretical ethanol yields from 301.7 L to 307.5 L per dry Mg of biomass (USDOE, 2009). Assuming a yield of 20 Mg ha^{-1} of dry biomass, this results in an additional 116 L of ethanol per ha.

Published research examining sorghum GxE interactions for compositional traits relevant to biofuels is limited. Significant compositional trait GxE interactions have been identified in sweet sorghums and grain sorghum x sweet sorghum RILs (Corn, 2009; Murray et al., 2008). But data examining GxE interactions for compositional

traits in a wider diversity of sorghums, including high-biomass sorghums, are currently unavailable.

Data for sorghum compositional trait GxE interactions would aid researchers in determining the feasibility of modifying these traits via breeding, provide insight into testing requirements, estimate the potential stability and adaptability of modifications, and estimate the breeding resources required. Ultimately, the relative size and type of GxE interactions could be established and taken into consideration when establishing compositional breeding goals. With these factors in mind, the objectives of this research are to:

1. Estimate the quantity of cellulose, xylan (a proxy for hemicellulose), and lignin in a diverse array of sorghums, including high-biomass sorghums, from multi-environment trials using Near Infrared Spectroscopy (Hames, 2003).
2. Identify the presence, magnitude and patterns of genotype x environment interactions for cellulose, xylan, and lignin content in the sorghums studied.

Materials and Methods

Fifteen sorghum entries representing high-biomass hybrids, a grain sorghum, and various forage sorghums (photoperiod-sensitive, photoperiod-insensitive, *bmr* types, and sweet) were evaluated in field trials across five environments in 2008; Halfway, TX; Corpus Christi, TX; Weslaco, TX; and an irrigated and a dryland nursery in College

Station, TX (Table 5.1). Although fifteen entries were used total, only 12 at a time were evaluated within an environment.

Table 5.1 Sorghum genotypes evaluated across five environments for biomass compositional traits and yield. Genotypes include photoperiod-sensitive (PS) types, photoperiod-insensitive (PI) types, and brown midrib (bmr) mutants. Not all genotypes were evaluated in every environment as indicated by missing Xs in the columns

Genotype	Type	Weslaco	Corpus Christi	College Station Irrigated	College Station Dryland	Halfway
TAMUXH8001	High-biomass hybrid	X	X	X	X	X
TAMUXH8002	High-biomass hybrid	X	X	X	X	X
TAMUXH8003	PS forage	X	X	X	X	X
TAMUXH8004	PS forage	.	.	X	X	X
TAMUXH8005	PS sudangrass	X	X	X	X	X
TAMUXH8006	PS sudangrass	.	.	X	X	X
TAMUXH8007	PI sudangrass	.	.	X	X	X
Sugar T	Sweet forage	X	X	X	X	X
Graze-n-Bale	PS forage	X	X	X	X	X
GrazeAll 3	PI sudangrass	X	X	X	X	X
22053	PS forage (bmr)	X	X	X	X	X
84G62	Grain sorghum	X	X	X	X	X
R07008	High-biomass pollinator	X	X	.	.	.
R07018	High-biomass pollinator	X	X	.	.	.
R07020	High-biomass pollinator	X	X	.	.	.

A randomized complete block design with four replications was used. Plots consisted of four rows, each 6.7 meters long with 75 cm row spacing. Plots were planted in mid-February in Weslaco, mid-March in Corpus Christi and College Station, and late May in Halfway with 3 grams of seed per row. Seed weight varied per entry and thus plot plant populations also varied. Plots were managed with standard sorghum agronomic practices including fertilization, cultivation, and pesticide and herbicide

applications. Supplemental irrigation was provided in Halfway (55 cm), Weslaco (26.2 cm), and in the irrigated College Station environment (15 cm).

Plots were harvested for biomass yield in mid to late October. The entire lengths of the two middle rows of each plot were harvested. Plots in College Station were harvested by a self-propelled forage harvester with a row crop head. Harvested material was collected in a silage wagon modified with an Avery Weigh-Tronix system with weigh spindles to obtain plot weights. Plots in Halfway, Weslaco, and Corpus Christi were harvested by a tractor-mounted PTO-driven forage harvester with a row crop head. Harvested material was collected in a suspended bucket mounted to the forage harvester modified with an Avery Weigh-Tronix system with a weigh bar underneath the bucket to collect plot weights.

For each plot, a ~500g sample of harvested material was immediately taken at harvest, placed in a paper bag, and weighed. The samples were oven dried at 50° C for approximately one week and then weighed to estimate dry weight. Samples were then prepared for near-infrared spectroscopy analysis using procedures described by the NREL Laboratory Analytical Procedures (Hames et. al, 2008) for NIR biomass sample preparation. This included grinding dry samples in a Wiley mill to pass through a 2mm sieve and afterwards placing the ground samples in sealed plastic bags with silica gel moisture absorbing packets for storage.

NIR spectra from 400 to 2500nm were collected for each sample using a FOSS XDS Rapid Content Analyzer and scanning operations were managed with ISIscan v.3.1 (Infrasoft International). An NIR predictive model developed by the National

Renewable Energy Laboratory and Texas A&M University was used to estimate the compositional profile of the samples based on their NIR spectra. This model is based on a calibration set of over 100 diverse sorghum samples analyzed for cell wall composition with an Uppsala dietary fiber analysis. The R^2 values for the predictive ability of this model were 0.93 for lignin, 0.71 for xylans (hemicellulose proxy), and 0.79 for cellulose. Results of this analysis were reported as percentages of ash, protein, sucrose, lignin, xylan, cellulose, water extractable sugars, and ethanol extractable sugars

The estimated lignin, xylan, and cellulose contents obtained were analyzed individually using ANOVAs, rank correlations, and additive main effects and multiplicative (AMMI) analyses. For analyses across environments, a reduced dataset that only included entries represented across environments was used. ANOVAs with all random effects and rank correlations were performed with SAS 9.1 using the PROC MIXED and PROC CORR procedures, respectively. Two-component AMMI analyses were performed using IRRISTAT 5.0 (IRRI, 2005).

Results and Discussion

Lignin

Across environments, the mean lignin content was 13.4 % (Table 5.2). Weslaco was the environment with the highest mean lignin content (16.5%) and Halfway had the lowest mean lignin content (10.6 %). These values are consistent with those reported in sweet sorghums by Corn (2009) but Murray et al. (2008) reported a lower average lignin content of 6.0% for stems in a sweet sorghum x grain sorghum RIL population.

However, comparisons are complicated by the use of different NIR predictive models in each study. In maize stover, average lignin contents of 11.6% and 13.3% have been reported (Lorenz et al., 2009; Templeton et al., 2005). Among genotypes, lignin concentrations ranged from a low of 6.9% to a high of 20.1%. When analyzed within environments, a highly significant genotype effect was observed for each environment in ANOVAs (Table A.1).

Large and frequent rank shifts for lignin concentrations were observed for several genotypes between the environments (Table 5.3). For example, genotype 11 had the 3rd highest mean lignin content in Weslaco but the lowest mean lignin content in Corpus Christi. Rank correlations between the environments were non-significant except for Weslaco and the College Station dryland environment. These two environments had a highly significant and moderately large rank correlation of 0.81. The size and frequency of the rank shifts and the overall lack of significant rank correlations are evidence of significant genotype x environment interactions for lignin.

Table 5.2 Average lignin, xylan, and cellulose contents with biomass yield (Mg ha^{-1}) for a diverse set of sorghum genotypes. Additionally, their standard deviation, and range for each value are presented. Genotypes were evaluated in 5 environments: Weslaco, TX; Corpus Christi, TX; Halfway, TX; and an irrigated and dryland environment in College Station, TX.

Environment	Mean % Lignin†	SD	Min	Max
Weslaco	16.5 a	1.6	14.1	20.1
Corpus Christi	14.6 b	1.0	12.3	16.6
College Station-Irrigated	14.4 b	0.9	12.3	16.5
College Station-Dryland	11.2 c	1.0	9.4	13.8
Halfway	10.6 c	1.7	6.9	13.9
Across Locations	13.4	2.5	6.9	20.1

Environment	Mean % Xylan†	SD	Min	Max
Weslaco	17.7 a	0.9	16.26	18.79
Corpus Christi	17.6 a	0.6	16.4	18.98
College Station-Irrigated	16.9 b	0.5	15.76	18.02
College Station-Dryland	16.2 c	0.5	14.84	17.21
Halfway	15.7 d	0.8	14.33	17.35
Across Locations	16.8	1.0	14.33	18.98

Environment	Mean % Cellulose†	SD	Min	Max
Weslaco	29.5 a	2.1	25.3	33.0
Corpus Christi	28.8 a	1.7	24.8	32.1
College Station-Irrigated	26.6 b	1.8	22.6	30.0
College Station-Dryland	26.1 b c	1.7	21.4	29.7
Halfway	25.2 c	2.5	21.5	30.7
Across Locations	27.3	2.6	21.4	33.0

Environment	Mean Biomass Yield (Mg ha^{-1})†	SD	Min	Max
Weslaco	21.2 a	6.0	8.8	38.2
Corpus Christi	17.1 b	6.9	3.3	29.7
College Station-Irrigated	15.2 b	5.4	6.3	27.7
College Station-Dryland	15.2 b	6.3	5.3	25.5
Halfway	10.1 c	5.2	2.2	25.0
Across Locations	16.0	6.9	2.2	38.2

† Means sharing the same letter are not different at $\alpha = 0.05$

Table 5.3 Lignin content (%) and ranks of 15 sorghum genotypes evaluated in 5 environments; Weslaco, TX; Corpus Christi, TX; Halfway, TX; and irrigated and dryland environments in College Station, TX. Decreasing rank values represent lower mean percent lignin values.

					College Station		College Station				Across
	Weslaco		Corpus Christi		Irrigated		Dryland		Halfway		Locations
Genotype	Lignin	Rank	Lignin	Rank	Lignin	Rank	Lignin	Rank	Lignin	Rank	Lignin
TAMUXH8001	15.1	1	14.3	6	13.6	1	10.8	5	11.3	7	13.1
TAMUXH8002	16.4	7	14.0	4	14.6	8	10.2	1	11.0	6	13.0
TAMUXH8003	15.9	5	14.3	5	14.4	6	10.8	4	10.2	5	13.1
TAMUXH8004	.	.	.	.	15.1	12	10.8	6	11.5	9	12.5
TAMUXH8005	15.4	2	13.9	3	14.3	5	11.0	7	11.6	10	13.2
TAMUXH8006	.	.	.	.	13.9	3	10.4	2	12.4	11	12.3
TAMUXH8007	.	.	.	.	15.0	11	11.9	10	13.2	12	13.4
Sugar T	17.0	9	13.8	2	14.5	7	10.6	3	8.7	2	12.4
Graze-n-Bale	15.7	4	15.0	9	15.0	10	11.4	8	11.4	8	13.7
GrazeAll 3	18.7	11	15.7	11	14.9	9	12.8	12	8.3	1	14.0
22053	17.3	10	12.8	1	13.7	2	11.7	9	9.5	4	13.0
84G62	18.9	12	15.2	10	14.0	4	12.6	11	8.8	3	13.9
R7008	16.0	6	14.7	7	.	.	.	.	.	.	15.4
R7018	15.7	3	14.9	8	.	.	.	.	.	.	15.3
R7020	16.5	8	15.8	12	.	.	.	.	.	.	16.1
Average	16.6		14.6		14.4		11.3		10.6		13.4

An ANOVA analysis for the lignin data across environments identified a highly significant environmental effect and genotype x environment interaction (Table 5.4). Most of the variability for lignin content can be attributed to the differences between environments as evidenced by the relative size of the environmental effect mean square. These results are consistent with what others have reported, highlighting the predominant role of the environment in sorghum biomass and maize stover lignin variability (Corn, 2009; Murray et al., 2008; Templeton et al., 2005).

The first interaction principle component axis (IPCA) of an AMMI analysis of the data was highly significant and accounted for 76.5% of the interaction variance while a second IPCA accounted for 11.0%. An AMMI-2 plot is presented to interpret the GxE

interaction (Figure 5.1). In this plot, the College Station environments and Corpus Christi were grouped together. Weslaco and Halfway are at extremes from the origin and represent the environments with the most unique genotypic evaluations. Considering that Halfway is the only truly temperate environment represented and Weslaco was buffeted by Hurricane Dolly in July of 2008, these results seem congruous.

Per these results, 3 of these environments would be required to obtain accurate evaluations of relative lignin content; Halfway, Weslaco, and an environment in College Station or Corpus Christi. However, these results are based on a single year of data, and multiple years are needed for their confirmation.

Several of the genotypes demonstrate specific adaptations in the AMMI-2 plot. For example, because of its distance in the biplot, genotype 10 would be superior in Halfway for low lignin while not in the other environments. Genotype 6 has a more specific adaptation for low lignin in the College Station-Corpus Christi environments

Table 5.4 Analysis of variance results for 15 sorghum genotypes evaluated for lignin, xylan, and cellulose content across 5 environments. All effects are random.

	Lignin		Xylan		Cellulose	
Variance Source	df	MS	df	MS	df	MS
Genotype	14	3.8	14	1.6*	14	15.2*
Environments	4	246.6**	4	32.5*	4	155.3*
Rep (Env)	15	1.4**	15	0.37*	15	3.8*
G x E	41	4.4**	41	0.81**	41	7.8**
Error	151	0.6	151	0.17	151	1.8

* Statistically significant at $\alpha = 0.05$
** Statistically significant at $\alpha = 0.01$

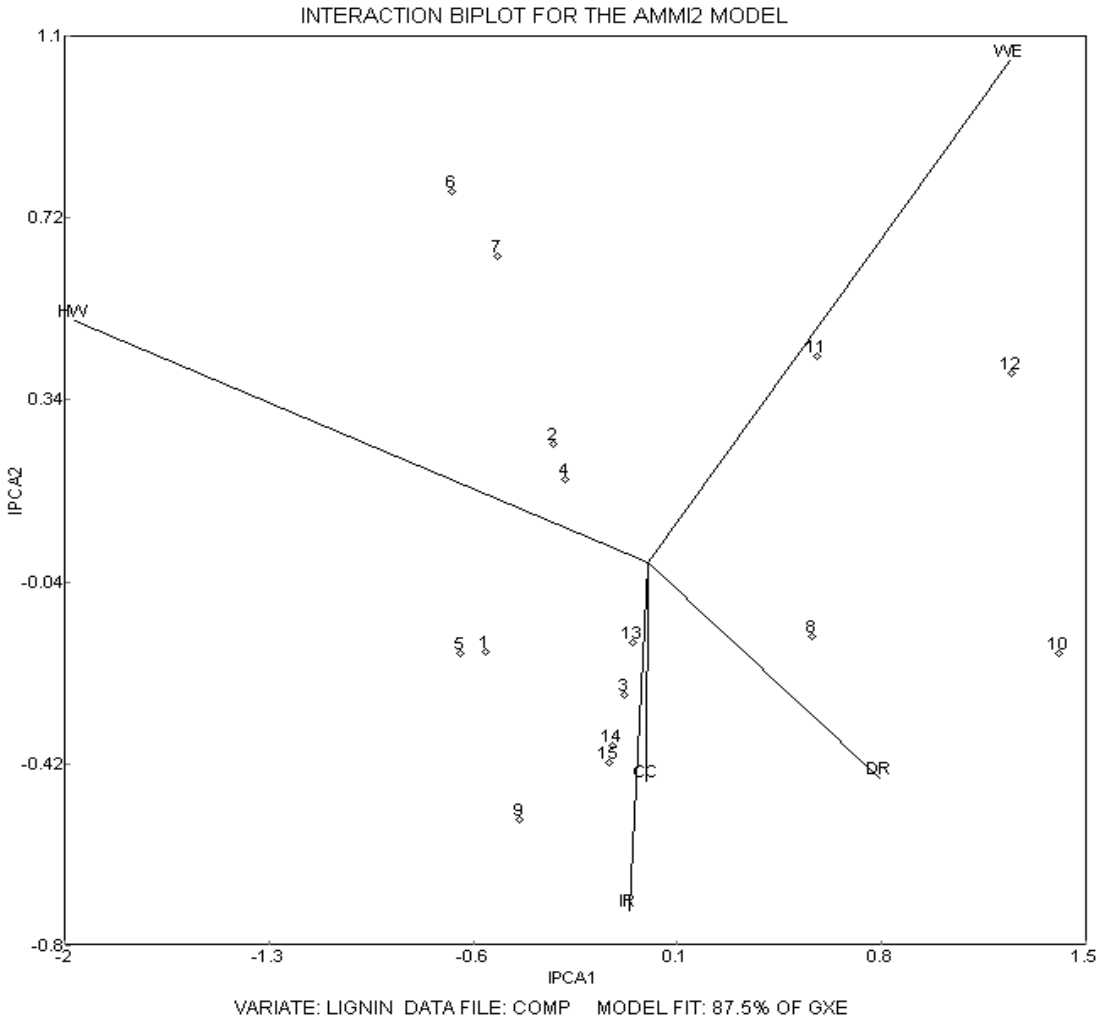

Figure 5.1 AMMI-2 plot (biplot of IPCA1 x IPCA2 scores) of 15 sorghum genotypes and 5 environments analyzed in a two-component AMMI analysis for lignin content. Environments are represented by their letter abbreviations and genotypes by numerals.

rather than for Halfway. Genotypes with relatively stable lignin rankings, such as genotype 3, can also be identified in the AMMI-2 plot. Genotype 3 was evaluated in all of the environments and is relatively close to the plot origin. These specific and general adaptations are confirmed in the rankings (Table 5.3).

In an AMMI-1 plot that simultaneously displays mean lignin content with specific and general genotypic adaptations, Weslaco and Halfway represent the extremes for lignin evaluations as in the AMMI-2 plot (Figure 5.2). College Station-dryland has greater distance from College Station-irrigated and Corpus Christi in the AMMI-1 plot, but this seems largely due to its lower mean lignin. The AMMI-1 plot also confirms several of the specific adaptations, such as those of genotypes 10 and 6. Genotypes 3, 14, 13, 2, and 1 appear to combine rank stability with lower lignin values. Indeed, these genotypes are consistently in the lower half of the ranks for lignin content across the environments they are evaluated in (Table 5.3).

The overall results of the data indicate that significant genotype x environment interactions exist for lignin content in sorghum. The presence of significant GxE interactions for lignin content are consistent with what others have reported in other sorghums and maize stover (Corn, 2009; Murray et al., 2008; Templeton et al., 2005). Differences between the environments for their relative genotypic evaluations were identified, with two environments, Weslaco and Halfway producing the most unique results. Genotypes with general adaptations and specific adaptations were identified. Genotypes with consistently lower lignin across environments were identified.

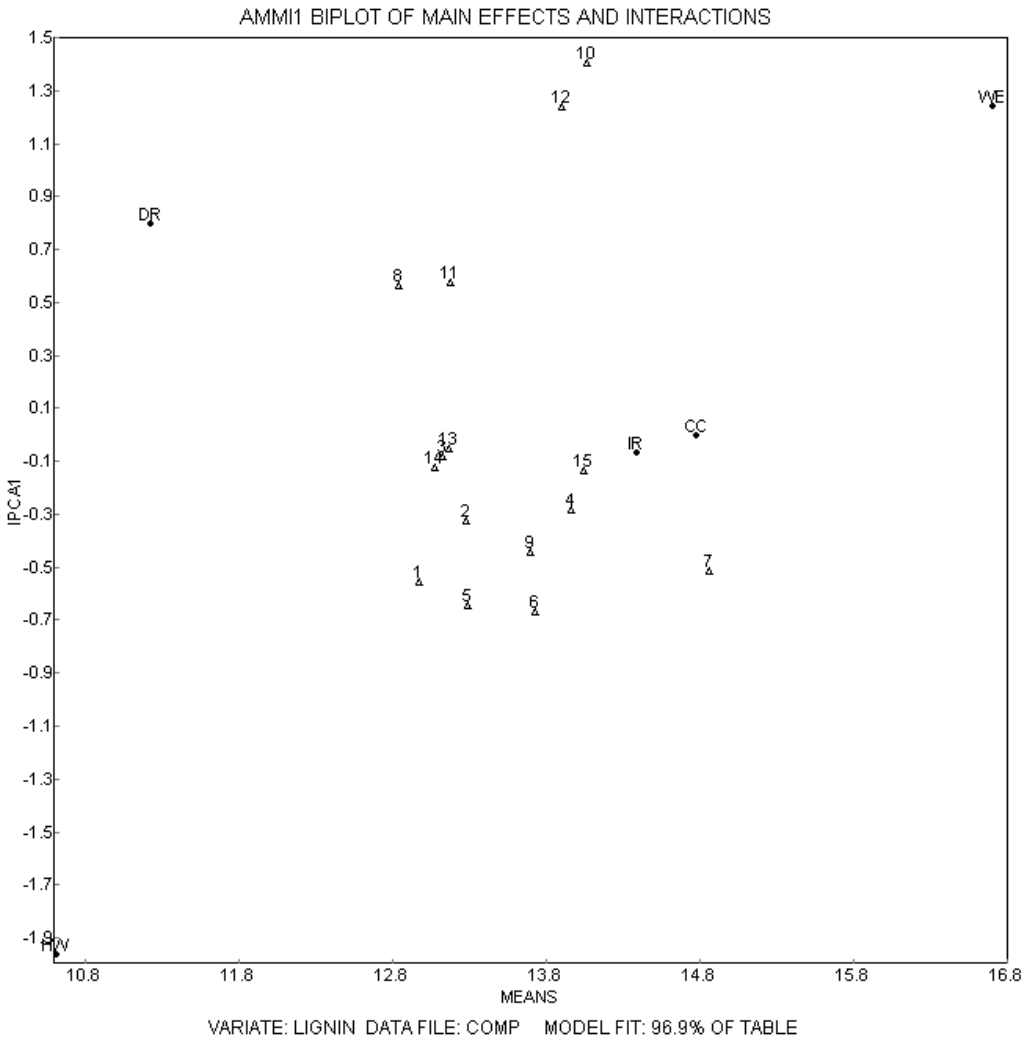

Figure 5.2 AMMI-1 plot (biplot of IPCA 1 scores x mean percent lignin) of 15 genotypes and 5 environments analyzed for lignin content. Environments are represented by their letter abbreviations and genotypes by numerals.

Xylan

Across the environments, the mean xylan content of the entries was 16.8% (Table 5.2). College Station irrigated was the environment with the highest mean xylan content (17.71%) and Halfway had the lowest mean xylan content (15.73%). These xylan values are higher than those reported by Corn (2009) for sweet sorghums (8.5%-13.9%) but less than the reported mean hemicellulose content of 23.1% in Murray et al. (2008) for the stems of sweet sorghum x grain sorghum RILs. In maize stover, average xylan contents of 18.9% and 17.7% have been reported (Lorenz et al., 2009; Templeton et al., 2005). Individual xylan values ranged from a low of 14.3% to a high of 18.9%. Xylan values exhibited less variability than lignin or cellulose values. Templeton et al. (2005) and Corn (2009) reported xylan values with less variability then cellulose or glucans but slightly more variability than lignin content in maize stover. When analyzed within environments, a highly significant genotype effect was observed in ANOVAs for each environment except College Station irrigated (Table A.2).

Prominent rank shifts for xylan values were observed between the environments for several genotypes (Table 5.5). Rank correlations between the environments were all non-significant. The presence of large rank shifts combined with the lack of significant rank correlations are indicative of significant genotype x environment interactions for xylan.

Table 5.5 Xylan content (%) and ranks of 15 sorghum genotypes evaluated in 5 environments; Weslaco, TX; Corpus Christi, TX; Halfway, TX; and irrigated and dryland environments in College Station, TX.

					College Station		College Station				Across
	Weslaco		Corpus Christi		Irrigated		Dryland		Halfway		Locations
Genotype	Xylan	Rank	Xylan	Rank	Xylan	Rank	Xylan	Rank	Xylan	Rank	Xylan
TAMUXH8001	16.8	12	16.7	10	17.2	11	16.1	7	15.9	5	16.6
TAMUXH8002	17.6	7	16.8	7	17.5	8	15.8	10	15.9	6	16.7
TAMUXH8003	17.2	10	16.9	6	17.6	7	15.9	9	15.3	8	16.6
TAMUXH8004	.	.	.	.	18.2	2	16.0	8	16.1	3	16.7
TAMUXH8005	16.9	11	16.3	11	17.3	10	16.3	6	15.9	4	16.6
TAMUXH8006	.	.	.	.	17.2	12	15.6	12	16.9	2	16.6
TAMUXH8007	.	.	.	.	18.3	1	16.6	3	17.1	1	17.4
Sugar T	17.5	8	16.7	8	18.0	3	15.7	11	14.5	12	16.5
Graze-n-Bale	17.4	9	17.1	5	17.9	5	16.4	5	15.8	7	16.9
GrazeAll 3	18.4	1	17.5	1	18.0	4	17.0	1	15.3	9	17.2
22053	17.8	5	16.2	12	17.7	6	16.4	4	15.1	10	16.7
84G62	17.8	6	16.7	9	17.4	9	16.7	2	14.9	11	16.7
R7008	18.0	3	17.3	4	.	.	.	.	.	.	17.6
R7018	17.8	4	17.5	2	.	.	.	.	.	.	17.7
R7020	18.4	2	17.4	3	.	.	.	.	.	.	17.9
Average	17.6		16.9		17.7		16.2		15.7		16.8

An ANOVA analysis for the xylan data across locations identified a significant genotype effect and a highly significant environmental effect and genotype x environment interaction (Table 5.4). Most of the variability for xylan content can be attributed to the differences between environments as evidenced by the relative size of the environmental effect mean square. As with lignin, others have also reported the predominant role of the environment in xylan or hemicellulose variability in sorghum biomass and maize stover (Corn, 2009; Murray et al., 2008; Templeton et al., 2005).

The first interaction principle component axis (IPCA) of an AMMI analysis of the data was highly significant and accounted for 68.7% of the interaction variance and a

second IPCA accounted for 16.9%. An AMMI-2 plot is presented to interpret the GxE interaction (Figure 5.3). In the AMMI-2 plot, the environments formed three general clusters. Weslaco, Corpus Christi, and the College Station irrigated environment grouped together while Halfway and College Station dryland remained distinct. Given these results, accurate evaluation of genotypes for xylan content would require evaluation in a temperate environment (Halfway), and two subtropical environments, each differing for water stress levels. But as with the lignin data, these results are based on a single year of data and would require more years for their confirmation.

Also like the lignin data, several of the genotypes demonstrate specific adaptations in the AMMI-2 plot. If the production of high xylan is desired, genotype 6 has a specific adaptation to Halfway and genotype 8 has a specific adaptation to College Station irrigated. For stability, genotypes 9, 3, and 2 are near the biplot origin and their stability can be confirmed in the rankings. Genotypes 13, 14, and 15 are also near the biplot origin, but they were evaluated in only two of the five environments.

In an AMMI-1 plot to simultaneously identify mean xylan content with specific and general genotypic adaptations, Halfway and College Station dryland represent the extremes for xylan evaluations (Figure 5.4). Although it appears that the separation of College Station-dryland is largely based on differences for mean xylan content. The AMMI-1 plot confirms several of the specific adaptations, such as genotype 6 with Halfway. Genotypes 9 and 4 seem to combine moderate xylan values with stability and genotypes 3 and 2 combine lower xylan content with stability.

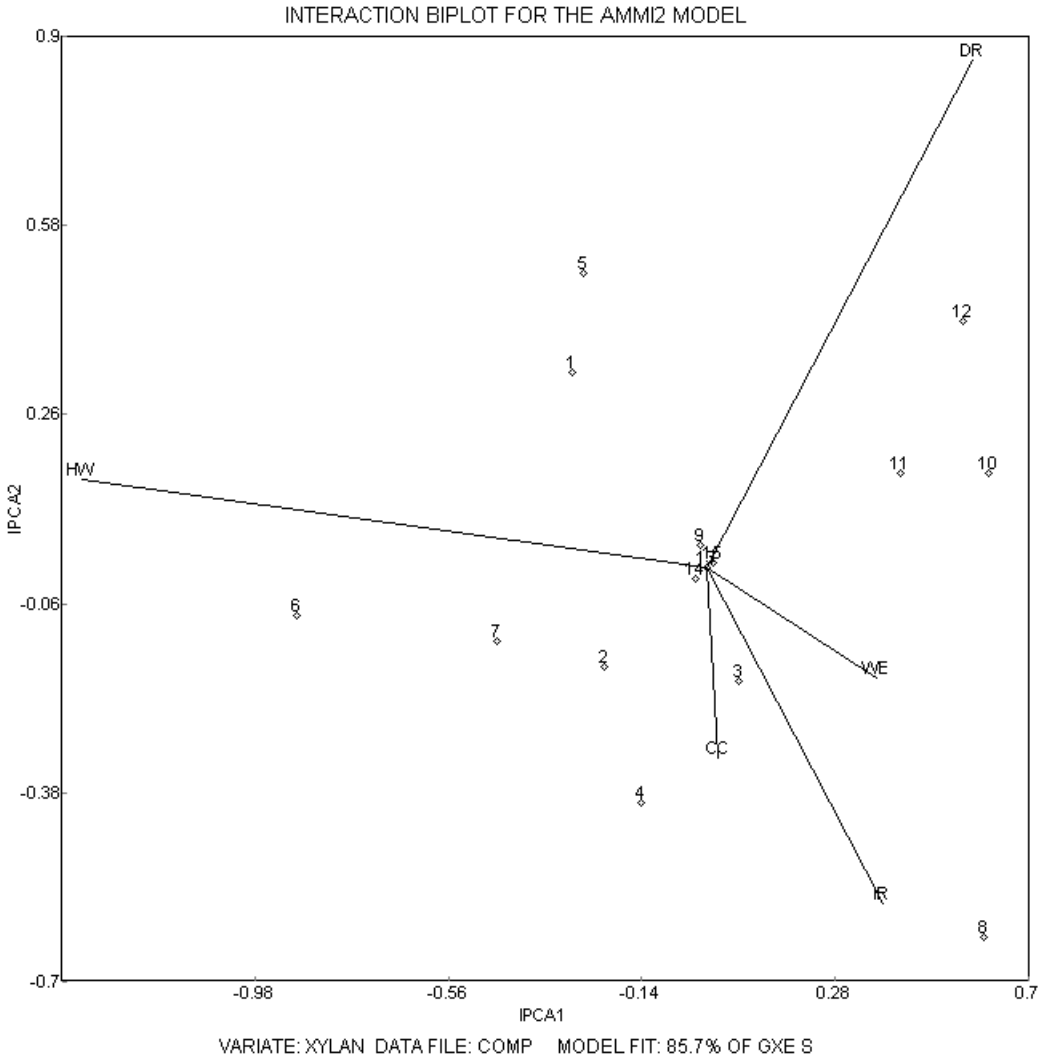

Figure 5.3 AMMI-2 plot (biplot of IPCA 1 x IPCA 2 scores) of 15 sorghum genotypes and 5 environments analyzed in a two-component AMMI analysis for xylan content. Environments are represented by their letter abbreviations and genotypes by numerals.

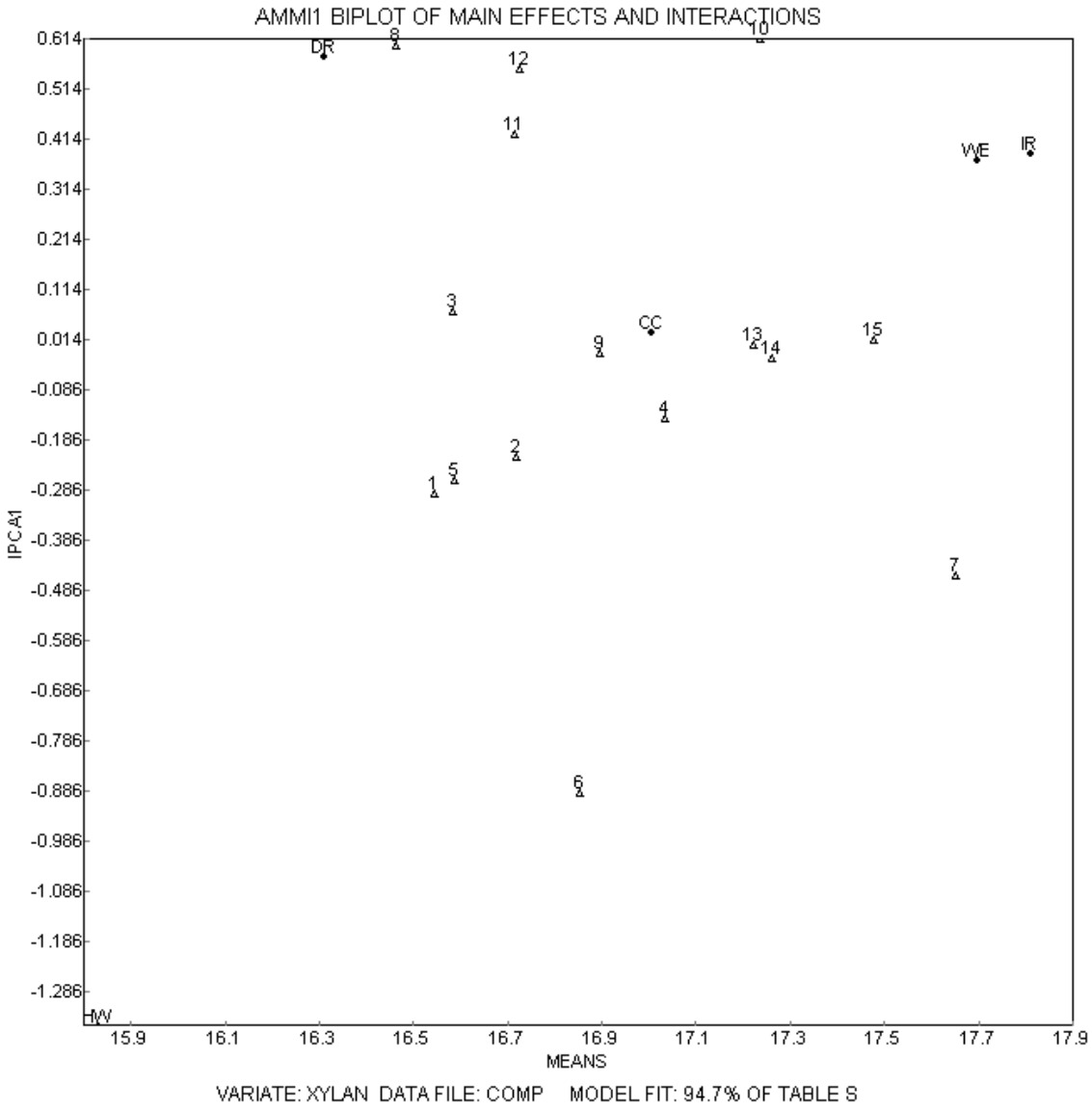

Figure 5.4 Biplot of AMMI analysis IPCA 1 scores and mean percent xylan content of 15 genotypes in 5 environments analyzed for xylan content. Environments are represented by their letter abbreviations and genotypes by numerals.

Cellulose

Across the environments, the mean cellulose content of the entries was 27.3% (Table 5.2). Entries in the College Station irrigated environment had the highest mean cellulose content (29.51%) while entries in the Halfway environment had the lowest mean cellulose content (25.25%). These estimates of cellulose content are lower than the 35.7% average cellulose content reported by Murray et al. (2008) for the stems of sweet sorghum x grain sorghum RILs and lower than the average glucan contents (24.7% - 38.5%) reported by Corn (2009) in sweet sorghums. As was stated with lignin, the use of different NIR predictive models complicates these comparisons. In maize stover, Lorenz et al. (2009) reported average cellulose contents of 36.8% and Templeton et al. (2005) reported average glucan content of 31.9%. Individual cellulose values ranged from a low of 21.4% to a high of 33.0%. When analyzed within environments, a highly significant genotype effect was observed in ANOVAs for each environment except College Station irrigated (Table A.3).

Prominent rank shifts for cellulose values can be observed between the environments for several genotypes (Table 5.6). Rank correlations between the environments were all non-significant except for a moderately large (0.81) and highly significant rank correlation between Weslaco and College Station dryland. The size and frequency of the rank shifts and the overall lack of significant rank correlations are evidence of significant genotype x environment interactions for cellulose.

Table 5.6 Cellulose content (%) and ranks of 15 sorghum genotypes evaluated in 5 environments; Weslaco, TX; Corpus Christi, TX; Halfway, TX; and irrigated and dryland environments in College Station, TX. Decreasing rank values represent lower mean percent lignin values.

					College Station		College Station				Across
	Weslaco		Corpus Christi		Irrigated		Dryland		Halfway		Locations
Genotype	Cellulose	Rank	Cellulose	Rank	Cellulose	Rank	Cellulose	Rank	Cellulose	Rank	Cellulose
TAMUXH8001	26.0	12	25.8	7	27.8	12	25.4	8	25.6	1	26.1
TAMUXH8002	28.2	10	25.5	9	28.2	10	24.4	11	25.2	7	26.3
TAMUXH8003	28.2	9	27.2	6	29.0	7	25.2	10	24.6	9	26.8
TAMUXH8004	.	.	.	.	31.3	2	25.3	9	25.5	6	27.4
TAMUXH8005	28.1	11	25.3	10	28.2	8	26.0	6	25.6	4	26.6
TAMUXH8006	.	.	.	.	28.2	9	23.9	12	28.9	2	27.0
TAMUXH8007	.	.	.	.	31.8	1	27.8	3	29.4	1	29.7
Sugar T	28.7	7	25.8	8	30.5	3	25.4	7	21.9	12	26.4
Graze-n-Bale	28.3	8	27.2	5	30.2	6	26.8	5	26.1	3	27.7
GrazeAll 3	31.2	1	28.2	2	30.4	4	28.5	1	22.9	10	28.2
22053	30.8	2	24.9	11	30.3	5	28.1	2	24.8	8	27.7
84G62	28.8	6	24.8	12	28.2	11	27.2	4	22.7	11	26.3
R7008	29.4	4	27.5	4	.	.	.	.	.	.	28.4
R7018	28.9	5	27.7	3	.	.	.	.	.	.	28.3
R7020	30.0	3	28.2	1	.	.	.	.	.	.	29.1
Average	28.9		26.6		29.5		26.2		25.2		27.3

An ANOVA analysis for the cellulose data across locations identified a significant genotype effect and a highly significant environmental effect and genotype x environment interaction (Table 5.4). Most of the variability for cellulose content can be attributed to the differences between environments as evidenced by the relative size of the Environment effect mean square. As with lignin and xylan, others have also reported the predominant role of the environments in cellulose or glucan variability (Corn, 2009; Murray et al., 2008; Templeton et al., 2005).

The first interaction principal component axis (IPCA) of an AMMI analysis of the data was highly significant, accounting for 68.7% of the interaction variance and a second IPCA accounted for 16.9%. An AMMI-2 plot is presented interpret the GxE interaction (Figure 5.5). In the AMMI-2 plot, the environments assorted themselves into three groups. Weslaco and College Station dryland grouped together as did Corpus Christi and College station irrigated, Halfway remained separate from all of the other environments. As has been stated, these results are based on a single year of data and more years will be required to confirm these results. Of special interest is whether the College Station irrigated environment continues to associate with the typically water-stressed environment of Corpus Christi. Establishing multi-year environmental patterns for compositional traits will aid in determining the role of environmental factors such as water availability in compositional variability. But given the results here, accurate evaluation of genotypes for cellulose content would require evaluation in a temperate environment (Halfway) and then at two subtropical environments, each differing for water stress levels.

Genotypic differences for specific and general adaptations can be identified in the AMMI-2 plot. For high cellulose content, genotypes 11 and 12 had specific adaptations to College Station dryland, genotype 8 with College Station irrigated and genotype 6 with Halfway. Genotype 10 was highly adapted to all of the environments except Halfway, where it had one of the lowest cellulose values. For stability, genotypes 9, 2, and 3 are near the biplot origin. These general and specific adaptations are

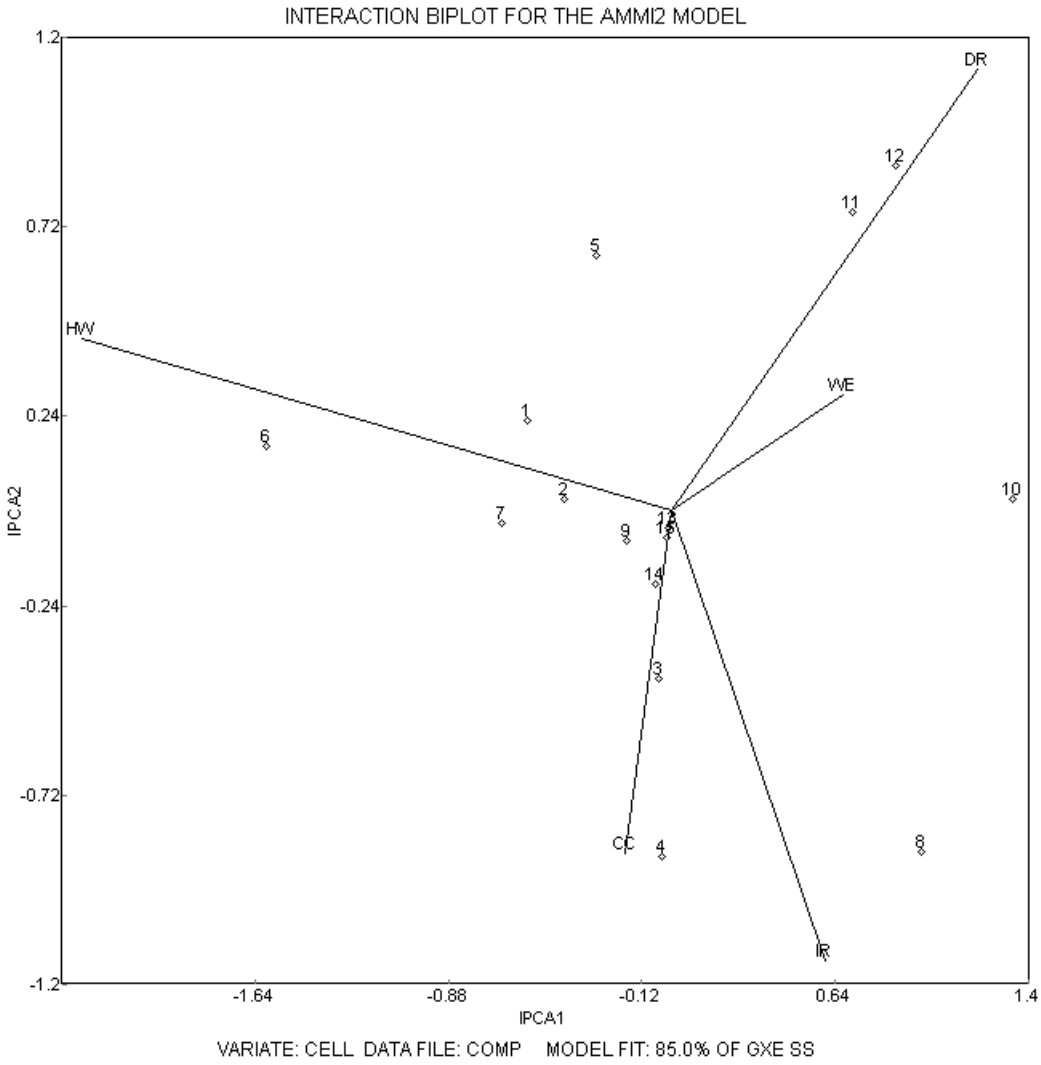

Figure 5.5 AMMI-2 plot (biplot of the IPCA 1 x IPCA 2 scores) of 15 sorghum genotypes and 5 environments analyzed in a two-component AMMI analysis for cellulose content. Environments are represented by their letter abbreviations and genotypes by numerals.

confirmed in the rankings (Table 5.6). Genotypes 13, 14, and 15 are also near the biplot origin, but they were evaluated in only two of the environments.

An AMMI-1 plot can simultaneously identify mean cellulose content with specific and general genotypic adaptations (Figure 5.6). In this plot, Halfway was again very distinct while the other environments appear to differ mostly for mean cellulose content. In the AMMI-1 plot, many of the specific and general adaptations of the AMMI-2 plot are reflected. Genotype 6 is again associated with Halfway and genotype 10 combines high mean cellulose content with adaptation to all of the environments except Halfway. Genotype 7 has high mean cellulose content with moderate stability, but because it was only evaluated in 3 of the 5 environments, its stability may not be entirely comparable. However, it maintained rank stability across disparate environments such as Halfway and College Station irrigated. Genotype 9 combines moderate cellulose content with stability. The confirmation of these patterns can be seen in the rankings of the genotypes (Table 5.6).

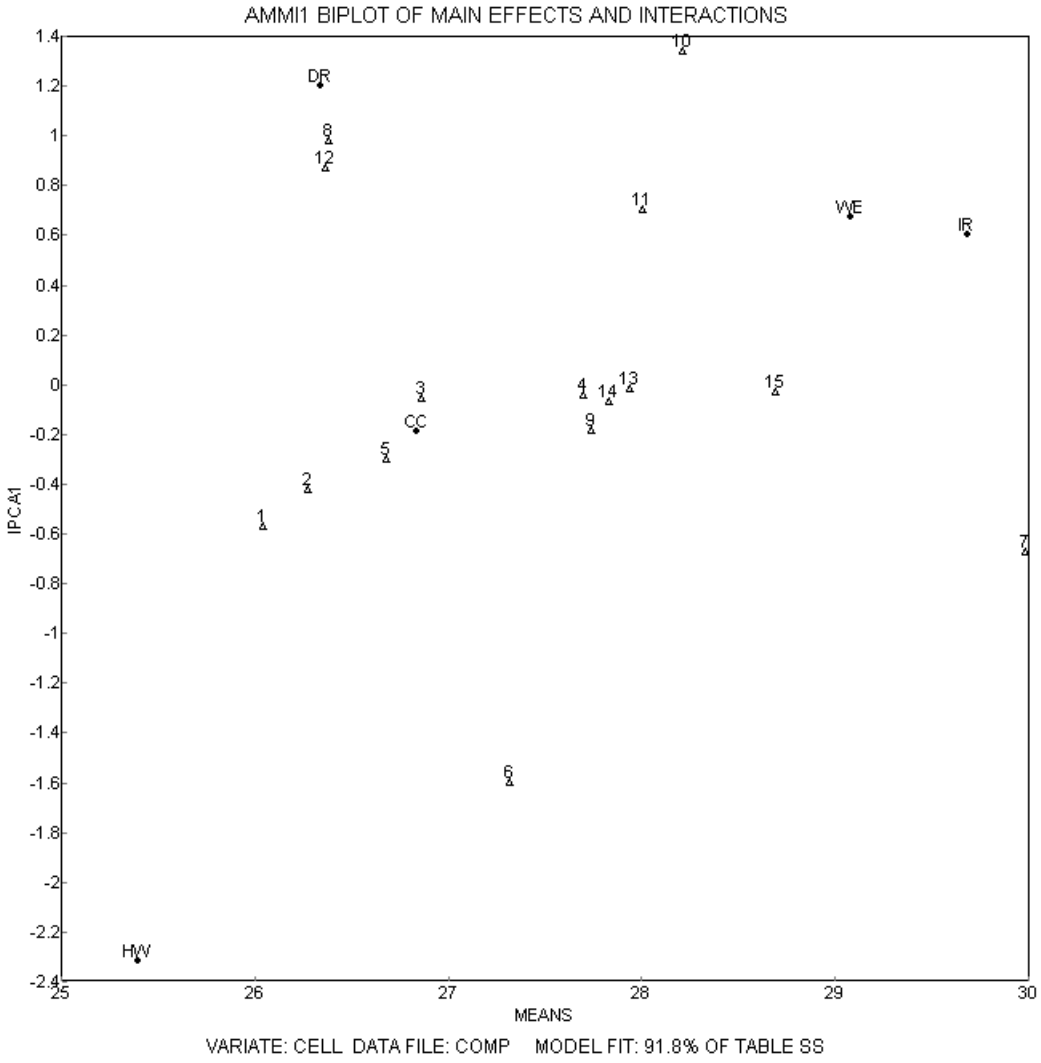

Figure 5.6 AMMI-1 plot (biplot of IPCA 1 scores x mean percent cellulose content) of 15 genotypes and 5 environments analyzed for cellulose content. Environments are represented by their letter abbreviations and genotypes by numerals.

The overall results of this data indicate that significant genotype x environment interactions exist for cellulose content in sorghum, particularly when considering the reactions of the genotypes in Halfway. The presence of significant GxE interactions for cellulose content are consistent with what others have reported in other sorghums and maize stover (Corn, 2009; Murray, 2008; Templeton 2005). Differences between the environments for their relative genotypic evaluations were identified. Differences between genotypes for adaptation (general and specific) were also identified.

Biomass Yield and Composition

As stated in the introduction, a consensus on compositional ideotypes has not been established. Thus the relative importance of the compositional differences seen here remains to be determined. But the importance of yield for reducing land and transportation requirements and thereby improving the economic viability and sustainability of biomass biofuels is well established (Granda et al., 2007; Towler et al., 2004). With this in mind, the biomass yields of the genotypes in this study are presented here. However, given the diverse array of sorghums used (ranging from high-biomass hybrids to grain sorghum) direct comparisons of genotypic yields are not presented

The genotypic means for dry biomass yields across environments ranged from a high of 21.5 Mg ha^{-1} to a low of 10.0 Mg ha^{-1} (Table 5.7). Halfway had the highest mean yield (21.3 Mg ha^{-1}) and Corpus Christi had the lowest mean yield (10.1 Mg ha^{-1}). The top three yielding genotypes averaged 19.9 Mg ha^{-1} of dry biomass across environments. The reported yields of current sorghums with high-biomass potential

range from 15.6 Mg ha^{-1} to 40.3 Mg ha^{-1} from many different environments (Habyarimana et al., 2004; McCollum et al., 2005; Rooney, 2007; Venuto and Kindiger, 2008). It should be noted that the genotypes used here have had only limited or no selection for total biomass production, including the high-biomass hybrids. For comparison, recent U.S. maize grain yields averaged approximately 9.3 Mg ha^{-1} (Egli, 2008) McCollum et al., (2005) reported average silage maize dry matter yields of 19.0 Mg ha^{-1} under irrigated conditions in the Texas Panhandle, and Burns et al. (2008) released a switchgrass cultivar with superior dry matter yields of 15.7 Mg ha^{-1}. GxE interactions for biomass yield were not identified in ANOVAs (Table A.4). But this was expected given that the inclusion of inherently lower and higher yielding genotypes would minimize GxE interactions.

Correlation analyses between the compositional traits and biomass yield within the genotypes were all non-significant. Thus, breeding for biomass yield and various altered compositional profiles may be plausible. Lewis et al. (2010) stated that simultaneous breeding for grain yield and stover quality could be achieved in maize. Burns et al. (2009) successfully released a switchgrass cultivar that had been selected for both superior yield and conversion efficiency. Among the limited genotypes evaluated here, several of the higher yielding genotypes combined high biomass yield with moderate lignin and cellulose levels (Tables 5.7, 5.3, and 5.6). Reduced lignin is generally associated with lower biomass yields (Pederson et al., 2005; Pauly and Keegstra, 2008), but this may vary by genetic background (Sattler et al., 2010; Pederson

Table 5.7 Dry biomass yields (Mg ha^{-1}) of genotypes evaluated in 5 environments over 1 year: Weslaco, TX; Corpus Christi, TX; Halfway, TX; and an irrigated and a dryland environment in College Station, TX.

					College Station		College Station				Across
	Weslaco		Corpus Christi		Irrigated		Dryland		Halfway		Locations
Genotype	Yield	Rank	Yield	Rank	Yield	Rank	Yield	Rank	Yield	Rank	Yield
TAMUXH8001	18.1	5	14.0	3	18.7	6	18.8	2	26.6	2	18.9
TAMUXH8002	16.3	7	16.2	1	21.9	3	15.2	6	24.3	5	19.1
TAMUXH8003	20.9	1	15.2	2	20.0	4	15.1	7	25.3	4	19.3
TAMUXH8004	.		.		19.5	5	19.5	1	16.7	10	18.5
TAMUXH8005	18.2	4	7.8	9	22.1	2	15.5	4	26.8	1	18.6
TAMUXH8006	.		.		26.6	1	15.3	5	22.6	6	21.5
TAMUXH8007	.		.		9.4	11	11.4	11	19.3	8	13.7
Sugar T	15.6	8	4.5	10	17.3	7	11.2	12	19.1	9	13.6
Graze-n-Bale	19.3	2	13.3	4	16.2	8	18.6	3	25.5	3	18.6
GrazeAll 3	7.2	12	4.2	11	8.8	12	13.7	8	15.3	11	10.1
22053	10.3	10	2.4	12	13.4	9	13.2	9	20.6	7	13.1
84G62	7.6	11	8.2	8	10.1	10	12.6	10	14.3	12	10.6
R7008	16.9	6	10.3	5	.		.		.		14.3
R7018	18.3	3	9.5	7	.		.		.		13.9
R7020	15.4	9	9.7	6	.		.		.		12.1
Average	15.3		9.6		17.0		15.0		21.4		15.7

et al., 2005; Oliver et al., 2005a, b). Given past success in altering composition for forage quality with yield, simultaneous improvement of composition and yield, while challenging, may be realistically achieved.

Conclusions

Fifteen diverse sorghum genotypes were evaluated in 5 Texas environments. Entries were harvested for biomass and their lignin, cellulose, and xylan (hemicellulose proxy) contents were estimated using Near-Infrared Spectroscopy. With this data, significant genotype x environment interactions were identified for each compositional

trait. However, most of the variability for composition was due to the differences between environments. Prominent rank shifts for each trait were readily observed for several genotypes. For each trait, the environments generally formed three groups, each providing distinct genotypic evaluations. The only truly temperate environment (Halfway, TX) was especially distinct. Future compositional breeding will require multi-environment testing for accurate evaluations. For each trait, genotypes with specific adaptations and more general adaptations were identified. Thus, genotypes with broader applicability or that maximize performance in specific environments can be identified for sorghum compositional traits.

The relative importance of the GxE interactions identified remains to be determined. Most of the differences between genotypes for each trait ranged from 1-3%. Large-scale biomass processing for fuels will determine the importance of these differences and the amount of breeding resources they warrant. Given that most of the compositional variability was due to the environments, altering composition through agronomy rather than breeding may yield more rapid initial progress. However, environmental effects governing composition and their control remain to be determined.

Regardless of the compositional ideotypes established and their importance, biomass yield will retain its importance. Alterations to composition, whether through agronomy or breeding, must keep yields intact and allow for improvement. Simultaneously breeding for composition and yield may be possible. But given the complexity of cell wall composition, its fitness roles, and GxE interactions, compositional breeding may be a challenging addition to biomass yield breeding.

However, given the success of breeding sorghum for forage quality and the relatively unselected nature of sorghum composition and biomass yield specifically for biofuels, their simultaneous improvement seems highly plausible.

VI. CONCLUSIONS

Several issues regarding the development of high-biomass sorghums specifically for energy applications were addressed.

Molecular markers for alleles at the *Ma1*/*Ma5* sorghum maturity loci were compared to testcrossing for the identification of PI high-biomass sorghum experimental lines that produce PS hybrids in the *Ma1*/*Ma5*/*Ma6* seed production system. *Ma1*/*Ma5* marker selections for experimental lines producing PI hybrids were reliable and could be used to discard such lines. *Ma1*/*Ma5* marker selections for experimental lines producing PS hybrids were not reliable and identification of such lines will require testcrossing or potentially, genotyping at *Ma6* or other additional loci.

An attempt was made to determine whether meaningful relationships exist between the passport data (geographic origin) of exotic sorghum accessions and high-biomass desirability. Significant relationships between passport data and high-biomass desirability were identified within environments, but because of large GxE interactions, they were not applicable across environments. A larger sampling of environments than those used here will be needed to understand these GxE patterns and establish reliable patterns. Until then, the utility of passport data for prioritizing exotic sorghum accessions for high-biomass desirability will be limited.

Moderate levels of high-parent heterosis for biomass yield were widely available in hybrids created with high-biomass pollinators and grain sorghum females. Thus the

production of high-biomass hybrids with grain sorghum females enables both commercial seed production and the capture of high-parent heterosis for superior biomass yields. Heterosis and biomass yields were maximized in specific hybrid combinations and were subject to GxE interactions.

Significant GxE interactions for the biomass composition (% cellulose, xylan, and lignin) of high-biomass sorghums were identified. However, most compositional variability was attributable to environmental differences. Thus, compositional breeding will require multi-environment testing for accurate evaluations and genotype recommendations. Differences between genotypes for compositional traits were small (1-3%), but these may prove important with large-scale biomass processing.

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APPENDIX

Table A.1 Analysis of variance results for 15 sorghum genotypes evaluated for lignin content in 5 environments: Corpus Christi, TX; Halfway, TX; Weslaco, TX; and irrigated and dryland environments in College Station, TX. 12 genotypes were evaluated per environment. All effects are random.

			College Station		College Station					
Variance	Corpus Christi		Irrigated		Dryland		Halfway		Weslaco	
Source	df	MS	Df	MS	df	MS	Df	MS	df	MS
Genotype	11	2.13**	11	1.08	11	2.81**	11	9.61**	11	6.02**
Rep	3	0.92	3	1.21	3	0.48	3	0.26	3	4.30**
Error	26	0.60	33	0.64	33	0.33	32	0.84	28	0.9

** Statistically significant at $\alpha = 0.01$

Table A.2 Analysis of variance results for 15 sorghum genotypes evaluated for xylan content in 5 environments: Corpus Christi, TX; Halfway, TX; Weslaco, TX; and irrigated and dryland environments in College Station, TX. 12 Genotypes were evaluated per environment. All effects are random.

			College Station		College Station					
Variance	Corpus Christi		Irrigated		Dryland		Halfway		Weslaco	
Source	df	MS	df	MS	df	MS	Df	MS	df	MS
Genotype	11	0.62**	11	0.54	11	0.79**	11	2.33**	11	0.89**
Rep	3	0.14	3	0.83	3	0.16	3	0.15	3	0.58**
Error	26	0.16	33	0.31	33	0.10	32	0.18	28	0.10

** Statistically significant at $\alpha = 0.01$

Table A.3 Analysis of variance results for 15 sorghum genotypes evaluated for cellulose content in 5 environments: Corpus Christi, TX; Halfway, TX; Weslaco, TX; and irrigated and dryland environments in College Station, TX. 12 genotypes were evaluated per environment. All effects are random.

			College Station		College Station					
Variance	Corpus Christi		Irrigated		Dryland		Halfway		Weslaco	
Source	df	MS	df	MS	df	MS	Df	MS	df	MS
Genotype	11	5.86**	11	7.86**	11	8.82**	11	20.07**	11	7.28**
Rep	3	4.51	3	6.17	3	1.78	3	1.47	3	5.03**
Error	26	2.38	33	2.81	33	0.88	32	1.95	28	0.94

** Statistically significant at $\alpha = 0.01$

Table A.4 Analysis of variance results for the dry biomass yield of 12 diverse sorghum genotypes evaluated in 5 environments: Corpus Christi, TX; Halfway, TX; Weslaco, TX; and irrigated and dryland environments in College Station, TX. All effects are random.

Variance Source	df	MS
Genotypes	14	190.4 **
Environments	4	680.3 **
Rep (Env)	14	16.4
G x E	41	32.8
Error	146	23.7

** Statistically significant at $\alpha = 0.01$

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