INTRODUCTION AND SELECTION OF PHOTOPERIOD SENSITIVE SORGHUM GENOTYPES FOR AGRONOMIC FITNESS AND BIOMASS COMPOSITION

A Dissertation

by

LEO HOFFMANN JR.

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

August 2012

Major Subject: Plant Breeding

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ABSTRACT

Introduction and Selection of Photoperiod Sensitive Sorghum Genotypes for Agronomic Fitness and Biomass Composition. (August 2012)

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Co-Chairs of Advisory Committee: Dr. William L. Rooney
Dr. John Mullet

In 2007, U.S. Congress created the "Energy Independence and Security Act" with primary goals focused on increasing the knowledge in production of renewable fuels, increasing the percentages of renewable fuels in the transportation sector and decreasing the emissions of greenhouse gases from fossil fuel sources. To achieve these goals, many species have been pointed as sources of feedstock for the biofuel industry. Photoperiod sensitive (PS) biomass sorghum for the lignocellusosic based conversion is one. In this study, three main objectives were addressed regarding the relative performance for biomass yield and biomass composition of PS biomass sorghum.

First, genetic and environmental variation effects on the biomass yield and biomass composition, and usefulness of pre-classification of genotypes by biomass lignin content were evaluated. On the set of genotypes and locations tested, the environmental effect had the largest influence on the biomass composition, yield and its components. Although smaller, the genetic variation effect was significant for most of the traits, some traits had significant genotype by environment GXE interaction. The

pre-classification of genotypes according to lignin content proved to be an efficient system of separating genotypes as groups, but failed to be efficient in separating on the entries bases.

Assessment of growth patterns for biomass yield and composition, characterized photoperiod sensitive sorghum as capable of producing a harvestable crop as soon as 4 months, but variations in the concentration of constituents and moisture percentage, pointed to a harvest window that can be extended up to the 7^{th} month after planting. Genetic variation was observed in this trail for most agronomic and composition traits, but a strong environmental effect was also observed.

Lastly, the influence of three diverse cytoplasm male sterility (CMS) systems in biomass sorghum hybrids was assessed. The presence of A1, A2 or A3 CMS in the hybrids tested in this study had no influence on the biomass yield performance or in the biomass composition. Therefore, any of the CMS systems can be used in the production of biomass sorghum hybrid seed. Also, in this trial the environmental effects were significant and strong for most traits evaluated.

DEDICATION

To my wife and family

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Rooney, for the investment of time on my education, teaching me plant breeding on a magnificent crop that is sorghum. As well I would like to thank my committee members, Dr. Mullet, Dr. Murray and Dr. Hague. Also, I thank Dr. Odvody for his help on the Corpus Christi station.

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NOMENCLATURE

CC	Corpus Christi
CMS	Cytoplasm male sterility
CS	College Station
HW	Halfway
NIR	Near infrared
PS	Photoperiod sensitive
WE	Weslaco

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CHAPTER I

INTRODUCTION

In the past ten years, oil prices have increased over 760% while consumption has also increased (EIA, 2010). There are several reasons for this increase, but the occasional limited supply of crude oil and the continued growth of new economies in countries such as China, India, Russia, and Brazil is one reason that the demand and price of oil is not expected to drop in the future.

Among all countries, the USA has and continues to lead the world in the consumption of crude oil. To meet fuel needs, domestic oil production is approximately 2 billion barrels annually with the balance (4.1 billion barrels) being imported (EIA, 2010). To reduce the U.S. dependence on foreign oil, reduce greenhouse gases emissions, and alternate the source fuel for automobiles, the Energy Independence and Security Act of 2007 was signed into law (Publ. L. No. 110-140–Dec. 19, 2007). Goals of the Energy Independence and Security Act of 2007 are to (1) reduce greenhouse gas emissions by at least 20%, (2) increase the volume of biofuel from 0.6 billion gallons in 2009 to 21 billion gallons by 2022, (3) increase the volume of cellulosic biofuel from 0.1 billion gallons in 2010 to 16 billion gallons by 2022, and (4) improve knowledge on renewable fuel products through research (Congress, 2007; Sissine, 2007). Proposed production of alternate fuel sources relies heavily on biomass production for conversion to liquid transportation fuel.

This dissertation follows the style of Crop Science.

A study conducted by the United States Department of Agriculture (USDA) indicates that the U.S. has potential to annually produce 1.3 billion tons of dry biomass to be used for cellulosic biofuel production (Perlack et al., 2005). Currently the primary fuel product targeted for production from biomass is ethanol. Ethanol production has increased dramatically in the past five years. In 2011, the U.S. ranked first with 13 billion gallons (USDA, 2011), and Brazil in second with 7 billion gallons of ethanol production. Together both countries produce 88% of world's ethanol.

There are major differences between the two ethanol producing systems. Ethanol in the U.S. is starch-based, using corn (*Zea mays* L.) and other cereal grains as feedstocks. In Brazil, sugar extracted from sugarcane (*Saccharum* ssp.) is the base for ethanol. While each of these production systems are slightly different, they both use crops that are also used for food and this does cause some problems with the perceived food versus fuel issue (Pimentel, 2003).

In 2010, approximately 30 percent of U.S. corn production was used for ethanol production. However, corn alone cannot be used to meet ethanol production goals of the "Energy Independency and Security Act of 2007". Alternate feedstock for biofuel is needed and dedicated bioenergy crops will be inevitable for the successful production of biofuels. Currently, biomass production in the U.S. is up to 190 million dry tons, but only 25% of that comes from cropland (Perlack et al., 2005). In order to optimize biomass production, improved feedstocks are needed that will utilize cropland more efficiently than crops currently grown. Improved feedstocks will lessen the impact on food production in the country.

Dedicated bioenergy crops are defined as a crop used specifically as a source of feedstock for energy production and has limited use as food or feed. Several plant species have been identified as potential dedicated bioenergy crops. Lewandowski et al. (2000) described the use of miscanthus clone (*Miscanthus* x *giganteus*) as a novel lignocellulosic bioenergy crop for Europe. Another proposed dedicated bioenergy crop is switchgrass (*Panicum virgatum*) which is a species native to the U.S (McLaughlin and Adams, 2005). Sugarcane and now energycane (*Sacharum spp.*) has been important as a sugar-based bioenergy crop but its biomass production potential is important for cellulosic ethanol production as well (Alexander, 1985). Another bioenergy crop, originally from the continent of Africa, is sorghum (*Sorghum bicolor* L. Moench) (Rooney et al., 2007).

Among these crops, sorghum is unique in this group for several reasons. First, it is a crop with a long established breeding and improvement history (Rooney, 2004). Because there are established sorghum breeding programs, a seed industry is in place readily being applicable to energy sorghum production. Second sorghum is known as a drought tolerant crop, able to produce under water limited situation where other crops such as corn would decrease production (Beadle et al., 1973). Also, sorghum as a C4 grass has the physiological and morphological ability of continuing the photosynthesis under heat stress conditions longer than C3 plants.

Sorghum as a crop can be divided into three types; grain, forage and most recently bioenergy. Each of these groups has a distinctive plant phenotype (Rooney et al., 2007) Grain sorghum has been used by humans as food and feed for long time, more

recently starch ethanol mills have used it together with grain corn to produced ethanol fuel. Forage sorghums are used for animal feed specially ruminates that can digest its grains and vegetative parts for energy and protein. Most commonly forage sorghum is stocked and feed to the cattle as silage and or hay.

Among energy sorghums, Rooney et al. (2007) divided this category into two distinct types: energy and sweet sorghum. Sweet sorghum is known as good forage by its palatability, because it has high sugar accumulation in its stem, this same sugary juice can be extracted for syrup production or fermented into ethanol (Rooney et al., 2007). High biomass sorghum is characterized by its capacity to remain in a vegetative growth phase for long periods, because it is photoperiod sensitive, and will not initiate reproductive growth until daylengths are reduced to an equatorial defined length (Rooney and Aydin, 1999). Structural biomass from any of these sorghums can be used as combustion fuel for electricity or converted into ethanol. The grain and sweet sorghums can fit in this last category as well, when vegetative parts of the plants are used in similar manner in ethanol production.

Sorghum Origins and Genetics

The center of origin for sorghum is believed to be Northeast Africa; consequently, the continent of Africa is the primary source for diversity within the species (Kimber, 2000). The species is divided into five primary races: Kafir, Caudatum, Durra, Bicolor and Guinea (De Wet and Harlan, 1971). Furthermore,

landraces are spread throughout the tropical regions of the World, extending from Africa to Asia, Indonesia and Australia after colonization (De Wet and Huckabay, 1967).

Because sorghum evolved and was domesticated in tropical and subtropical regions, much of the sorghum germplasm based is photoperiod sensitive. When grown in temperate regions changes in photoperiod through the seasons can have significant effects on the growth and development of the plant. Photoperiod sensitive sorghum does not initiate reproductive development until daylengths shorten below a specific and defined time period. Until this condition is met, the plant grows vegetatively. In sub-equatorial regions of sorghum domestication, the PS trait was an adaptive trait to mitigate drought or coordinate flowering with a rainy season. The cultivation of these PS sorghums in temperate climates results in a vegetative crop that may never flower because the critical day length is not achieved until it is too late for further growth.

Maturity and photoperiod sensitivity are genetically controlled; there are distinct genes with major effects that have been identified. The majority of sensitivity is controlled by a series of at least six maturity genes; Ma1, Ma2, Ma3, Ma4 (Quinby, 1967), and Ma5 and Ma6 (Rooney and Aydin, 1999). Of these genes, Ma1, Ma5 and Ma6 influence photoperiod sensitive responses; mutations at other loci are maturity related and provide relative degrees of earliness (Quinby, 1967). In addition, the discovery that Ma3 of sorghum and PHYB of Arabidopsis are syntenic confirmed that pathways to flowering are similar in both groups of plants (Childs et al., 1997; Murphy et al., 2011).

As in most crops, increasing the duration of the vegetative growth phase increases total biomass yields. Quinby (1967) noted this phenomenon in two different hybrids, one having the genotype ma1Ma2ma3Ma4 (49 days to anthesis) and the other Ma1Ma2ma3Ma4 (102 days to anthesis). The difference in biomass production between the two was 245g*plant^{-1}, with the higher biomass weight coming from the genotype with three dominant loci and longer vegetative stage. In another study, sets of crosses between grain types (ma1Ma5ma6) and photo period sensitive (Ma1ma5Ma6) types demonstrated moderate levels of high-parent heterosis for biomass yield around 40% across environments (Packer, 2011).

Existence of maturity genes is important regarding biomass production on a commercial scale. They allow breeders to generate variability for maturity that can be used to maximize biomass accumulation in different regions of the world. In addition, these complementary genetic systems can be used to efficiently produce seed of hybrids with distinct photoperiod requirements (Rooney and Aydin, 1999). Specifically, hybridization of ma5ma5Ma6Ma6 x Ma5Ma5ma6ma6, (both of which are photoperiod insensitive) result in a photoperiod sensitive hybrid heterozygous (Ma5ma5Ma6ma6). Another reason to have a hybrid seed system is the incapability of photoperiod sorghum lines to flower in U.S. making it impossible to have inbred seed production. Use of this of the genetic method to produce PS hybrid seed from two flowering parents should facilitate the establishment of sorghum as a bioenergy crop.

Sorghum Biomass Composition

Biomass yield is the most important trait for an energy crop and a biofuel conversion operation, but composition of the biomass is also important. To convert biomass to biofuels using biochemical approaches requires that the biomass be deconstructed into simple carbohydrates. This is usually completed by a combination of chemical, heat, pressure and enzymatic treatments. Thus, crops with adequate proportion of biomass components may lead to more efficient and profitable conversion process.

Structural plant biomass is composed of different compounds that are present in the cell wall of panicles, stalks, leave and roots of sorghum plants. The primary components of this biomass are structural polymers which include cellulose, hemicelluloses, pectin and lignin. Cellulose is composed of a 30 to 36 glucose molecule chain linked by hydrogen bonds which are organized to form fibrils approximately 7 μm in length. Hemicellulose is a branched polysaccharide having neutral sugars with hydrogen bonds linked to the cellulose fibrils around it. Similarly to reinforced concrete, where steel bars (cellulose microfribils) are tied to each other by wires (hemicelluloses chains) to improve stability and strength (Fig. 1). Pectin is formed mostly by uronic acids (Somerville et al., 2004). Although present in small amounts, pectin is responsible for forming a gel matrix that suspends the cellulose-hemicellulose web. Usually, lignin is observed in the middle lamella between cell walls and a major component of vascular tissue in plants (Taiz and Zeiger, 2004).

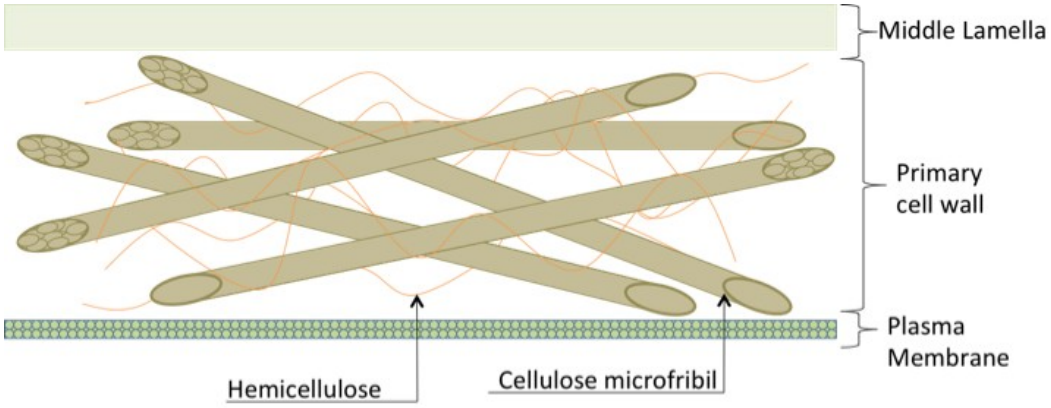

Figure 1.1: Plant cell wall scheme the main source of carbohydrates to be converted in biofuel, cellulose and hemicelluloses are situated in the primary cell wall and interact to each other promoting strength to the plant cell wall, adapted from Somerville et al., 2004.

Lignin is a phenolic polymer mainly found in plant secondary cell walls, xylem cell walls and it has particular physiological function in water migration throughout the plant as source of hydraulic strength in the vascular cells known as tracheids. Furthermore, the complex structure of this polymer is resistant to biological degradation, serving as a defense mechanism for plants against bacterial and fungal infection (Campbell and Sederoff, 1996). Lignin levels influence digestibility (bacterial degradation) in forage plant species such as sorghum (Akin et al., 1986). Lignin itself is not amenable to fermentation and its presence inhibits the hydrolysis of both cellulose and hemicellulose. Thus, it retards the swelling of cellulose fibrils and binds to enzymes intended to degrade cellulose and/or hemicelluloses fractions, reducing efficiency and ethanol yield (Vermerris et al., 2007). While not fermentable, lignin can be used in combustion or gasified to produce electricity and is actually desirable for this purpose

compared to cellulose or hemicelluloses as lignin is more energy dense on a BTU basis (White, 1987).

Both cellulose and hemicelluloses are amenable to fermentation and ethanol production, but the ligno-cellulosic structure must be deconstructed to allow access to the compounds. This is accomplished using some form of heat, pressure and enzymes (DOE, 2012). Second, the cellulose and hemicellulose portions of biomass need to be enzymatically hydrolyzed to breakdown cellulose and hemicellulose into smaller sugar units of glucoses and pentoses. These smaller sugar molecules can then be fermented and distilled into ethanol. Modification of the relative concentration of these different compounds may be useful in increasing efficiency or yield of processed components. However, there has been little to no screening of sorghum germplasm for compositional traits as they related to bioenergy conversion.

Compositional Analysis Methods

The composition of sorghum biomass can be measured using several different methods, such as Kjeldahl, crude fiber and dietary fiber. In Kjeldahl protein analysis, samples are artificially digested and the nitrogen value found by this procedure is multiplied by 6.25 to estimate the crude protein value of the samples (Wall and Blessin, 1970; AOAC, 1984). Detergent fiber analysis measures neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin. These methods are gravimetric where samples are submitted to a neutral digestion period and an acid digestion period and the lignin is calculated by the difference. This methodology is commonly used in

forage, and silage analysis for ruminant animals feed. Similarly, dietary fiber is a gravimetric digestion process but the digestion is done using the physiological enzymes pepsin and pancreatin to simulate the normal digestion, it is mostly used by food industry and monogastric animals feed analysis, (AOAC, 1984; Olivier et al., 2005). These composition analyses vary in cost and time. For example, to analyze a sorghum plant dry matter using the dietary fiber method requires at least a month and costs approximately $500 per sample (outsourced). Finally, the estimated values from each method may not be highly correlated (Wolfrum et al. in review).

Because of the time required and cost associated with these methods, faster and cheaper methods to estimate composition have been developed. Among these, near infrared reflectance (NIR) spectroscopy is fast and inexpensive relative to other methodologies (Sanderson et al., 1996; Roberts et al., 2011). NIR technology is based on the ability of low frequency (near infrared) light to react to molecular bonds. Molecular bonds have unique energy vibration signatures of stretching or bending. These vibrations can absorb near infrared light when a nick of energy from a different wavelength (800-2500 nm) is encountered, producing a signature spectrum that can be quantified and correlated to the composition of the sample exposed to the near infrared light treatment. The correlation of the sample spectrum and the sample composition is usually calculated through multivariate statistics. A calibration curve is developed using a minimum of 30 to 100 unique samples, where each sample is submitted to conventional wet chemistry and NIR spectroscopy analysis. Both measurements are then combined using statistical techniques such as principal component analysis and

multi-linear regression software. The result is a regression equation that can predict the wet chemistry composition of similar samples using NIR spectroscopy data (Hames et al., 2003; Vermerris et al., 2007).

The biomass composition and yield of energy sorghum plants varies depending on the genotype and environment in which it is grown, but the extent of this as opposed to variation from genotype is not known. This variation of composition and yield will directly reflect energy production. In a study containing six genotypes of sorghum, ranging from grain to forage sweet types, McBee et al. (1988) found a difference in biomass yield of 82%, indicating the existence of genetic variability for biomass production within the sorghum types. Rooney et al. (2007) explored the composition variation of various types of sorghum currently available as potential candidates for improvement of biomass energy production. In sweet sorghums study, Corn (2009) demonstrated variation in composition traits such as glucans (cellulose) ranging from 24.7% to 38.5%, xylans (hemicellulose) from 8.5% to 13.9% and lignin from 9.3% to 13.0%. Packer (2011), reported variation in biomass composition across 15 genotypes of photo period sorghum and five environments, cellulose range from 26.9% to 31.8%, xylan from 14.9% to 18.4% and lignin from 8.3% to 18.9%.

Identification of Sorghum Bioenergy Germplasm

Breeding bioenergy sorghums requires selection for some unique traits. While forage sorghum provides one logical source of germplasm, selections for forage have some inherent differences to what would be desirable in energy sorghum. In the case of forages, a higher leaf to stem ratio, thinner stems, and higher protein content are

desirable. For bioenergy production, a smaller leaf to stem ratio, with essentially no leaves in the harvest and low protein or N levels are desirable.

The sorghum germplasm collections represent an extensive source of genetic variation that has been extremely valuable in the sorghum breeding community (Rooney, 2004). There are sorghum germplasm collections at ICRISAT and in the USDA-GRIN, and each collection holds approximately 40,000 accessions. Most of these accessions are photoperiod sensitive and most breeding efforts were focused on photoperiod insensitive germplasm (Rooney, 2004). The sorghum conversion program was one approach to tapping into the exotic sorghum germplasm collection (Stephens et al., 1967) but this program evaluated and utilized the germplasm within insensitive breeding program. A vast array of photoperiod sensitive sorghum germplasm is directly applicable to bioenergy sorghum breeding but it has not yet been evaluated from that perspective.

Over the past five years, the Texas AgriLife sorghum breeding program at College Station, Texas, has been systematically screening sorghum germplasm for its potential use for breeding. To identify elite germplasm for biomass yield and provide biomass composition samples, non-replicated plantings of entries, from the USDA GRIN germplasm bank database located in Griffin Georgia, were completed in College Station. Sorghum germplasm selected for this study was expected to be photoperiod sensitive cultivars/landraces of various types, origins and races. Each accession was visually evaluated in a single experimental unit; in total, more than 7,000 entries were evaluated over three years at College Station (2007-2009). For each entry 100 seed were

planted in April and harvested in October. Plots were one row (5.5 meter) in length with 76 cm between rows. Plant density was approximately 216,000 plants per hectare, at a calculated germination rate of 80%. Agronomic data was collected on flowering date, height, and general agronomic desirability. Flowering date (days to anthesis) was marked when half of the plants in the plot attained mid-anthesis, this parameter was used to select out genotypes that where less photoperiod sensitive. Desirability was a measurement of the visual breeding quality of the plot with a scale ranging from 1-9 with 1 being a desirable plot and 9 being a plot with no desirable characteristics such as lodging susceptible and flowering ability. Plant height was measured in increments of 15 cm and taken only on plots with a recorded desirability of between 1 and 4. Samples for composition analysis were harvested from plots receiving a desirability rating between 1 and 4 and with no signs of flowering. These samples were composed of 1.5 meter sections cut from the plant stems of three random individual plants per plot. Drying of samples was done in a forced-air dryer set at ~52^{o}C. Once dried, samples were weighed and ground using a Wiley mill to the point where material can pass through a 2mm sieve. Ground samples were stored in air-tight plastic bags until analysis were done. Composition analyses were performed using a FOSS XDS near infrared spectroscopy instrument. Spectrum data were converted into composition prediction data using a calibration curve which has been developed through cooperation between the sorghum research team at Texas A&M University Sorghum Quality Lab and National Renewable Energy Laboratory (Wolfrum et al. in review). Prediction parameters were percent glucans (cellulose), xylans (hemicellulose), lignin, and solubles. Based on single

plot observation trials from 2007, 2008 and 2009, a set of 56 entries categorized as either low or high lignin content were selected and an additional 24 lines were selected based on agronomic desirability only (Table 1.1). Seed of all 80 lines were increased in a winter nursery located in Guayanilla, Puerto Rico, and harvested in March of 2010. This seed was used for phenotypic evaluations addressing objectives 2 and 3 (Table A.1).

Although there was no plot replicates in the three years of selections made, there were trends observed during pre-selection. First the variation for lignin concentration varied from year to year. For example, the basis for selecting low lignin concentrations in 2008 was similar to the selection line for high lignin. The standard deviation varied within each year for the entries selected. In 2007, standard deviations were double the standard deviations in 2008 and 2009. While it is not testable, the environment is likely the reason for this variation. To validate the efforts of pre-classification on PS sorghum genotypes for lignin concentration, further investigation with proper experimental design was necessary. That was accomplished in the objective 2 and discussed in the chapter 2 of this dissertation.

Table 1.1. List of photoperiod sensitive sorghum selections made from phenotypic evaluation in College Station in 2007, 2008 or 2009 based on lignin concentration and agronomic desirability.

2007 selections		2008 selections		2009 selections		2009 selections based on desirability
High lignin						
Entries	**Lignin**	**Entries**	**Lignin**	**Entries**	**Lignin**	**Entries**
R.09075	18.08	R.09091	19.05	R.09105	14.21	R.09051
R.09076	20.32	R.09092	17.67	R.09106	11.45	R.09052
R.09077	20.74	R.09093	16.20	R.09107	13.81	R.09053
R.09078	18.95	R.09094	16.59	R.09108	13.05	R.09054
R.09079	19.71	R.09095	16.36	R.09109	12.73	R.09055
R.09080	16.39	R.09096	18.52	R.09110	12.30	R.09056
R.09081	20.57			R.09111	13.11	R.09057
R.09083	20.32					R.09058
						R.09059
Low lignin						R.09060
Entries	**Lignin**	**Entries**	**Lignin**	**Entries**	**Lignin**	R.09061
R.09083	12.60	R.09097	13.88	R.09113	10.41	R.09062
R.09084	11.96	R.09098	14.19	R.09114	10.46	R.09063
R.09085	13.09	R.09099	14.22	R.09115	10.51	R.09064
R.09086	13.39	R.09100	14.39	R.09116	10.61	R.09065
R.09087	12.06	R.09101	14.56	R.09117	11.01	R.09066
R.09088	13.07	R.09102	14.64	R.09118	11.03	R.09067
R.09089	12.67	R.09103	14.65	R.09119	11.29	R.09068
R.09090	13.14	R.09104	14.67	R.50002	11.44	R.09069
R.50001	13.38	R.09105	14.79	R.09053	12.02	R.09070
		R.09106	14.85	R.50003	11.08	R.09071
		R.09107	16.15	R.50004	12.23	R.09072
		R.09108	16.08			R.09073
		R.09109	15.68			R.09074
		R.09110	16.37			
Total	17		20		19	24
Average	11.96		13.88		10.00	-
Maximum	20.74		19.05		14.21	-
Minimum	15.91		16.16		11.98	-
STDV	3.54		1.47		1.18	

* Entries with classified as low lignin% in 2009 also.

Objectives

The objectives of this dissertation study were to; (1) determine the effects of genotype and environment on composition and agronomic productivity in selective photoperiod sensitive sorghum genotypes; (2) follow the evolution of composition and yield accumulation for biomass of six photoperiod sensitive sorghum lines throughout a growing season at the location of Corpus Christi and College Station, Texas; (3) Determine the effect of three different cytoplasms (A1, A2 and A3) on the composition and yield of biomass in photoperiod sorghum.

CHAPTER II

GENOTYPIC AND ENVIRONMENTAL VARIABILITY FOR COMPOSITION AND YIELD IN PHOTOPERIOD SENSITIVE BIOMASS SORGHUM

Introduction

Higher demand and lower supplies of crude oil prices have increased interest in the production of liquid transportation fuels using biomass. To promote the development of a biomass-based renewable fuel industry, the U.S. Congress approved the energy independence and security act of 2007. This legislation mandates that 30% of U.S. transportation fuel should be derived from renewable resources by the year 2030. Increased use of renewable fuels is projected to reduce greenhouse gases emissions by 20% and reduce U.S. dependency of oil from foreign countries which would elevate the security and sustainability of the U.S. energy sector. Currently, starch-based ethanol comprises about 10% of the volume and 7% of the energy content of domestic gasoline consumed (2011). While this production (> 13.5 billion gallons annually) is significant, the U.S. simply does not produce enough corn to meet EISA mandates. Thus, additional feedstock sources must be developed to produce the volumes mandated by the EISA in 2022.

A study conducted by the United States Department of Agriculture (USDA) has shown that the U.S. has the potential to produce 1.3 billion tons of dry biomass annually. Much of this biomass will be derived from herbaceous energy crops and the Department of Energy (DOE) has identified potential bioenergy crop species (Lewandowski et al.,

2000), including Miscanthus (*Miscanthus x giganteus*), switchgrass (*Panicum virgatum*) (McLaughlin and Adams, 2005), energycane (*Saccharum spp.*) and sorghum (*Sorghum bicolor* (L.) Moench).

Of these crops, sorghum is unique because three distinct types of sorghum (grain sorghum, sweet sorghum and high biomass) are or can be utilized as feedstock for biofuel production (Rooney et al. 2007). Currently, approximately 30% of the U.S. grain sorghum is fermented to ethanol. In addition, sorghum can contribute with ethanol production as feedstock of fermentable sugars. Sweet sorghums are genotypes of sorghum that are 2 to 4 meters tall and able to accumulate fermentable sugar in its stems. The advantages of sweet sorghum are rapid crop growth cycles (can produce a crop in 4 months), drought tolerance, and processing is available (sugar cane processing can be readily adapted) for this type of sorghum. In the US sweet sorghum could be used in areas where sugar cane is not adapted, but more importantly, it can complement sugarcane to extend mill seasons throughout the world. In Brazil, this approach is being testing and sweet sorghum could potentially extend the operation of sugar mills of that country. The third type high biomass sorghum and the focus of this paper is suited for second generation ethanol production also known as lignocellulosic conversion; this technology is base on the use of plant biomass composed by structural carbohydrates that are treated, fermented and distillated in to ethanol.

Biomass sorghum

Biomass sorghum [*Sorghum bicolor* (L.) Moench] has an extended vegetative stage when cultivated in temperate growing environment. Usually the plants are taller than the grain or sweet types of sorghum with elongated stem internodes and capable of accumulating large amounts of biomass. This extended vegetative growth phase is the results of photoperiod sensitivity and PS sorghums require short days to initiate reproductive growth. While the exact time varies, a strongly PS sorghum usually requires daylengths of 12.5 hrs or less to initiate reproductive growth (Rooney and Aydin, 1999).

Maturity and PS are controlled by numerous genetic loci, but a series of six maturity genes (Ma1, Ma2, Ma3, Ma4 (Quinby, 1967) and Ma5, Ma6 (Rooney and Aydin, 1999)) have been described. In temperate latitudes, PS sorghum has extremely late flowering or may not flower at all. This extended vegetative state consistently increases total biomass yield (McBee et al., 1988). Quinby (1967) noted this phenomenon in two different hybrids having $ma_1Ma_2ma_3Ma_4$ (which requires 49 days to anthesis) and Ma1Ma2ma3Ma4 (which requires 102 days to anthesis). The difference in biomass production between the two was 245g*plant^{-1}, with the higher biomass weight coming from the genotype with three dominant loci and 102 days to anthesis. In another study, sets of crosses between grain types (ma1Ma5ma6) and photoperiod sensitive types (Ma1ma5Ma6), demonstrated moderate levels of high-parent heterosis (40%) for biomass yield across environments (Packer, 2011). In addition to increased yield, the PS trait also enhances pre-flowering drought tolerance. While not directly associated with

any particular sorghum genotype, plants in a vegetative growth phase are inherently more drought tolerant than those in reproductive growth (Blum, 1973; Smith and Frederiksen, 2000). These genotypes can slow growth and stay latent for an undetermined period of time and when moisture is available, the plants resume growth.

Composition

Structural plant biomass is composed primarily of different compounds present in the cell wall of stalks, leaves, roots and panicles. In most plants, including sorghum, the majority of the biomass is composed of structural carbohydrates which include cellulose, hemicelluloses, pectin and lignin. Cellulose is organized in 30 to 36 glucose molecules linked in a chain of hydrogen bonds which form fibrils approximately 7 μm in length. Hemicellulose is a branched polysaccharide composed of neutral sugars with hydrogen bonds linked to the cellulose fibrils around it (Somerville et al., 2004). Although present in small amounts, pectin is responsible for forming a gel matrix that suspends the cellulose-hemicellulose web. Lignin is usually observed in the middle lamella between cell walls and it is a major component of vascular tissue in plants (Taiz and Zeiger, 2004). Hence, lignin molecules bind to cellulose and hemicellulose as well, given plant cell walls strength and stiffness helping as defense mechanism against pathogens and weathering.

Both cellulose and hemicellulose can be catalyzed to simple sugars and fermented into ethanol. This deconstruction is usually accomplished by using some form of chemical, heat, pressure and enzymatic treatment (DOE 2010; Wu et al., 2010).

Lignin itself cannot be converted to ethanol, but it can be combusted or gasified to produce electricity. If genotypes with variations for these compounds can be identified, these would be useful for enhancing desirable or reducing undesirable carbohydrates compounds in the plants. Due to these reasons, there is a significant interest in assessing the composition variation present within existing sorghum germplasm.

The composition of sorghum biomass can be measured using several different methods, including the Kjeldahl protein analysis, where samples are artificially digested and the nitrogen value found by this procedure is multiplied by a conversion factor of 6.25 to estimate the crude protein value of the samples (Wall and Ross, 1970; AOAC, 1984). The crude fiber analyses are separated in neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) process. The fiber and lignin analysis are classified as gravimetric, where samples are submitted to a neutral digestion period and an acid digestion period and the lignin is calculated by difference. Similarly, dietary fiber is a gravimetric digestion process but the digestion is done using the physiological enzymes pepsin and pancreatin to simulate the normal digestion (AOAC, 1984; Olivier et al., 2005).

Because traditional analytical methods to estimate composition are time consuming and expensive, alternative "high-throughput" methods are essential for selection and breeding. Near infrared reflectance (NIR) spectroscopy is a method of biomass analysis known for being fast and inexpensive relative to other methodologies (Sanderson et al., 1996; Roberts et al., 2011). NIR technology is based on the ability of low frequency (infrared) light to react to molecular bonds. Molecular bonds have unique

energy vibration signatures of stretching or bending. These vibrations can absorb near infrared light when a nick of energy from a different wavelength (800-2500 nm) is encountered, producing a signature spectrum that can be quantified and correlated to the composition of the sample exposed to the near infrared light treatment. The correlation of the sample spectrum and the sample composition is usually calculated through multivariate statistical tools. A calibration curve is developed using a minimum of 30 to 100 unique samples that can vary depending on homogeneity of the population in question and accuracy required. Each sample is then submitted to conventional wet chemistry and NIR spectroscopy analysis. Both sets of data are then combined using statistical techniques, such as principal component analysis and multi-linear regression software. The result is a regression equation that can predict the composition of similar samples using NIR spectroscopy data (Hames et al., 2003; Vermerris et al., 2007).

The biomass composition and yield of sorghum plants varies depending on the genotype and environment in which it is grown. This variation of composition and yield will directly reflect energy production. In a study containing six genotypes of sorghum, ranging from grain to forage sweet types, McBee et al. (1988) reported differences in biomass yield of 82%, indicating extensive existence of genetic variability for biomass production within the sorghum types. In sweet sorghum, Corn (2009) demonstrated variation in composition traits with glucan content ranging from 24.7% to 38.5%, xylan content ranging from 8.5% to 13.9% and lignin concentration ranging from 9.3% to 13.0%. Packer (2011) reported variation in biomass composition across 15 genotypes of PS sorghum and across five environments, with cellulose ranging from 26.9% to 31.8%,

xylan from 14.9% to 18.4% and lignin from 8.3% to 18.9%, for these three compositional traits the different environments proved to have greater effect than genotype. Additionally, Dien (2009), studying the effects of *bmr6* and *bmr12* mutations in forage isogenic lines of sorghum, found that reduction of lignin content by these mutations was correlated to increased overall ethanol production of forage sorghum lines.

Genetic by environment interactions

The potential composition of plant biomass is genetically defined, but the actual composition is strongly influenced by the environment and the genotype x environment interaction as well. In reviewing the quality of maize and sorghum grain for monogastric and ruminant animal feeding, O'Brien (1999) reported that grain composition was as affected by environments as much, if not more than by genotype. Packer (2011) reported that environment was the largest single source of variation on the composition of biomass sorghum. Murray et al (2008a) Murray et al (2008b) and Corn (2009) found similar effects of environment on the composition of sweet sorghum juice and biomass. In addition to genotype and environment, the maturity of the crop strongly influences biomass composition (Murray et al., 2008a).

The genotype by environment interaction (GXE) refers to genotypes responding differently to different test environments. Three basic scenarios can occur regarding the relative response of the genotypes across the environments. Ouyang et al. (1995) illustrates these GXE scenarios; (1) genotypes increase or decrease performance but

similar rates of increasing or decreasing are observed and there is no change in ranking, this situation is a no interaction relationship; (2) genotypes increase or decrease performance in different rates but no change in ranking is observed, this situation is a GXE relationship; (3) genotypes increase or decrease performance in equal or different rates and change in ranking of the genotypes is observed. In plant breeding, the latter situation is the most significant, because there is genetic variability in the tested population and selection on genotypes for target environments can happen. Furthermore, the use of data to identify superior genotypes with stability across environments is a useful tool for selection. One manner to explore the GXE interaction is the utilization of a biplot graph, where a data summary is expressed through relative position of genotypes and environments on a Y by X plot. In this type of graphic representation genotypes and environments with best means will be plotted furthest to the right side. In a study with cassava, Bokanga et al. (1994.) found that HCN was strongly affected by GXE interactions; genotypes responded and ranked differently to rainfall regime and soil moisture. In sweet sorghum (Murray et al., 2008a) found GXE interactions significant on composition of structural carbohydrates in biomass of inbreed lines, similarly (Packer, 2011) found GXE interactions for composition on stems of PS sorghum.

Objectives

Using a set of pre-selected sorghums we sought to; (1) determine the relative effect of genotype, environment and genotype x environment on compositional traits in bioenergy sorghum, (2) determine if pre-selection for high and low lignin content in the

biomass sorghums is effective for the identification of lines that vary in lignin content and (3) verify the stability of sorghum agronomic and compositional traits across location.

Materials and Methods

Plant germplasm

A set of 39 sorghum lines were selected from a group of over 7000 sorghum genotypes USDA/GRIN germplasm system that was visually evaluated in College Station, Texas over a period of three years (2007, 2008 and 2009). Genotypes in this set were selected based on agronomic performance and lignin composition. The agronomic performance was the first criteria of the screening, and individual non-replicated plots were evaluated based on photoperiod sensitiveness, lodging and a general phenotypic desirability. In each year, approximately 10% of the entries that did not flower or lodge and had good desirability were selected for composition analysis. Composition was estimated using NIR spectroscopy (Wolfrum et al. in review). Data for structural carbohydrates was taken from biomass samples from 1.5 meters sections of three random plant stems of each respective plot. Two main groups were created based on low lignin and high lignin genotypes, each group with 18 lines from the USDA/GRIN sorghum collection. For comparisons, two PS inbred lines and a PS hybrid with established agronomic performance were included as checks (Table 2.1).

Experimental design and data collection

The experiments were planted in a randomized complete block design with three replications. The experimental units were two rows of 7.92 meter in length and spaced 76 cm apart. Plant density was maintained at 160,000 plants ha^{-1}. The trials were planted in 2010, in three locations in Texas, College Station (CS) planted in April 13th, Corpus Christi (CC) planted in April 26th and Halfway (HW) planted in June 1st. Corpus Christi is located in the Coastal Bend of Texas and in a subtropical and dry climate. College Station is in South Central Texas in the Brazos River bottom and typically has warm and humid days and nights. The Halfway location is a temperate environment on the Texas High Plains with warm, dry days and cool nights during the cropping season. In CC, the soil is classified as Orelia fine sandy loam (Of) with a top 9 cm layer of fine sandy loam and bottom layer of 140 cm of sandy clay loam. In CS the soil is classified as Ships Clay (Sha) and the soil type in Halfway is a Pullman Clay Loam.

Table 2.1. List of genotypes, pedigrees, composition classification by NIR spectroscopy and sorghum races classification.

Entry #	Pedigree	Classification	Race
1	R.09075	High Lignin	Caudatum
2	R.09076	High Lignin	Unknown
3	R.09077	High Lignin	Unknown
4	R.09078	High Lignin	Guinea-caudatum
6	R.09080	High Lignin	Unknown
7	R.09081	High Lignin	Kafir-bicolor
8	R.09083	Low Lignin	Kafir-durra
9	R.09084	Low Lignin	Caudatum
10	R.09085	Low Lignin	Durra
11	R.09087	Low Lignin	Unknown
12	R.09088	Low Lignin	Unknown
13	R.09089	Low Lignin	Unknown
14	R.09090	Low Lignin	Unknown
15	R.09091	High Lignin	Caudatum-bicolor
16	R.09092	High Lignin	Guinea-kafir
17	R.09093	High Lignin	Caudatum
18	R.09094	High Lignin	Guinea-caudatum
19	R.09095	High Lignin	Guinea-bicolor
20	R.09096	High Lignin	Caudatum-bicolor
21	R.09098	Low Lignin	Caudatum
22	R.09099	Low Lignin	Durra-bicolor
23	R.09101	Low Lignin	Durra-caudatum
24	R.09102	Low Lignin	Unknown
25	R.09103	Low Lignin	Durra-caudatum
26	R.09104	Low Lignin	Durra
27	R.09105	High Lignin	Caudatum
28	R.09106	High Lignin	Caudatum
29	R.09108	High Lignin	Guinea
30	R.09109	High Lignin	Guinea-caudatum
31	R.09110	High Lignin	Durra-caudatum
32	R.09112	Low Lignin	Durra-caudatum
33	R.09114	Low Lignin	Caudatum-bicolor
34	R.09115	Low Lignin	Durra-caudatum
35	R.09116	Low Lignin	Kafir
36	R.09117	Low Lignin	Unknown
37	ATx2928/BTx2752//R07020	Hybrid	Tester
38	R.08028	Agronomic fit	Tester
39	R.07020	Agronomic fit	Tester

At all locations, agronomic production practices standard to the region were followed with irrigation available as needed in CS and HW; the CC environment was dryland. The measured agronomic traits were plant height, lodging, stem diameter and biomass yield. Height of the plant was an average of the plot height from the soil level to the top of the point of growth of the canopy. Lodging measurements were recorded in the fall and were based on a scale from 1-9 with completely standing plots receiving a 1 and completely lodged plots receiving a 9. Stem diameter was taken from four random plants per plot at the second node from the base of the plants. The agronomic traits data was taken just prior to harvest at each location. At harvest, all the genotypes remained in a vegetative growth stage and none had signs of differentiation. Biomass fresh yield was measured by weighing harvested biomass at the end of the growing season. In College Station, plots were harvested on September 23th 2010, using a John Deere forage harvester Model 5460 with a three row header and weigh wagon equipped with an Avery Weigh-Tronics model 640 electronic scale capable of measuring 500g increments. In Corpus Christi, yield was measured on September 30th 2010, by hand harvest where a two meter section of the plot was chopped and weighed using a Cardinal HSCC-500 scale with increments of approximately 50g. In Halfway September 14th 2010, harvest was performed with a one-row forage harvester, New Holland model 707, and the plot biomass was collected and weighed on an attached bin equipped with an Avery Weight-Tronics scale system model 640 with increments of 500g. At each location, a sample of the whole plant biomass was collected to estimate dry biomass yields, moisture content and compositional analysis.

These samples were dried in an air-forced flux drier at 52°C +- 1°C until weight stabilization. Once dried, samples were weighted to measure percent of moisture loss and to calculate the dry yield by multiplying the percent of dry matter with the biomass fresh yield. Then, samples were ground (2mm mesh) and stored in plastic air-tight bags for compositional analysis of ash%, lignin%, xylan% (hemicellulose), cellulose%, and soluble% (fermentable sugars). The NIR spectroscopy composition predictions were performed using procedures described in Chapter I.

Statistical data analysis were performed using JMP 9 software for the ANOVA of each individual location and contrast analysis between the groups and Pearson's correlation among traits, the data for the two pre-classified groups of genotypes (high and low lignin classification columm in Table 2.1) from all locations was combined to do the contrast analysis. SAS version 9.2 software (SAS institute Inc. 2002) was used for the combined analysis of variance and the "Type III" output was used for having the sources of variance.

The mean separation test used was the least significant difference (LSD) and the formula follows;

$$LSD = (t\alpha)\left(\frac{\left(\sqrt{2 * \sigma^2}\right)}{r}\right)$$

t= tabular T value with alpha at 0.05 of significance and at degree of freedom of the error.

σ^2= mean square of error (MSE).

r= number of replications.

For each location, the basic statistical model was;

Y= mean + reps + genotypes + error

For the combined analysis (ANOVA type III) a mixed model was adopted;

Y = mean + reps(location) + genotype + location + genotype*location +error

With genotypes as a fixed effect and all others sources of variation as random effects.

Data were analyzed by environment and tests of homogeneity (Levene, 1960) revealed that some of the data were homogeneous (lignin, soluble and stem diameter) while others were not (ash, xylan, cellulose, lodging, dry matter, fresh biomass yield and dry biomass yield). Transformations of those that were not homogeneous did not improve the dataset regarding homogeneity of error variance. As we were interesting in the effect of locations as an environmental source of variance, the data were combined and analyzed.

Repeatability was calculated from the relationship between the genetic and the location variances to the error variance and its formula follows;

$$Repeatability = \frac{\sigma^2 G}{\sigma^2 GL + \sigma^2 G + \sigma^2 E}$$

$\sigma^2 G$ = genotype variance

$\sigma^2 GL$ = genotype x location interaction variance

$\sigma^2 E$ = error variance

GGE biplot software (Yan and Tinker, 2006) was used for stability visualization purposes and only performed for the traits lignin% and dry biomass yield. The stability graphs are based in the variance within the three environments tested. The stability graphs are based in the variance within the three environments tested.

Results and Discussion

Analysis of variance

In each location, the effect of genotype was significant for all traits with the exception of solubles in CS, lodging in both CC and CS and dry matter in CS (Table 2.2). The results indicate that variation exists among the genotypes evaluated in this study for most of these traits that were measured. While the heritability of these traits could be measured in this set of germplasm, variation among genotypes opens the possibility of further improvement for these traits.

In the combined analysis, ANOVA detected significant effects ($p<0.05$) due to both genotype and location for most traits (Table 2.3). The genotype effect was significant for all traits except for lodging; location was significant for all traits except cellulose and stem diameter. For some traits a significant Genotype x Location interaction (GXE) was detected for many of the traits (lignin, xylan, cellulose height, dry matter, fresh yield and dry yield), indicating that genotypes responded differentially to the environments (Table 2.3).

Table 2.2. Mean squares from the analysis of variance of compositional traits for 39 sorghum genotypes grown in Corpus Christi (CC), College Station (CS) and Halfway (HW), in Texas, 2010.

Trait	Ash			Lignin			Xylan			Cellulose		
Location	**CC**	**CS**	**HW**	**CC**	**CS**	**HW**	**CC**	**CS**	**HW**	**CC**	**CS**	**HW**
Genotype	1.3**	0.7**	1*	1.5**	0.7*	1.1**	1.3**	0.8**	1.5**	5.8**	3.3**	7.7**
Rep	0.1	0.2	0	8.8**	12.2**	1.2	6.4**	2.2**	5.1*8	22**	1	33.2
Error	0.6	0.3	0.3	0.6	0.4	0.5	0.4	0.2	0.4	2.1	1.1	2.6
Root MSE	0.8	0.5	0.5	0.8	0.6	0.7	0.6	0.5	0.7	1.4	1	1.6
G. Mean	7.8	8	8.9	15.7	15.3	14	18.2	17.4	17.5	32.8	32.4	32.2
CV%	9.7	6.3	5.9	4.9	4.2	5.2	3.5	2.8	3.8	4.4	3.2	5

Trait	Solubles			Stem Diameter			Lodging			Height		
Location	**CC**	**CS**	**HW**	**CC**	**CS**	**HW**	**CC**	**CS**	**HW**	**CC**	**CS**	**HW**
Genotype	6.1**	4.6	5.8**	22.21**	14.56**	16.58**	0.8	5.75	0.96**	6315**	4452**	2208**
Rep	74.1**	20.5*	5.8	20.42	18.94	82.18**	1.04	15.23	0.21	7891	10847**	14108**
Error	2.6	3.7	2.7	10.79	6.39	6.56	0.93	4.63	0.41	2005	897	530
Root MSE	1.6	1.9	1.6	3.28	2.53	2.56	0.96	2.15	0.64	44.78	29.94	23.03
G. Mean	17.5	17	21.3	20.41	20.71	18.85	8.46	6.64	1.3	314.15	372.48	310.81
CV%	9.2	11.3	7.7	16.09	12.21	13.59	11.4	32.38	49.46	14.25	8.04	7.41

Trait	Dry matter			Fresh biomass			Dry Yield		
Location	**CC**	**CS**	**HW**	**CC**	**CS**	**HW**	**CC**	**CS**	**HW**
Genotype	102.05**	20.37	47.04**	1921.99**	4245.68**	662.95**	140.62**	239.7**	38.54**
Rep	0.05	19.69	3.39	4177.31**	1690.69	1336.06**	235.86*	34.92	9.41
Error	17.06	15.00	10.87	591.84	1504.63	149.59	50.88	91.53	12.2
Root MSE	4.13	3.77	3.3	24.33	38.79	12.23	7.13	9.57	3.49
G. Mean	26.5	23.57	20.81	72.79	106.71	79.42	19.05	24.56	16.58
CV%	15.59	15.98	15.85	33.42	36.35	15.4	37.45	38.96	21.07

*Significant difference at level of 0.05% of error probability

**Significant difference at level of 0.01% of error probability

Table 2.3. Analysis of variance (Type III mean squares), Shapiro Wilk normality test value (W), CV% and repeatability of 39 PS-biomass sorghum genotypes evaluated in three location Corpus Christi (CC), College Station (CS) and Halfway (HW), in Texas, 2010.

Source of Variation	Ash	Lignin	Xylan	Celullose	Solubles	Stem dia.	LG†	Height	Dry matt.‡	Fresh yield	Dry yield
Genotype	2.03*	1.84*	2.29*	10.53*	11.86*	32.74*	2.95	8464.04*	110.20*	3819.32*	237.44*
Error Genotype	0.45	0.74	0.61	3.01	2.40	10.14	2.30	2238.53	24.32	1544.31	78.53
Location	35.26*	79.81*	18.04*	8.33	568.67*	111.72	1622.88*	122086*	736.02*	30827*	1273.34*
Error Location	0.73	4.74	3.44	13.48	19.48	17.76	5.68	7620.26	51.82	2100.56	110.78
Rep[Loc]	0.63	4.57*	3.23*	12.59*	20.32*	23.54*	5.31*	6592.11*	42.23*	1332.07	87.91
Genotype x Location	0.46	0.75*	0.61*	3.02*	2.40	10.17	2.30	2252.92*	24.61*	1552.47*	79.30*
Error	0.35	0.49	0.34	1.88	2.92	7.80	1.89	1082.75	13.33	744.30	52.82
W	1.00	0.97	0.97	0.97	0.98	0.96	0.91	0.99	0.93	0.96	0.96
CV%	7.19	4.67	3.30	4.22	9.15	14.00	25.21	9.90	15.31	31.64	36.16
Repeatability	0.26	0.14	0.25	0.22	0.22	0.18	0.03	0.27	0.31	0.17	0.18
Grand Mean	8.26	14.99	17.71	32.46	18.65	19.95	5.45	332.35	23.85	86.23	19.73
Minimum	6.42	12.91	15.43	27.64	13.75	10.00	1.00	127.00	12.20	17.56	3.96
Maximum	10.37	17.59	19.55	36.62	24.21	27.00	9.00	446.19	48.02	187.03	43.27

*Significant different at the level of 5% of probability or less.
†LG=Plant lodging.
‡Dry matt. = percentage of dry matter in the plants.

Of the effects in the combined analysis, the location effect was proportionally larger than any other source of variation partitioned in the analysis. For lignin the location effect was approximately 30 times greater than other significant sources (genotype and genotype x location). For dry biomass yield, the location effect was four times greater than either the genotype or genotype x location effects. The results indicate that while genotype does influence yield and quality potential, the location ultimately has greater influence (Table 2.3). Similar findings regarding the large effect of environment on compositional traits of sorghum are reported by (Murray et al., 2008a; Corn 2009; Packer 2011).

The genotype x environment interaction was significant for the compositional traits of lignin, xylan and cellulose as well as the agronomic traits plant height, dry matter, fresh yield and dry yield (Table 2.3). In this study, several interactions were of significant importance as well. For example, the lignin composition for the hybrid genotype (ATx2928/BTx2752//R07020) was among the highest of any entries in CC and HW, but in CS it was one of the lowest ranking entries (Table 2.3). This interaction is important in that it demonstrates the complexity of composition in genotypes and it points to the importance of selection of environments and screening methodologies for these traits.

For most traits, estimates of repeatability were low (Table 2.3). The average repeatability for the composition traits was 22%, with a range of 14% (lignin concentration) to 26% (ash concentration). For the agronomic traits, the average repeatability value was 19%, with a range of 0% (no lodging in Corpus Christi and College Station) and a 27% (plant height) (Table 2.2). The repeatability of fresh and dry biomass yield were 17 and 18 percent respectively, because this traits in sorghum are considered to be quantitative traits (Murray et al., 2008a), controlled by multiple genes, and were affected by environmental conditions, this generally reduce the scores of repeatability. The low repeatability values reflect the significant GXE effect on performance for these traits.

Contrast analysis and correlation

Contrast analysis detected statistical differences between the high and low-lignin groups for several traits (Table 2.4). Lignin concentration was statistically higher in the high lignin selection group indicating that prescreening can delimit genotypes into categories that are repeatable in future environments. In addition to the difference in lignin, the groups selected based on lignin also varied for other traits (Table 2.4). For example, the high lignin group had higher xylan and cellulose concentrations than the low lignin group. Given that these compounds are all structural carbohydrates, plant cell wall and have high level of molecular interaction (Somerville et al., 2004), thus it is not surprising that they would trend similarly. This association also implies that increasing one of these compounds concomitantly increases the others, making it difficult to breed

specifically for one trait and not the other. The high lignin group was taller and produced greater fresh and dry biomass yields than the low lignin group (Table 2.4). Across all environments, the high lignin lines averaged 4.42 $Mg*ha^{-1}$ more than the low lignin group. Some of the increase in biomass was associated with height; the high lignin group was 13cm taller than the low lignin group.

Several correlations were detected among measured traits (Table 2.5). Positive correlations existed among lignin, xylan, and cellulose. Interestingly, a positive correlation was present between these traits and lodging as well. This correlation suggests that plants with more lignin concentration would have higher levels of lodging and is opposite of what would predicted for this relationship. One explanation is that genotypes with more lignin become less flexible and under high wind conditions they would break more than genotypes with intermediate lignin content, more studies need to be done in order to verify this. An alternative explanation is that these plants were generally taller.

Table 2.4. Contrast analysis of two groups with 18 high against 18 low lignin genotypes of PS sorghum, regarding composition and field traits, harvested in three locations Corpus Christi, College Station and Halfway, in Texas 2010.

High vs. Low	Ash	Lignin	Xylan	Cellulose	Solubles	Stem Dia.	LG	Height	Dry Matter	Fresh Yield	Dry Yield
Sum Square	0.68	8.68	15.19	38.71	0.35	0.01	1.26	14085.00	11.02	17965.00	1185.00
F Ratio	0.83	6.83	20.02	11.58	0.04	0.00	0.11	4.81	0.29	12.59	13.35
Prob>F	0.36	0.01	<.0001	0.00	0.83	0.98	0.74	0.03	0.59	0.00	0.00

Means of	%	%	%	%	%	mm	(1-9)	cm	%	$Mg*ha^{-1}$	$Mg*ha^{-1}$
High Lignin	8.23 A	15.15 A	17.91 A	32.79 A	18.75 A	20.06 A	5.35 A	335.30 A	23.90 A	92.55 A	21.68 A
Low Lignin	8.33 A*	14.81 B	17.46 B	32.08 B	18.82 A	20.05 A	5.48 A	321.66 B	23.49 A	77.30 B	17.44 B

*Means in a column followed by different letters are significant different at a probability of 0.01.
LG=lodging

Table 2.5. Person's correlation (r) from combined data of CC, CS and HW locations for compositional and field related traits on biomass PS sorghum genotypes, in Texas, 2011.

Ash	-0.63*	-0.12*	0.00	0.43*	0.00	-0.42*	-0.37*	-0.05	-0.22*	-0.26*
	Lignin	0.68*	0.48*	-0.75*	0.07	0.58*	0.29*	-0.04	0.07	0.21*
		Xylan	0.79*	-0.29*	0.05	0.22*	-0.05	-0.15*	-0.16*	-0.06
			Celullose	-0.28*	0.03	0.13*	-0.10	-0.14*	-0.21*	0.02
				Solubles	-0.03	-0.60*	-0.39*	-0.05	-0.18*	-0.31*
					Stem Dia.	0.25*	-0.29*	0.23*	0.09	0.23*
						LG	0.31*	0.02	0.14*	0.17*
							Dry Matter	-0.10	0.27*	-0.04
								Fresh Yield	0.90*	0.39*
									Dry Yield	0.41*
										Height

*Statistically significant at the level of probability of 0.05%.

Lignin content was not correlated to fresh or dry biomass yield and modest negative correlations were detected for xylan and cellulose with fresh and dry biomass yield. These results appear opposite to the observations in the contrast analysis. Taken together, it may indicate that high biomass yield potential is independent of lignin concentration. Further study is required to confirm this observation and individual genotypes may be identified or bred that break this relationship.

Lignin concentration in the whole plant was highest in CC and lowest in HW (Table 2.6). The difference demonstrate the wide range of variation caused by environment and similar to those reported by Packer (2011) where lignin concentration vary across locations and at CS lignin levels in average were (14.4%), CS dryland (11.3%), CC lignin (14.6) and HW (10.6%) in that study.

While the overall significant differences in lignin concentration appear small (1.15% difference between the high and low groups), small shifts in composition can have profound impacts on performance and productivity. For example, a 1 percent change in lignin content amounts to 10 $kg*T^{-1}$ of the compound. If average of biomass production is 20 $Mg*ha^{-1}$, that equals 200 $kg*ha^{-1}$ of lignin that will be in the feedstock loads and that cannot be converted to ethanol using existing technologies.

Table 2.6. Least significant difference means separation test of percent lignin of 39 different PS sorghum genotypes, color code yellow pre-classified as low lignin and green as high lignin content, in three locations Corpus Christi (CC), College Station (CS) and Halfway (HW), in Texas 2010.

Lignin%								
CC			CS			HW		
LSD	1.25%		LSD	1.02%		LSD	1.12%	
Genotype	Mean		Genotype	Mean		Genotype	Mean	
R.09101	13.64		R.09094	14.21		R.09116	12.91	
R.09092	14.35		R.09117	14.32		R.09085	12.96	
R.08028	14.44		R.08028	14.36		R.09114	13.11	
R.09089	14.55		R.09114	14.48		R.09112	13.14	
R.09087	14.76		R.09085	14.69		R.09117	13.19	
R.09078	14.94		R.09098	14.77		R.08028	13.28	
R.09088	14.98		R.09101	14.85		R.09075	13.31	
R.09116	15.00		ATx2928/BTx2752//R07020	14.85		R.09103	13.46	
R.09104	15.02		R.09077	14.90		R.09092	13.50	
R.09102	15.19		R.09076	14.91		R.09093	13.67	
R.09103	15.22		R.09088	15.00		R.09101	13.72	
R.09114	15.32		R.09087	15.08		R.09102	13.77	
R.09090	15.39		R.09075	15.11		R.09094	13.78	
R.09095	15.53		R.09110	15.12		R.09077	13.78	
R.09077	15.56		R.09078	15.15		R.09089	13.79	
R.09075	15.59		R.09106	15.20		R.09104	13.79	
R.09098	15.59		R.09116	15.22		R.09079	13.86	
R.09112	15.59		R.09102	15.24		R.09078	13.95	
R.09109	15.59		R.09089	15.31		R.09088	13.97	
R.09094	15.68		R.09095	15.42		R.09110	14.05	
R.09093	15.70		R.09115	15.43		R.09106	14.1	
R.09106	15.71		R.09080	15.44		R.09099	14.16	
R.09115	15.82		R.09092	15.45		R.09090	14.17	
R.09099	15.85		R.09109	15.49		R.09087	14.26	
R.09079	15.88		R.09090	15.49		R.09095	14.3	
R.09083	15.96		R.09103	15.50		R.09076	14.31	
R.09110	15.97		R.09079	15.51		R.09109	14.35	
ATx2928/BTx2752//R07020	16.08		R.09105	15.56		R.09096	14.40	
R.09084	16.10		R.09099	15.58		R.09080	14.41	
R.09096	16.15		R.09084	15.58		R.09084	14.49	
R.07020	16.29		R.09112	15.76		R.09098	14.53	
R.09105	16.33		R.09081	15.78		R.09083	14.57	
R.09117	16.35		R.09096	15.82		R.09115	14.58	
R.09108	16.43		R.09108	15.83		R.09081	14.66	
R.09091	16.51		R.09104	15.85		R.09108	14.68	
R.09080	16.73		R.09093	15.93		R.09091	14.84	
R.09081	16.81		R.07020	15.98		R.09105	14.90	
R.09076	17.11		R.09083	15.98		ATx2928/BTx2752//R07020	15.24	
R.09085	17.59		R.09091	16.37		R.07020	15.52	
Location mean	15.67	A*		15.30	B		14.04	C

*Means followed by different letter are significant different at level of 5% of probability, student's-T test.

Consistency of entry based on pre-classification

While the preselected high and low lignin groups could be differentiated, there was overlap for lignin concentrations on an entry mean basis (Table 2.6). For each location, entries originally classified as high were reported as low in that environment. Some of these inconsistencies are likely due to environment variation both in the pre-selection evaluation and in the currently reported environments. Even though the initial evaluation was completed in CS, results from CS in the following year indicate that any given entry was still subject to variation across environments. The results further confirm the importance of multi-location testing and that pre-classification of a set of germplasm based on single replication data is possible but that pre-classification of a single entry is not likely feasible.

Genotype variation for dry biomass yield was present and with significant level of GXE interaction, there were changes in the ranking of lines at each location for this trait (Table 2.7). The biomass yield ranged from a low yield of 3.96 $Mg*ha^{-1}$ (for the line R09116 at CC) to a high yield of 43.27 $Mg*ha^{-1}$ (for the line R09088 at CS) (Table 2.7). Across locations, CS was the best, with an average yield of 24.56 $Mg*ha^{-1}$, followed by CC and HW with average yields of 19.09 and 15.58 $Mg*ha^{-1}$ of dry biomass, respectively. Yields in CC were slightly lower because this trial was rainfed only and periods of dry weather reduced total growth. In addition, the test was planted later than optimal to ensure vegetative growth resulting from a photoperiod sensitive response. In HW, yields likely were lower due to the shorter duration growing season.

The biomass yield averages observed herein are comparable to the biomass yield of other potential energy crops such as *Miscanthus x gigantus* with reported yields of 20-30 $Mg*ha^{-1}$ (Somerville et al., 2010) and switchgrass (*Panicum virgatum*) reported to yield around 6-11 $Mg*ha^{-1}$ (Lemus et al., 2002; McLaughlin and Adams, 2005). In a study comparing diverse types of bioenergy crops the sorghum PS variety 1990CA had dry biomass average yield of 24.6 Mg ha-1 (Propheter, 2009). In our study with season duration of 4-5 months, some of the PS sorghum lines were able to yield dry biomass in the same range found in other biomass dedicated crops (Table 2.7). This indicates the level of dependence of this crop if used as a feedstock for bioenergy production.

The variation and effects of genotype and location combined with the GXE interactions are relevant information for future breeding and selection of PS sorghums for biomass yield. Environment variations include numerous natural and artificial factors, which are intrinsic to the managing of the crop at each location, duration of the season, distance between rows, and harvest methods. The difference in performance of environments (locations) can be explored to choose the best line or genotype to be used in each location. Furthermore, it is possible to breed such lines or genotypes for target environments (Yan et al., 2000).

Table 2.7. Least significant difference means separation test of dry biomass yield of 39 different PS sorghum genotypes, in three locations Corpus Christi (CC), College Station (CS) and Halfway (HW), in Texas 2010.

Dry Biomass Yield Mg*ha^{-1}								
CC			**CS**			**HW**		
LSD	11.59		**LSD**	15.47		**LSD**	5.76	
Genotype	**Mean**		**Genotype**	**Mean**		**Genotype**	**Mean**	
R.09116	3.96		R.09112	5.86		R.09112	8.99	
R.09085	4.35		R.09102	7.17		R.09085	10.04	
R.09101	4.47		R.09104	8.54		R.09104	10.60	
R.09117	8.12		R.09077	9.52		R.09117	11.02	
R.09077	9.24		R.09093	10.01		R.09084	11.55	
R.09112	9.37		R.09099	11.15		R.09116	12.04	
R.09104	10.29		R.09085	13.71		R.09078	12.38	
R.09114	10.48		R.09096	15.00		R.09102	12.82	
R.09076	11.84		R.09116	15.65		R.09099	12.86	
R.09080	14.21		R.09114	16.45		R.09075	13.52	
R.09084	14.65		R.09087	17.40		R.09101	13.75	
R.09110	15.98		R.09117	18.41		R.09114	14.31	
R.09106	16.31		R.09084	19.42		R.09077	14.39	
R.09089	17.10		R.09080	20.11		R.09088	14.61	
R.09083	17.46		R.09101	21.34		R.09089	14.85	
R.09081	18.11		R.09078	22.19		R.09103	15.42	
R.09093	18.59		R.09098	22.55		R.09081	15.58	
R.09102	18.98		R.09091	22.83		R.09091	15.62	
R.09087	19.10		R.09081	22.90		R.09087	15.83	
R.07020	19.70		R.09106	23.85		R.09076	15.96	
R.09096	19.83		R.09075	24.14		R.09110	16.23	
R.09075	20.91		R.08028	25.05		R.09106	16.25	
R.09105	21.22		R.09083	26.13		R.09080	16.48	
ATx2928/BTx2752//R07020	21.79		R.09092	26.57		R.09108	16.95	
R.09108	21.87		R.09079	28.20		R.09096	17.28	
R.09090	22.58		R.09076	28.67		ATx2928/BTx2752//R07020	17.38	
R.09078	22.83		R.09089	28.70		R.09094	17.62	
R.09094	22.91		R.09105	28.89		R.09115	17.90	
R.09109	23.02		R.07020	29.83		R.09095	18.29	
R.09098	23.17		R.09103	29.95		R.08028	18.37	
R.09095	23.37		R.09090	33.50		R.09093	20.13	
R.09088	23.70		R.09095	34.32		R.09079	20.83	
R.09091	25.81		R.09094	35.29		R.09098	20.85	
R.08028	27.05		R.09115	36.87		R.09105	22.97	
R.09092	27.57		R.09109	38.65		R.09092	23.19	
R.09103	28.75		R.09108	39.71		R.09109	23.20	
R.09099	29.57		ATx2928/BTx2752//R07020	40.31		R.09083	23.73	
R.09115	30.10		R.09110	40.44		R.09090	23.76	
R.09079	30.65		R.09088	43.27		R.07020	24.63	
Location mean	19.05	B*		24.56	A		15.58	B

*Means followed by different letter are significant different at level of 5% of probability, student's-T test.

On average, PS sorghum genotypes were 3.32 meters tall (Table 2.3). When compared to other sorghum types (sweet, forage and grain), PS sorghum has been consistently the tallest group (Rooney and Aydin, 1999; Rooney et al., 2007; Corn, 2009; Packer, 2011). In this study, a different genotype was the tallest in each location (Table 2.8). The change in rank of genotypes confirms the existence of GXE interaction of importance. The location of CS had the highest mean for plant height and average was 372.48 cm (Table 2.8). This difference on averages among location could be explained by the differences in water supply and season length, CS had the longest season of the three environments and had irrigation to establish and maintain the crop. The changes in ranking across the three locations thus reveals the different levels of adaptation of each genotype with confirmed change in rank for the height trait, and expressing the existence of GXE interaction of importance for this trait.

Table 2.8. Least significant difference means separation test of plant height (cm) of 39 different PS-sorghum genotypes, in three locations Corpus Christi (CC), College Station (CS) and Halfway, in Texas 2010.

Plant Height cm							
CC			**CS**			**HW**	
LSD	73.05		**LSD**	48.78		**LSD**	37.45
Genotype	**Mean**		**Genotype**	**Mean**		**Genotype**	**Mean**
R.09112	127.00		R.09112	266.70		R.09112	220.13
R.09085	234.95		R.09102	279.40		R.09104	258.23
R.09080	262.47		R.09104	279.40		R.09102	266.70
R.09094	270.93		R.09093	317.50		R.09116	270.93
R.09078	273.05		R.09099	325.97		R.09091	287.87
R.09104	273.05		R.09114	330.20		R.09096	287.87
R.09116	279.40		R.09078	338.67		R.09103	287.87
R.09110	281.09		R.09101	347.13		R.09099	292.10
R.09106	287.87		R.09077	349.25		R.09075	296.33
R.09101	292.10		R.09105	355.60		R.09087	296.33
R.09114	292.10		R.09106	355.60		R.09093	296.33
R.09084	300.57		R.09075	357.29		R.09101	298.03
R.09105	300.99		R.09098	357.29		R.09090	300.57
R.09077	309.03		R.09076	364.07		R.09078	309.03
R.09079	309.03		R.09083	364.07		R.09085	309.03
R.09096	309.03		R.09096	364.07		R.09098	309.03
R.09102	309.03		R.09079	368.30		R.09108	309.03
R.09076	311.15		R.09095	368.30		R.09088	313.27
R.09108	311.15		R.09103	368.30		R.09095	313.27
R.09089	313.27		R.09087	376.77		R.09105	313.27
R.09090	313.27		R.09090	376.77		R.09106	313.27
R.09117	317.50		R.09084	377.61		R.09079	317.50
R.09099	321.73		R.09109	381.00		R.09084	317.50
R.09075	325.97		R.09108	385.23		R.09089	317.50
R.09083	329.35		R.09115	385.23		R.09080	321.73
R.08028	330.20		R.09092	389.47		R.09092	321.73
R.09088	330.20		R.09094	389.47		R.08028	325.97
R.09091	330.20		R.09089	397.93		R.09110	325.97
R.09087	334.43		R.09081	406.40		R.09076	330.20
R.09098	334.43		R.09085	406.40		R.09109	330.20
R.09103	342.90		R.09088	406.40		R.09114	330.20
R.09109	342.90		R.09110	406.40		ATx2928/BTx2752//R07020	334.43
R.09093	351.37		ATx2928/BTx2752//R07020	412.33		R.07020	334.43
R.09092	355.60		R.08028	419.10		R.09077	334.43
R.09095	359.83		R.09080	419.10		R.09094	334.43
R.09115	359.83		R.09117	419.10		R.09115	334.43
R.07020	385.23		R.09091	420.79		R.09081	342.90
R.09081	400.05		R.09116	427.57		R.09083	359.83
ATx2928/BTx2752//R07020	419.10		R.07020	446.19		R.09117	359.83
Location mean	314.15	B*		372.48	A		310.82 B

*Means followed by different letter are significant different at level of 5% of probability, student's-T test.

Stability and GGE analysis

For stability analysis a graphic representation of the variation was done with biplot graphs (Fig. 2.1 and 2.2). In this analysis, genotypes that performed with higher values are plotted to the right of the graph, for example on figure 2.1 the genotype with higher lignin concentration (R.07020) was plotted on the right end of the graph, on the opposite side the genotype with lower concentration of lignin across the three environments (R.08028). Furthermore, the genotypes that are far from the overall mean hypothetical line (red line) are less stable across all three environments (Yan and Tinker, 2006). In the graph for dry yield for example (Fig. 2.2) the line R.09108 was an example of high yield stability across environments and the line R.09117 was the least stable for this trait.

For lignin content in plants across the three locations there was a grouping of low lignin lines at (R.09101, R.09114, R.09116 and R.09117) and a grouping of high lignin content lines on the opposite end of the GGE biplot (R.09091, R09081, R.09108 and R.09105) (Fig 2.1). For the remain of entries there was blending of both high and low lignin genotypes, indicating that pre-classification may work for identifying a core group of material but is less accurate in delimiting specific genotypes. For dry biomass yield the biplot presents a specific grouping delineation (Fig. 2.2). Genotypes classified in the high lignin group tended to group in the higher yield area while genotypes classified as low lignin were mostly in the lower yielding region, confirming results of the contrast analysis (Table 2.2).

The relationship between lignin concentration and biomass yield was not strong, and the classification for lignin concentration has changed for some of the genotypes. Using the data collected in this set of trials it was possible to identify genotypes with both phenotypes low lignin and high lignin based in the actual score of lignin% (Fig. 2.1) and biomass yield performance and stability across environments (Fig. 2.2).

The stability of performance of dry biomass yield (Fig. 2.2) was variable for the different genotypes tested, because the existence of GXE interactions most genotypes had great variability in performance across locations. However, it was possible to define a group of genotypes with high biomass yield potential and stability across location. The genotypes R.0109, R.09090, R.09015, R.09079, R.09092 and the tester R.07020 were consistently high yielding across the three environments. These genotypes are considered good candidates for future breeding to further improve yield in biomass.

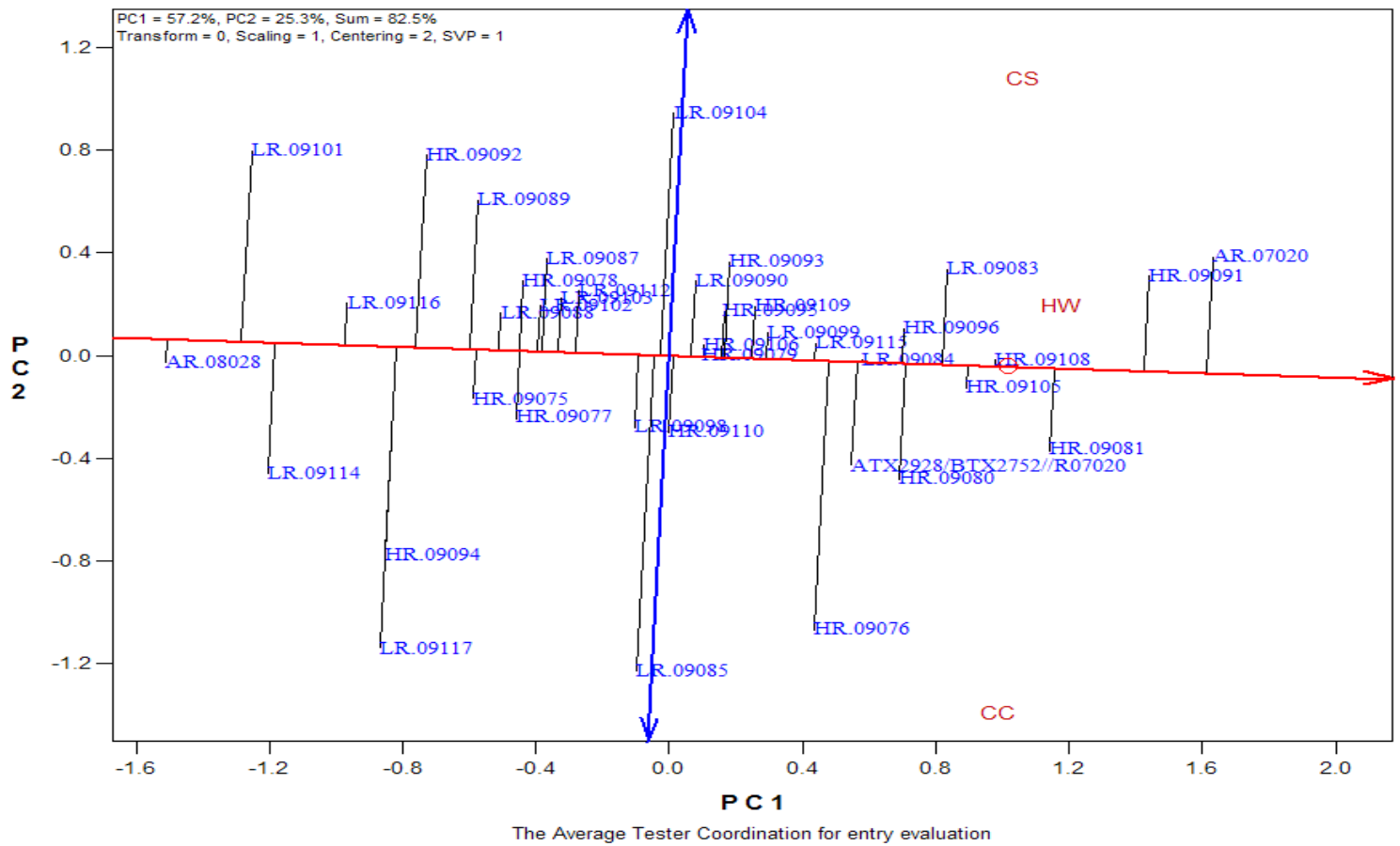

Figure 2.1. GGXE biplot of the lignin% of 39 different PS-sorghum genotypes pre-classified as low lignin (L) and high lignin (H) content and agronomic fit genotypes (A), in three locations Corpus Christi (CC), College Station (CS) and Halfway (HW), in Texas, 2010. The stability graphs are based in the variance within the three environments tested, the average-environment coordination (AEC) in the abscissa is represented by the red line in the graph and points towards the higher mean values in average. The blue double arrow line (ordinate) points to greater variability or poorest stability on both directions (Yan and Tinker, 2006).

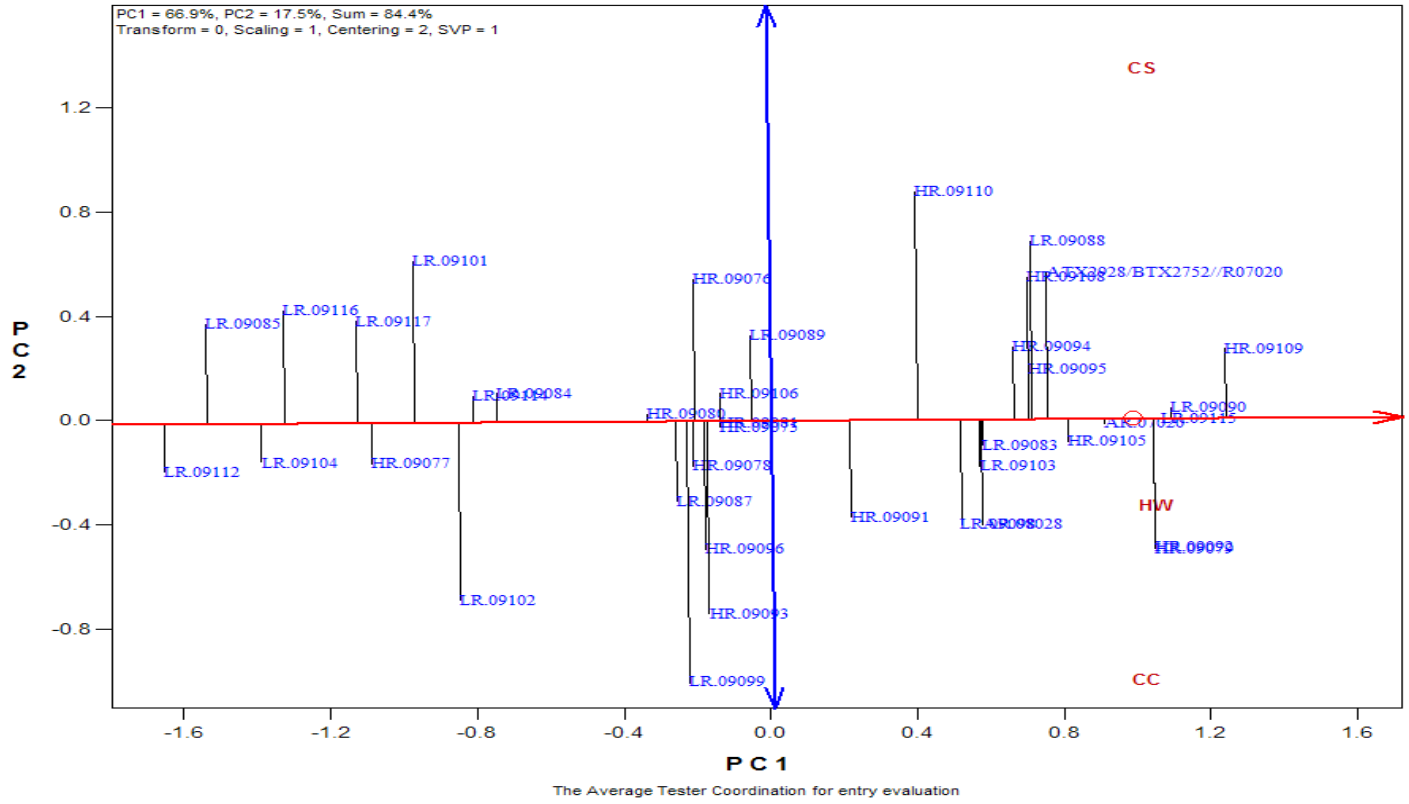

Figure 2.2. GGXE biplot of the dry biomass yield of 39 different PS-sorghum genotypes pre-classified as low lignin (L) and high lignin (H) content and agronomic fit genotypes (A), in three locations Corpus Christi (CC), College Station (CS) and Halfway (HW), in Texas, 2010. The stability graphs are based in the variance within the three environments tested, the average-environment coordination (AEC) in the abscissa is represented by the red line in the graph and points towards the higher mean values in average. The blue double arrow line (ordinate) points to greater variability or poorest stability on both directions (Yan and Tinker, 2006).

When analyzing both graphs (Fig. 2.1 and 2.2) genotypes were classified into two categories; (1) genotypes with high lignin content and high biomass yield (R.07020) and (2) genotypes with low lignin and high biomass yield (R.09092).

High lignin content in the plant is a problem when the biomass is intended to be used for biofuel conversion. If combustion or gasification is the conversion approach lignin content is less of a concern. In the latter methods, high lignin genotypes are even desirable as lignin has a higher BTU content. In breeding programs the two categories are useful depending on the conversion approach and if genetic studies are done to understand the lignin concentration trait the extreme genotypes can be used for such studies.

In summary, compositional and agronomic traits in PS sorghum had sufficient genetic variation to support breeding and selection. Similarly, traits were highly influenced by environment conditions showing the importance of agronomics and the need for multi-location experiments when using this crop. The pre-classification stage was helpful to identify the extremes for lignin content and good performing genotypes as a pre-stage of breeding systems. The relationship between lignin content and biomass yield exist; however it was not strong, allowing the identification of high and low lignin content genotypes and good yield and stability for biomass production. The identified superior genotypes can be used in sorghum breeding programs intended for biomass production as a source of variability for lignin content and biomass yield, although, low heritability (repeatability) of these traits may become a limitation.

CHAPTER III

BIOMASS ACCUMULATION AND COMPOSITION OF SIX PHOTOPERIOD SENSITIVE SORGHUM LINES

Introduction

The need for reliable, available and efficient sources of energy is critical to the long-term sustainability of modern society. The U.S. Congress enacted "Energy Independence and Security Act of 2007" with the goals to: (1) reduce greenhouse gas emissions by at least 20%, (2) increase the volume of biofuel from 0.6 billion gallons to 21 billion gallons by 2022, (3) increase the volume of cellulosic biofuel from 0.1 billion gallons in 2010 to 16 billion gallons by 2022, and (4) improve the knowledge on renewable fuel products through research (Congress, 2007; Sissine, 2007).

To achieve the goals mandated by this act, the U.S. DOE estimated that the U. S. could produce up to 1.3 billion tons of dry biomass for conversion to biofuels (Perlack et al., 2005). Of this biomass, a significant proportion is derived from biofuel crops which are crops grown specifically as a biomass source for biofuel production. This is critical because most ethanol is now derived from corn (*Zea mays* L.), but corn production alone is not sufficient to meet the goals established by the Act.

Several biofuel crops have been proposed by the scientific community. (Lewandowski et al., 2000) proposed the grass miscanthus (*Miscanthus x giganteus*) as a new lignocellulosic bioenergy crop in Europe. In Brazil, sugar extracted from sugarcane (*Saccharum* ssp.) is the base for ethanol. In the U.S., the perennial native grass

switchgrass (*Panicum virgatum*) has been identified as a dedicated bioenergy crop (McLaughlin and Adams, 2005). Rooney et al. (2007) proposed that specific types of sorghum (*Sorghum bicolor* L. Moench) could be useful as biofuel crop.

Sorghum was domesticated as a crop plant in Northeast Africa and it is well known as a cereal grain and forage crop. As a potential bioenergy crop, sorghum is unique because it has a long history of adaptation, cultivation, breeding and is well integrated in the seed industry (Rooney, 2004). As a crop, sorghum can be divided in five categories; grain sorghum, forage sorghum, broomcorn, sweet sorghum and high biomass sorghum (Rooney et al., 2007). Among these, sweet sorghum and high biomass sorghums are the most likely to make contributions to second generation (lignocellulosic) biofuels.

Biomass sorghums are highly productive because they are very photoperiod sensitive (PS) which allows them to grow in a vegetative stage for long periods. Vegetative growth allows increased biomass accumulation and enhanced drought tolerance. PS lines/hybrids are capable of long periods of vegetative growth before reproductive growth is initiated in response to a reduction in daylength, usually below 12.5 hours (Rooney and Aydin, 1999). Thus, for most temperate and subtropical climates, the initiation of growth does not occur until the fall season and therefore the plant never flowers prior to killing frosts.

As with most crops, maintaining sorghum in a vegetative growth stage increases biomass yields. Quinby and Karper (1945) noted this phenomenon in two sorghum hybrids that were had different gene set for maturity, one had ma1Ma2ma3Ma4 (which

requires 49 days to anthesis) and the other Ma1Ma2ma3Ma4 (which requires 102 days to anthesis). The difference in biomass production between the two was 245g*plant^{-1}, with the higher biomass weight coming from the genotype with three dominant loci and 102 days to anthesis. In another study, sets of crosses between grain types (ma1Ma5ma6) and PS sorghum (Ma1ma5Ma6) demonstrated moderate levels of high-parent heterosis for biomass yield around 40% across environments (Packer, 2011).

Because these biomass sorghums do not produce grain, the primary carbohydrate production is the structural carbohydrates of lignin, cellulose and hemicellulose in the forms of roots, leafs, and most important stems. Additionally, non-structural carbohydrates (sugars), protein and minerals make up the rest of the tissue. Lignin is a primary component of the biomass and acts as a matrix in the cell wall that integrates and surrounds cellulose and hemicelluloses compounds. Because cellulose and hemicellulose are tightly bound in this structure, they are not readily fermentable without a pre-treatment (heat, pH or enzymatic or a combination thereof) (DOE, 2010; Wu et al., 2010). During this pre-treatment, the lignin portion of biomass can bind enzymes and reduce their ability to deconstruct cellulose and hemicellulose to simple sugars, thus reducing efficiencies on multiple levels. For this reason, biomass composition becomes an important component in the development of energy crops.

To measure the composition of biomass, one of the fastest and cheapest methods is to use near infrared spectroscopy (NIR) (Sanderson et al., 1996; Roberts et al., 2011). This technology is based on the fact that infrared light interacts differently with different molecular bonds of substances and when combined with wet-chemistry measurements of

composition trough statistical multivariate analysis tools, it is possible to construct a prediction model for a training population (Vermerris et al., 2007). Thus, NIR-based compositional analyses are commonly used to estimate composition in many different crop and quality applications. These include the amino acid concentration of soybeans (Kovalenko et al., 2006), wheat dough quality (Alava et al., 2001), corn stover (Hames et al., 2003; Pordesimo et al., 2005) and sorghum stem, juice, leaf and grain composition (Murray et al., 2008a; Murray et al., 2008b; Corn, 2009; Packer, 2011).

Together with productivity, the quality of the biomass is important to biofuel conversion production systems. Biomass quality and its stability may be a limiting factor for profitability of the production system. In numerous studies plant biomass composition was found to be variable according to genotype, environment and stage of plant growth effects (Reeves, 1987; Murray et al., 2008a; Corn, 2009; Packer, 2011). An example of growth stage and composition variation is the successful attempt to isolate the effect of flowering time in sweet sorghum when studying quantitative trait loci (Murray et al., 2008b). In another study including various forage crops (alfalfa, corn, tall fescue, orchardgrass and wheat), Reeves (1987) reported changes in composition during the growing season, with increased lignin concentration, as the growing season advanced, implicating decreased digestibility for all forages considered. Pordesimo et al., (2005), during their study of evolution of the composition in corn stover, found similar trends regarding lignin concentration as the crop matures. If biomass sorghum is to become established as a dependable feedstock for biofuel conversion, an

understanding of biomass accumulation and biomass composition evolution over the growing season is key to increasing efficiency and profitability of this operation.

With these factors in consideration, the objectives of this study were (1) to assess the biomass yield accumulation and composition patterns of six photoperiod sensitive sorghum lines over the growing season in College Station and Corpus Christi, Texas, and (2) to identify differences in biomass accumulation and composition among the whole plant, stem and leaves of photoperiod sensitive sorghum while grown in College Station and Corpus Christi, Texas.

Materials and Methods

To assess the ontological effects of maturity on biomass accumulation and composition in biomass sorghums, a randomized complete block design with two replications was used. The variables in the design were maturity and genotype. For genotypes, six different sorghum accessions (R.09072, R.09093, R.09106, R.09084, R.09098 and R.09110) were selected based on desirable characteristics from previous evaluations. All of these lines were photoperiod sensitive with high yield potential but their variation for composition over a growth period was not known. For each genotype and at each location, 13 different harvests were completed with approximately 15 days between harvests at each location. Harvest dates are expressed as days after planting (DAP), College Station (CS) and Corpus Christi (CC), Texas.

Plant populations were overplanted and thinned to a density of 160,600 plants per hectare. Harvesting started at 60 days after planting (DAP) at both locations. For each

harvest date, a 2 meter section was cut, totaling an area of approximately 1.52m^2. Between harvest sections, a buffer section of 2 meters was left to decrease border effect. Harvests occurred in College Station at the dates; June 14 and 28, July 12 and 26, August 9 and 23, September 6 and 20, October 4 and 18, November 1, 15 and 29 in 2010. In CC, harvest dates were June 25, July 9 and 23, August 6 and 20, September 3 and 17, October 1, 15 and 29, November 12 and 26, and December 10. At each harvest date, agronomic data was collected for plant height (cm), total fresh weight, stem fresh weight, leaf fresh weight, and stem diameter (mm). After harvest, whole plot fresh weight was measured with a Cardinal HSCC-500 scale with increments of 50g. For the harvested plot, a sample of three random plants was taken to measure stem diameter and estimate moisture content and total dry weight. Stem diameter was recorded as the average of three random plants measured at the second node from the base of the plant. Three different tissues were sample on each harvest cut, whole plant, plant stems and plant leaves. The plot was processed as follows; 1st whole plot fresh weight was recorded, and then leaves were stripped from plants of the plot. Leaf portion and stem portion of the plot were weighed fresh. 2nd samples of stem and leaf portions were taken, fresh weight were recorded. 3rd each sample was dried separately (whole plant, stems leafs) an air-forced flux drier at 52^{o}C +- 1^{o}C to weight stabilization. Once the samples were dried, they were weighed again to estimate the dry weight of plots (whole plant, stem and leaf portions). After dry weight calculations, samples were ground in a Wiley mill (Thomas Scientific Inc.) so that they passed through a 2 mm sieve. These samples were stored in plastic air-tight bags until scanned using a Foss XDS NIR scanner.

Calibrations were used to estimate composition of ash, lignin, xylan and cellulose on a dry weight basis. The NIR spectroscopy composition analysis was performed using the methodology described methods in Chapter I of this dissertation.

Statistical data analysis was conducted using JMP 9 software (SAS Institute Inc. 2010) and the statistical model (all fixed effects) used was:

$$Y = mean + Rep + genotype + location + DAP + genotype * location + genotype * DAP + location * DAP + genotype * location * DAP + error$$

For the regression analysis of agronomic traits and composition traits best fit for the regression curves was achieved using a cubic model for agronomic data and a quartic model for composition data.

Results

Analysis of variance for agronomic traits

In the combined analysis, the genotypes varied for plant height, stem diameter, fresh and dry biomass yield for the whole plant and fresh and dry biomass yield for stem and leaf portion of the plant (Table 3.1). Location was a significant source of variation for many traits including fresh biomass yield for the whole plant, stem and leaves, and for the trait dry biomass yield of the leaves, but it was not significant for several other traits (Table 3.1). Also as expected, DAP accounted for the largest proportion of

variation and it influenced all the traits with the exception of stem diameter. The effects of all three factors reflect different main effects and their influence on agronomic productivity. Interactions of the main effect were few and relatively small. First order interaction were detected for stem diameter (genotype x location, and location x DAP) and for dry biomass yield and fresh and dry yield of leaves (location x DAP).

Analysis of variance for compositional traits

In the combined analysis of compositional traits, genotype was significant source of variation for ash content (leaves only) and lignin (whole plant, stem and leaf) xylan and cellulose (stem only) (Table 3.2). The significant source of variation for genotype indicates that the genetic background of the lines influences the performance of the line. Location had a profound effect on composition, affecting every trait except for cellulose (stem only).

Table 3.1. Summary of ANOVA, means square values for the field data collected from six different photoperiod sensitive sorghum lines, harvested at 11 different dates in two locations, Corpus Christi and College Station, in Texas, 2010.

		Whole plant				Plant Stem		Plant Leaf	
Source	**DF**	**Height**	**Stem Dia.**	**Fresh Yield**	**Dry Yield**	**Fresh Yield**	**Dry Yield**	**Fresh Yield**	**Dry Yield**
Error	130	1320.90	6.99	459.52	34.64	378.02	21.37	13.87	3.98
Genotype	5	4774.50*	235.32*	1566.02*	159.97*	1442.30*	86.89*	73.68*	82.75*
Location	1	1463.50	7.39	4080.83*	134.84	2386.55*	30.87	178.96*	73.83*
DAP	10	197679.90*	11.18	3812.11*	803.40*	3306.65*	676.10*	209.97*	276.38*
GxL	5	2997.00	19.07*	380.73	18.43	281.75	29.44	15.55	20.75
GxDAP	50	1642.50	9.79	362.40	33.68	274.31	27.22	12.92	146.60
LxDAP	10	1596.50	20.53*	715.38	140.85*	544.26	40.59	54.48*	91.81*
GxLxDAP	50	1590.50	6.42	263.30	21.04	183.47	15.58	14.59	214.23
Rep	1	1154.60	9.89	2604.13*	373.62*	2755.27*	402.49*	6.36	1.46
Mean		311.98 cm	20.29 mm	72.06†	17.46	60.49	12.74	11.40	4.68
R^2		0.93	0.73	0.61	0.76	0.61	0.80	0.73	0.65
CV%		11.65	13.03	29.75	33.71	32.14	36.28	32.69	43.32

* Significantly different at level of 0.05 of probability.

† Mean of yield data expressed in $Mg*ha^{-1}$

For most traits, location accounted for most of the variation in this experiment. For example, for whole plant ash content the location variation was twice that of the genotype effect while for lignin content, location variation was five times greater than genotypic variation, which has been previously reported (Murray et al., 2008a; Corn, 2009; Packer, 2011). Harvest date expressed as DAP was the most significant source of variation all except a few traits. While this phenomena is not new (Reeves, 1987), these observations confirm that sorghum biomass composition changes over the growing season even in the absence of reproductive growth.

The location x DAP interactions were significant for all traits measured emphasizing that both environment and stage of growth interact to effect composition. Interactions involving genotype were significant for xylan in the whole plant (G x L), ash in the stem (G x L) and lignin and xylan in the whole plant (G x DAP). These interactions were all smaller in magnitude then the L x DAP interactions, but the G x DAP, interactions changed the relative rank order of genotypes in the test as the season progressed. This would impact a plant breeding program as it attempts to identify desirable genotypes for advancement and development. The second order interaction was not significant for any composition trait.

Table 3.2. Summary of ANOVA, mean square values for the composition data collected with NIR spectroscopy from six different photoperiod sensitive sorghum lines for the whole plant, plant stem and plant leaf portions, harvested at 12 different dates in two locations, Corpus Christi and College Station, in Texas, 2010.

		Whole Plant				Plant Stem				Plant Leaf			
Source	**DF**	**Ash**	**Lignin**	**Xylan**	**Cellulose**	**Ash**	**Lignin**	**Xylan**	**Cellulose**	**Ash**	**Lignin**	**Xylan**	**Cellulose**
Error	132	0.82	0.7	0.32	2.25	0.81	1.45	1.07	4.05	0.34	0.34	0.12	3.28
Genotype	5	1.57	1.78*	0.44	2.79	0.4	10.05*	7.23*	17.13*	2.70*	1.75*	0.27	15.17*
Location	1	3.32*	3.10*	2.65*	46.04*	9.97*	51.72*	7.58*	1.15	2.96*	22.41*	9.96*	157.71*
DAP	11	24.01*	19.07*	3.08*	16.62*	28.71*	39.08*	13.08*	85.40*	17.97*	6.35*	1.08*	75.00*
GxL	5	0.32	1.50	0.86*	4.12	1.90*	2.43	1.72	5.38	0.47	0.11	0.04	1.84
GxDAP	55	0.72	1.01*	0.48*	3.19	0.9	1.28	0.95	2.94	0.28	0.3	0.12	2.14
LxDAP	11	4.81*	6.40*	2.88*	23.99*	5.87*	12.59*	6.25*	24.03*	3.89*	2.55*	0.64*	32.34*
GxLxDAP	55	0.69	0.43	0.26	2.71	0.83	0.99	0.65	3.36	0.46	0.33	0.13	3.34
Rep	1	0.75	5.53*	2.73*	12.50*	15.21*	16.21*	14.27*	39.39*	1.52*	2.30*	0.37	9.56
Mean		7.72†	14.31	16.95	31.13	6.61	14.31	16.51	31.14	10.22	12.74	16.48	30.6
R^2		0.79	0.81	0.74	0.75	0.82	0.8	0.71	0.76	0.87	0.79	0.74	0.80
CV%		11.73	5.85	3.34	4.82	13.57	8.42	6.28	6.46	5.69	4.55	2.12	5.92

* Significant different at level of 0.05 of probability.

† Mean of composition data expressed in % of dry biomass.

Effect of harvest date on agronomic productivity

Cubic regression model produced the best model for the effect of DAP on agronomic traits with varying levels of relative efficiency (as measured by the coefficient of determination (R^2))

Plant height and biomass accumulation patterns followed a standard growth curve shape with an initial lag phase as the plants develops, followed by a log phase of growth (fast and exponential) which eventually slowed and stabilized. For these biomass sorghums, the 60 to 120 DAP were the log phase where plants grew about 50 cm per week (Fig. 3.1) and between 120 and 150 DAP, the rate of growth slowed and stabilized.

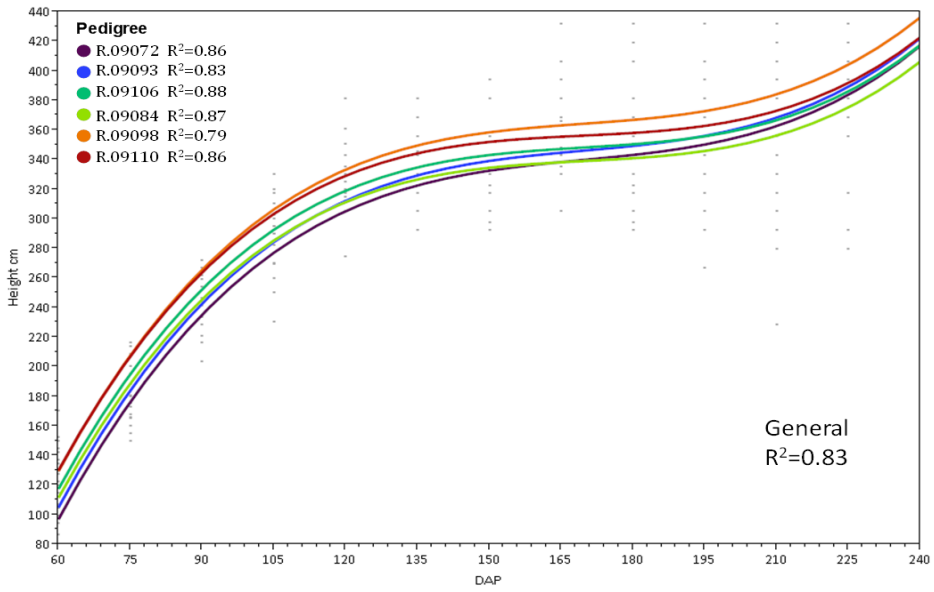

Figure 3.1. Cubic regression for plant height of six genotypes of biomass PS sorghum in combined CC and CS locations, in Texas, 2010.

These dates (120 to 150 DAP) coincided with typical late summer drought periods which are common in South and Central Texas. However, as rainfall occurred in late summer, there was a consistent trend among all genotypes to resume growth which ultimately further increased yields towards the end of the season.

Whole plant fresh biomass yield curves (Fig. 3.2) were presented separately due to significant location effects but a similar curve shape was observed on both locations. A peak in fresh yield for the whole plant was around 120 DAP, and values reached 96 Mg*ha^{-1} in CC and 92 Mg*ha^{-1} in CS. At 120 DAP, the approximate content of water was 77% of the fresh matter or 74 Mg*ha^{-1} of water. A decreasing slope was found to follow the mark of 120 DAP and lowest level for whole plant fresh biomass yield was at 210 to 225 DAP for both locations.

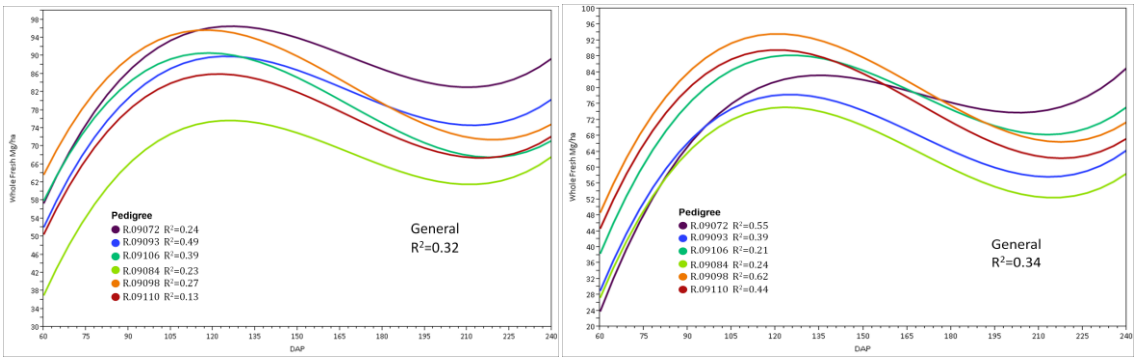

Figure 3.2. Cubic regression for whole plant fresh biomass yield of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010.

The whole plant dry biomass yield curve was similar to the plant height curve, but it had a defined flat period with little to no variation where dry biomass values did not changed. The dry biomass yield peaks at 150 DAP and is static until 195 DAP wherein a second increase on yield is observed that continues up to the final harvest. At 150 DAP the highest yield was approximately 22 Mg*ha^{-1} for R.09098. At 240 DAP, the highest dry yield was produced by genotype R.09072 at approximately 27 Mg*ha^{-1}, followed by the line R.09098 with a yield of 24 Mg*ha^{-1} (Fig. 3.3).

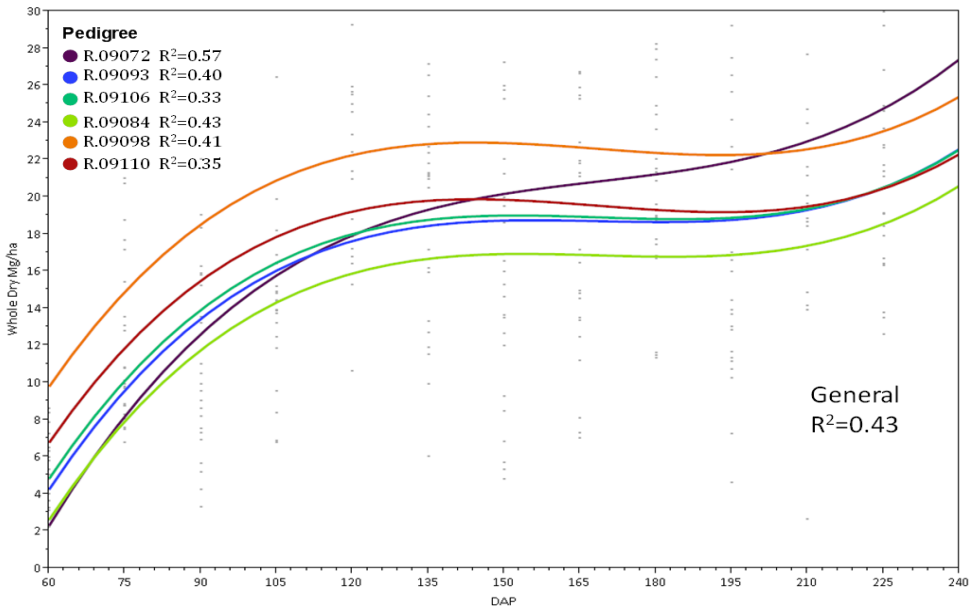

Figure 3.3. Cubic regression for whole plant dry biomass yield of six genotypes of biomass PS sorghum in combined CC and CS locations, in Texas, 2010.

The stem fresh biomass yield curve (Fig. 3.4) was similar to the whole plant fresh biomass yield (Fig. 3.2) likely because stem fresh tissue was approximately 83% of the plants fresh weight. For most genotypes, the main peak of production for stem fresh biomass was near to 120 DAP wherein fresh stem yield was approximately 75 $Mg*ha^{-1}$ at both locations. Because the stem portion of the plant represents between 80-85% of a PS sorghum plant, the curves of stem dry biomass yield (data not shown) had similar pattern to whole plant dry yield.

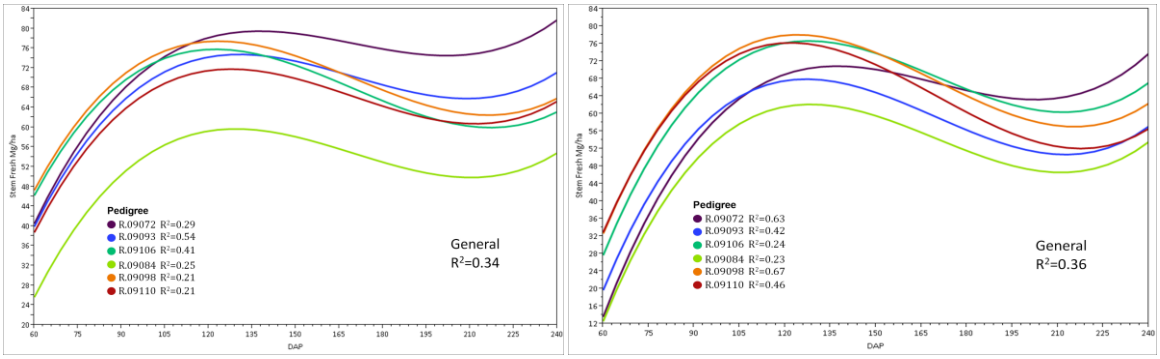

Figure 3.4. Cubic regression for stem plant fresh biomass yield of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010.

Maximum leaf biomass occurred earlier than either stem or total yields (Fig. 3.5). After 105 DAP, leaf yield did not increase and in some genotypes, it dropped over time. Once canopy coverage is achieved and most light is intercepted and captured, the plant would have no value in additional leaves and the older leaves are eliminated. Given that the plants continued to grow, it is surmised that lower leaves were simply dropped as

they were no longer useful in light capture. Similar results have been reported in previous studies (Olson et al., 2012) and are consistent with these observations.

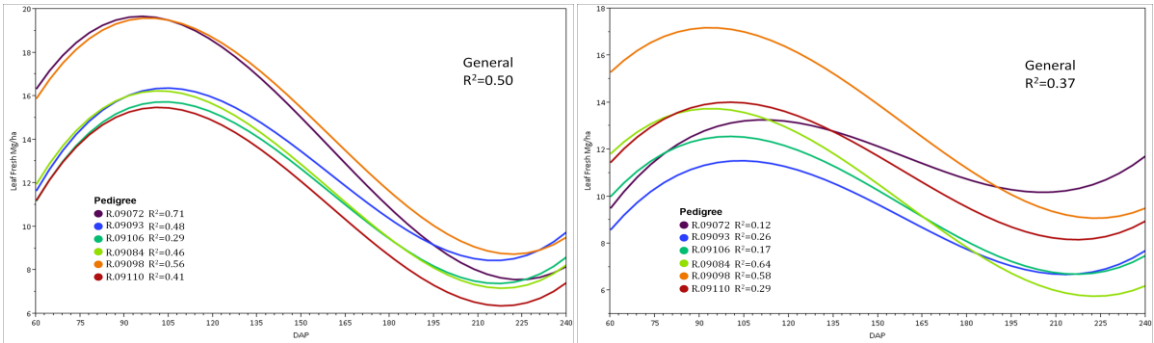

Figure 3.5. Cubic regression for leaf fresh biomass yield of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010.

Effect of harvest date on composition traits

As expected, compositional traits changed over the growing season (DAP), and for some most traits the genotype effect was significant. As seen previously, the location effect was significant for most of the analyzed traits. To access the variation that occurred during the season, the best fit was achieved with a regression curve of quadratic for all compositional traits.

Whole plant composition

As the season progressed, the ash content decreased (Fig. 3.6). Similar results were reported for elephant grass (*Pennisetum purpureum schum)* (Woodard and Prine, 1991), wheat (*Triticum aestivum* L.) and beans (*Vicia fava* L.) (Ghanbari-Bonjar and Lee, 2003). The reduction could be caused by the reallocation of mineral nutrients to the root system or because the ash content drops proportionally relative to the increase in other plant components.

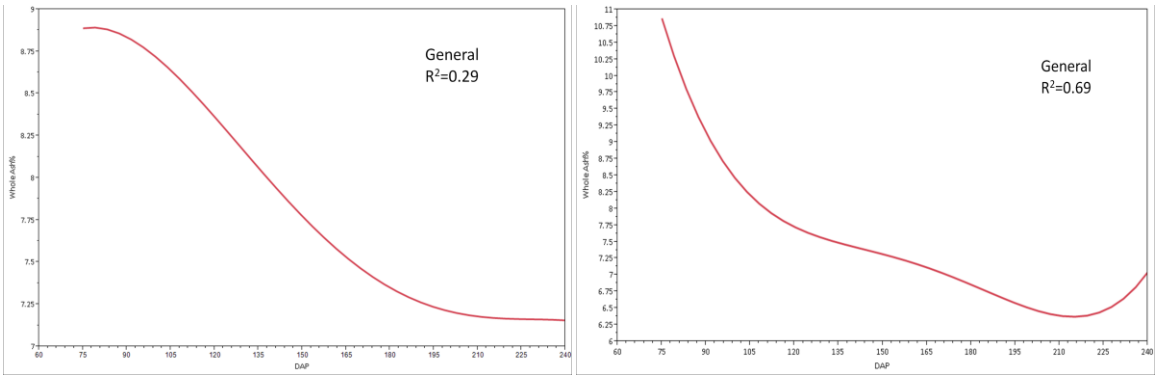

Figure 3.6. Quartic regression for ash percentage on the whole plant of six combined genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010. Data is based on percentage of dry matter.

While there were slight differences among genotypes for the rate of accumulation, lignin concentrations in the whole plant increased throughout the growing season (Fig. 3.7). The lignin content varied across locations, but lignin concentrations peaked concomitant with maximum yields. In CC, lignin concentration peaked (15.12% on R.09072 to 16.25% on R.09106) at 210 DAP while in CS, it peaked (14.62% on R.09106 to 15.25% on R.09098) slightly earlier at 195 DAP. In both locations, there was a slight drop in lignin concentration in the last one or two harvests, possibly due to plant degradation as growing conditions deteriorated.

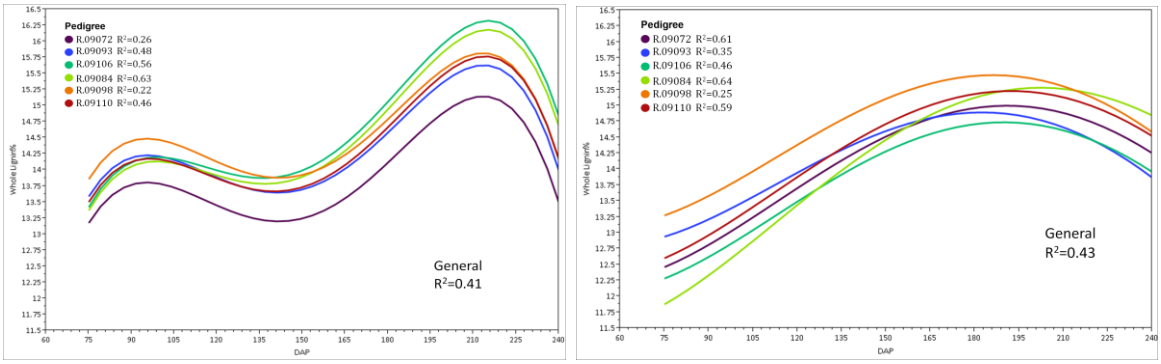

Figure 3.7. Quartic regression for lignin percentage on the whole plant of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010. Data is based on percentage of dry matter.

For xylan and cellulose content in the whole plant, effect of genotype was significant only for xylan at CC (Table 3.2). The concentrations of these two compounds were similar, likely because they are both associated with cell wall structure. (Pordesimo et al., 2005; Corn, 2009; Packer, 2011; Stefaniak et al., 2012). The slight drop in concentration during the middle of the growth phase may be a function of proportion (increase of other components) rather than an actual drop in quantity. In that growth phase, sugar concentrations are highest which would by definition decrease the proportions of other component. Like the lignin concentrations, the drop in both xylan and cellulose concentration at the end of the season is either an artifact of the regression model or due to degradation of the biomass in the field (Fig. 3.8 and Fig. 3.9).

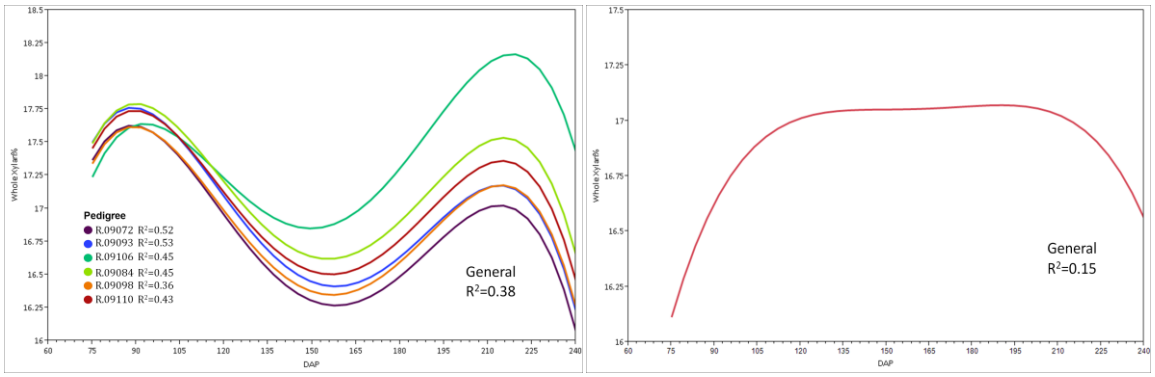

Figure 3.8. Quartic regression for xylan percentage on the whole plant of six genotypes of biomass PS sorghum in CC (left) and six combined genotypes of biomass PS sorghum in CS (right), in Texas, 2010. Data is based on percentage of dry matter.

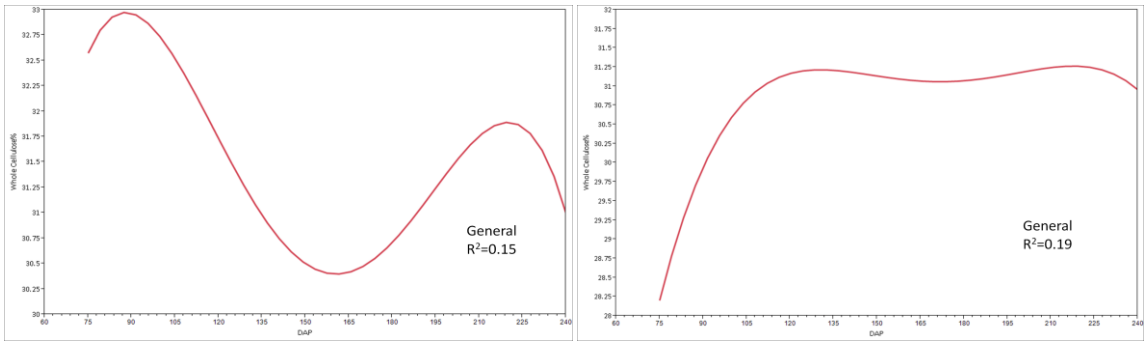

Figure 3.9. Quartic regression for cellulose percentage on the whole plant of six combined genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010. Data is based on percentage of dry matter.

Plant stem composition

Stems compose approximately 83% of the plant biomass and thus, stem compositions are similar to whole plant composition. In the CS location by the end of season, values for ash were the lowest observed between the two locations, approximately 5.75%, after the mark of 225 DAP (Fig. 3.10).

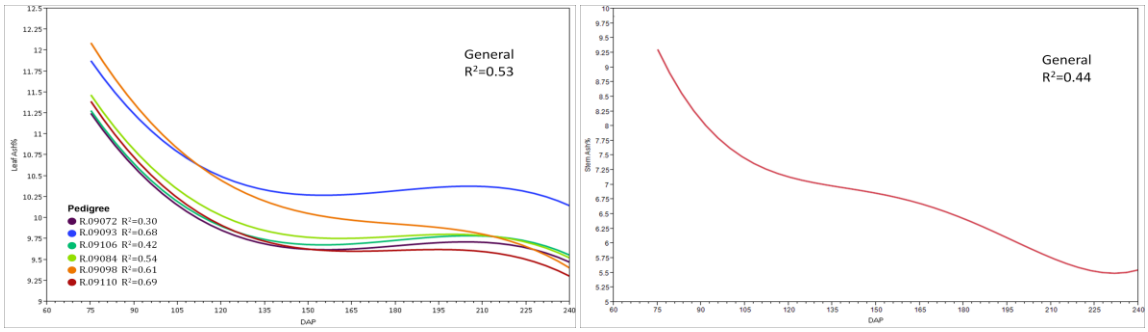

Figure 3.10. Quartic regression for ash percentage on plant stem of six genotypes of biomass PS sorghum in CC (left) and six combined genotypes of biomass PS sorghum in CS (right), in Texas, 2010. Data is based on percentage of dry matter.

Lignin percentage in the plant stem (Fig. 3.11) was similar to the whole plant lignin (Fig. 3.7), for both locations the curve shadows the shape found in the lignin whole plant at CC location. Genotypes are more distinguishable, the genotype R.09084 had higher levels of lignin, and the genotype R.09072 the lowest close to the 225 DAP mark at both location (Fig. 3.11).

The xylan concentration in the plant stem (Fig. 3.12) had significant genotype and location effects. In CC the pattern was not well defined with some fluctuation with two genotypes having higher xylan concentration at different time points than the other four. The genotype R.09106 (at 90 DAP) and R.09084 (between 210 and 225 DAP) demonstrated the G X DAP interaction with change in ranking for this composition trait. The genotypes with higher and lower production of xylan were the same for both locations. The line R.09084 presented the higher value of xylan percentage in the stem at the end of the season, with approximately 17.75% for both locations. The line R.09072 had the lowest xylan percentage in the stem at the end of the season with approximately 16.25% and 16% in CC and CS, respectively.

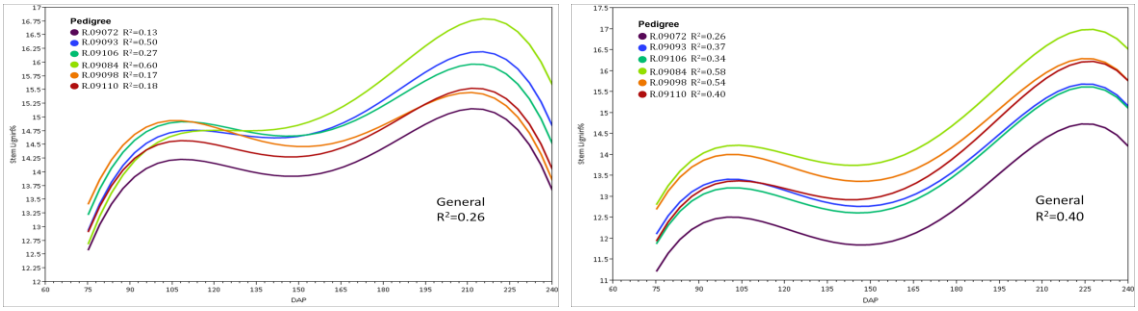

Figure 3.11. Quartic regression for lignin percentage on plant stem of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010. Data is based on percentage of dry matter.

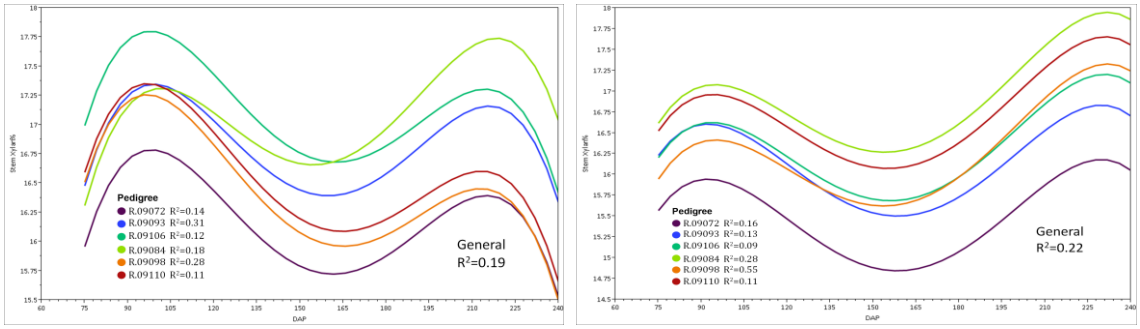

Figure 3.12. Quartic regression for xylan percentage on plant stem of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010. Data is based on percentage of dry matter.

No location effect was detected for cellulose percentage in the plant stem and cellulose values in the plant stem generally decreased as the season continued (Fig. 3.13). Cellulose peaked at 90 DAP, followed by a drops between 105 to 165 DAP. A second peak on cellulose in the plant stem was observed at the end of the season at the mark of 225 DAP; this was likely caused by new tillers. By this time, the highest value was recorded to be approximately 31% for the line R.09084 and the lowest value was identified for the line R.09072 with 29% cellulose in the dry stem.

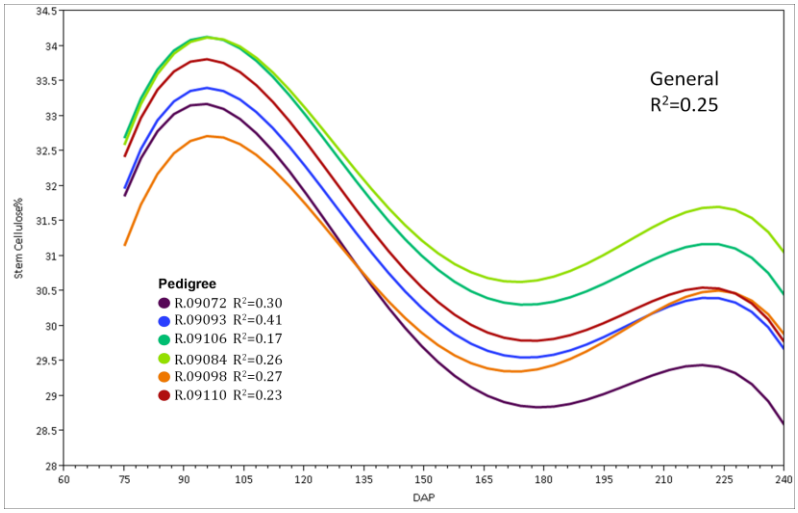

Figure 3.13. Quartic regression for cellulose percentage on plant stem of six genotypes of biomass PS sorghum combined for the two locations CC and CS, in Texas, 2010. Data is based on percentage of dry matter.

Plant leaf composition

Plant leaf composition ash concentrations were the only leaf composition curve shape and patterns that followed those observed on whole plant and plant stem tissue, however, the ash values in the leaf (Fig. 3.14) were approximately 2% higher than those observed in the stem and whole plant. This indicates leaf senescence at the end of season is an important way to recycle the mineral nutrients back to soil.

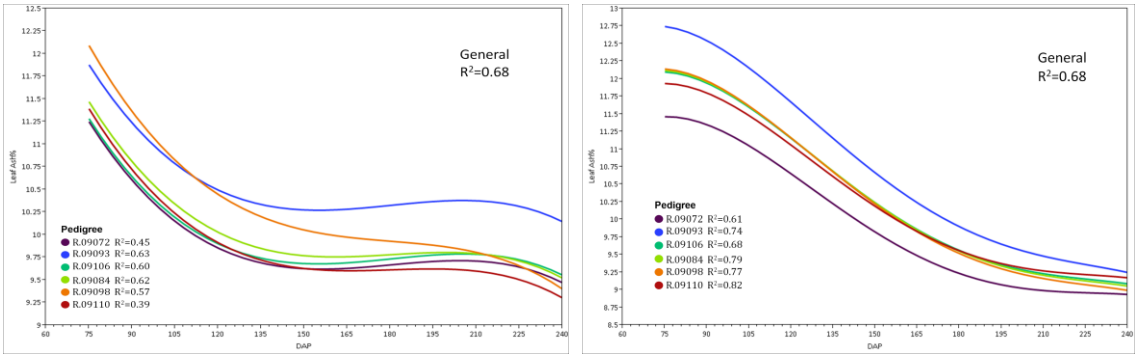

Figure 3.14. Quartic regression for ash percentage on plant leaf of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010. Data is based on percentage of dry matter.

Another difference among leaf composition and whole and stem plant composition was for on lignin concentration on the leaf (Fig. 3.15). Whole plant and plant stem lignin concentration (Figs.3.7 and 3.11 respectively) were approximately 3% higher than the leaf lignin values at the end of season. A different genotype (R.09093) had a low lignin profile throughout the season, this genotype had approximately 12.5% of lignin in the leaves close to 210 DAP mark.

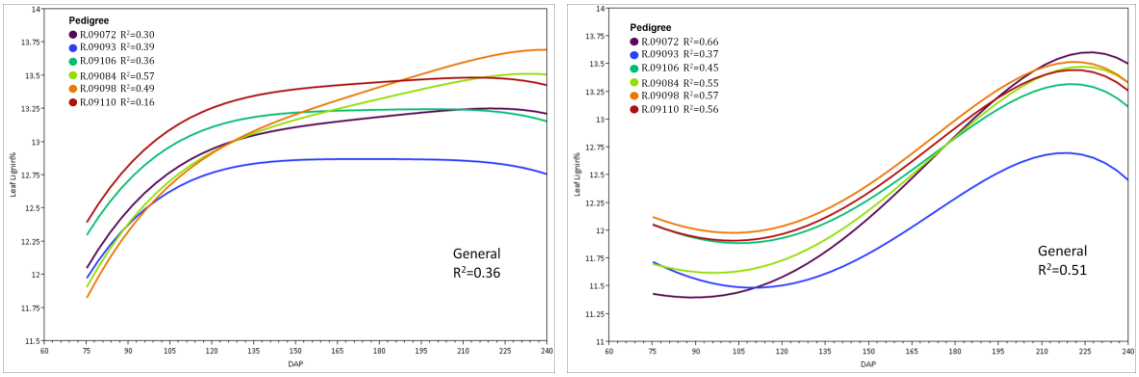

Figure 3.15. Quartic regression for lignin percentage on plant leaf of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010. Data is based on percentage of dry matter.

Xylan concentration in the leaves (Fig. 3.16) also had lower values over the season than the values observed on whole plant and plant stem (Figs 3.8 and 3.12 respectively). Xylan on the leaves never exceeded of 13.5% while in the whole plants and plant stem maximum values exceeded 18%. Another discrepancy was regarding the effect of genotypes, on leaf xylan it was significant, and no delineation of genotypes was possible for this trait.

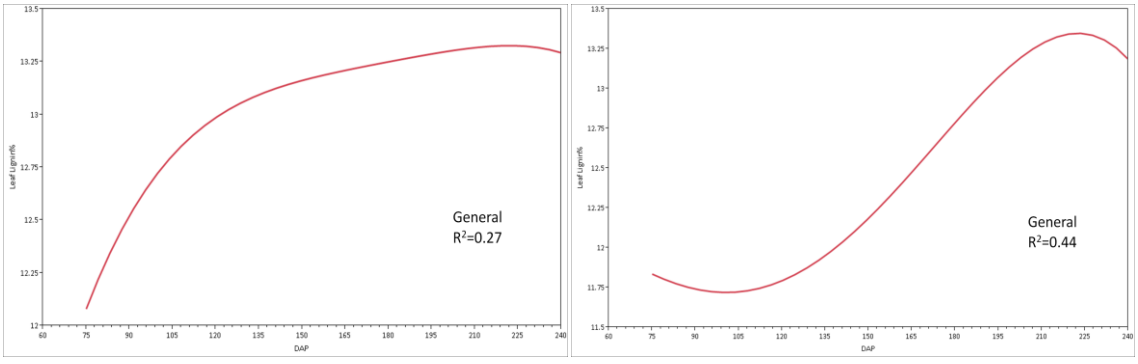

Figure 3.16. Quartic regression for xylan percentage on plant leaf of six combined genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010. Data is based on percentage of dry matter.

Leaf cellulose concentrations were similar to those in the whole plant and stem. In the case of whole plant cellulose in CC and stem plant cellulose (CC and CS) values dropped after 90 DAP then an increase was later observed. For case of cellulose on leaves, no drop in values was observed and variation was reduced after 120 DAP mark in CC, and in CS cellulose continue to increase up to the end of the season (Fig. 3.17).

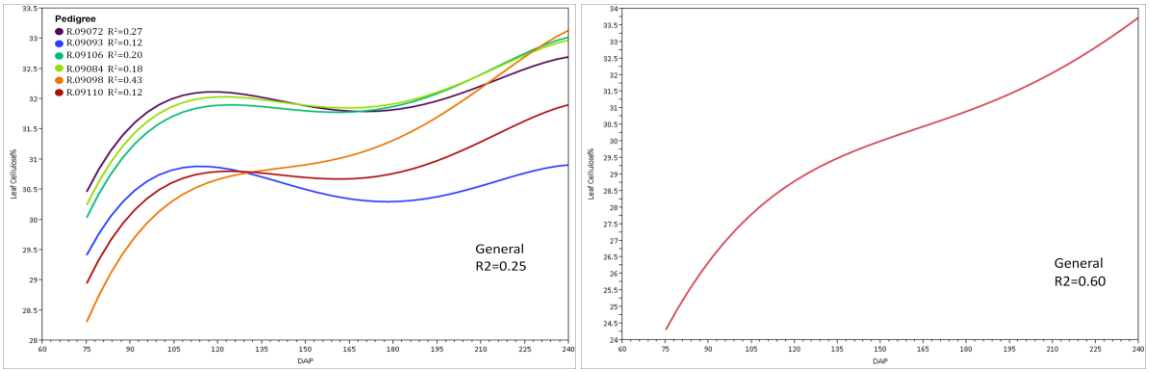

Figure 3.17. Quartic regression for cellulose percentage on plant leaf of six genotypes of biomass PS sorghum in CC (left) and six combined genotypes of biomass PS sorghum in CS (right), in Texas, 2010. Data is based on percentage of dry matter.

Discussion

Different genotypes growth curves

As reported above for most of the traits analyzed, the genotype effect was significant, and distinguished regression lines were used to represent each genotype. G x DAP interaction were significant for some of the traits, it is possible to identify the point where genotypes changed rank with regards to rate of growth and/or composition concentration. This information could be useful for breeding programs that are interested in selecting sorghum biomass based on growth rate and productivity. In addition the rates of development represented by the different regression curves is useful information for breeding programs looking for early and late harvesting types also a system for scattering of harvest. The genotypes had different rates of growth and composition; this can be further explored by producers and conversion mills to shorten or extend harvest as it would improve logistics and profitability for the operation.

Biomass harvesting and agronomics

All genotypes produced agronomic production curves of a similar pattern; peak biomass yields (both fresh and dry) occurred at approximately 120 DAP in both locations. Just after this time, yields stabilize or slightly drop (Figs. 3.2 and 3.3). While the reduction could be due to specific environments, a yield plateau is commonly observed in production, especially when periodic drought occurs in late summer as it did in both of these environments in 2010. As described by Rooney et al. (2007) the sorghum crop did respond to late season moisture, resulting in further yield increases.

These differences between these two sets of data (Figs. 3.2 and 3.3) give us important information related to reduction in water content in the biomass of sorghum.

Pattern of reduction in moisture content was also observed for the stem data and for the leaf data. For leaf biomass in CC, a steep decline in fresh biomass was reported just after 120 DAP, reaching a low between 210 and 215 DAP. For R.09098, the line with the greatest fresh leaf biomass throughout the season, this drop was approximately 9 $Mg*ha^{-1}$ (Fig. 3.5). Crop desiccation is a very import step prior to biomass harvesting intended for liquid biofuel, as it reduces the volume and total weight to be harvested, transported and processed. In addition, desiccation helps to reduce microbial activity that may cause degradation or rot of the biomass leading to reduction of the energy value per unit of weight. In general if less water is harvested it makes the operation potentially more profitable. Also the ratio of stem to leaf weight, genotypes with greater amount of leaves may have more volume per weight unit making transportation to conversion facilities more expensive.

In addition to crop desiccation, height and leaf senescence improves later during the season. These two factors are favorable in some aspects; height is a good indicator of crop maturity and production, but also can be associated with crop lodging, resulting in harvesting difficulties. Leaf senescence was characterized by reduction of leaf dry biomass (Fig. A.1), can be associated to better environment for the main stem to dry down this point needs additional study.

Biomass harvesting and composition

Analyses of biomass composition over time demonstrate that variation is caused by environment, maturity and genotypes. Of these sources, the stage of maturity and the environment account for the greatest amount of variation. While little can be done to minimize variation due to environment, agronomic management and a thorough understanding of composition due to maturity can be use to manage composition. Furthermore, variation among genotypes can be exploited to improve composition.

Growth stage had the largest effect on composition and different component changed over time. Some constituents decreased while others increased with increasing maturity. These patterns were not always well defined, but a general picture was formed with high levels of lignin, xylan and cellulose close to the last harvest cuts. This variation according to growth stage is not unique to biomass sorghums, and although biomass did not flower we were capable to identify such patterns.

The effect of the environment had the second greatest influence in composition on the biomass sorghum lines (Table 3.2), to this extend the variation in soil type, rain patterns, irrigation used, day and night time temperature and agronomic management represent a important factors that influence composition in this crop. Such large influence was reported in numerous studies (Murray et al., 2008a; Packer, 2011).

The factor with the least influence on the composition of the biomass sorghum was the genotype. Although significant effects due to genotype were observed for some of the compositional traits (Tables 3.1 and 3.2), evaluation of additional genotypes might

also increase this variation. Thus, crop management and environment will have the largest effects, but genotypes can be selected to maximize their genetic potential.

As the compositional quality of the biomass in the study was measured in percent, the influence of compositional traits not measured could have affected the data. For example, both protein and soluble sugars are present in varying levels in the biomass and this would likely affect any of the measured traits. For example, the level of cellulose (Figs. 3.9 and 3.13) dropped during the log phase of growth and this could have been due to a dilution of this parameter by an increase in protein and soluble sugar at the same time.

Ash content in the biomass of plants is related to nutrients and to the contamination of dust on the plant form soil during growth or harvest. As the plants start to senesce, nutrients in the plants tend to be reallocated to the roots. Low levels of ash in the plant are desirable at harvesting time, as when harvested plants are transported out of the field, ash can cycle back into the next crop. In addition, more ash in the biomass means more weight to be transported to the refinery and more byproducts to deal with during conversion to biofuel. The ash level results obtained in this study support a harvesting window that can start as soon as 120 days after planting (DAP) to the last harvest cut 240 DAP (Figs. 3.6, 3.10 and 3.14).

By constituent

Lignin is an essential constituent of the biomass that provides structural strength and support to cells and tissues of plants. The same value that is important for structural

integrity is undesirable as a compound in biochemical conversion as it binds enzymes intended to breakdown structural carbohydrates (cellulose and hemicellulose). This ability to bind to enzymes makes the conversion processes longer and less cost efficient. As reported for others crops (Reeves, 1987; Jung and Vogel, 1992), lignin levels in the biomass sorghum vary throughout the season. Commonly known as "lignification" seasonal grasses will harden by the end of the fall. This phenomenon seems to be true for biomass sorghum as well. For the three types of tissues sampled in this experiment the levels of lignin presented some fluctuation but terminated the season higher than at the begging. An example of this fluctuation was observed on stem lignin concentration, a low point was found at 150 DAP for all lines. If only lignin is counted as a parameter, the harvesting should occur around this point, because lignin levels rise again reaching its maximum at 210 DAP (Fig. 3.11).

Xylan, five carbon structural carbohydrates, had variable levels throughout the season. For the whole plant its main concentration peak for all genotypes was at 210 DAP at CC. Cellulose, six carbon structural carbohydrates, reduced as the season progressed (Fig. 3.13). This reduction in cellulose content helps to support the lignification status of the crop towards the end of season. By the end of season, forages will lose their nutritional value by lacking digestibility, which seems to be the case for the biomass sorghum, which presented increases in lignin levels and reduction in cellulose levels. If lower lignin is desirable then this situation is problematic but if high lignin is desirable then these observation indicate that harvest should be delayed for optimum composition.

In summary, the data indicate that harvest can start as early as 120 days after planting and continue until at least 240 days after planting. Over this harvest window there are compromises that must be made. For example, earlier harvest results in more leaf material, higher moisture content and higher ash content per unit of biomass. Later in the season, harvested biomass sorghum may have less water, lower ash content and leaf material, but the lignin concentration are likely higher.

Biomass yield and composition presented sufficient variation with throughout the measurement periods where cost of transportation together with economical/technical limitation of conversion of biomass to biofuel may play a role on adequate harvesting dates. Hence, genetic variation may be explored to have optimization of quality of biomass produced and yield performance throughout the time period.

CHAPTER IV

THE EFFECT OF CYTOPLASM ON TOTAL YIELDS IN BIOMASS SORGHUMS FOR ENERGY PRODUCTION

Introduction

To meet the anticipated need for increased plant biomass, plant breeding programs have initiated improvement programs in several dedicated bioenergy crops such as sorghum (*Sorghum bicolor* L. Moench). Sorghum was domesticated in Africa and is known throughout the world as a grain and forage crop. Because of this history, the development of bioenergy sorghum is accelerated by the presence of established production history, breeding programs and seed production systems (Rooney et al., 2007).

Regardless of the type of sorghum, commercial seed production is based on hybrids produced using cytoplasmic male sterility (CMS) systems. The CMS is essential because sorghum is a self-pollinated crop and hybrid seed production without a sterility system is not feasible. The first CMS was discovered, characterized and described by Stephens and Holland (1954). This system, designated as A1 CMS remains the predominant CMS system in hybrid sorghum seed production.

In 1970, the Southern Corn Leaf Blight (*Helminthosporium maydis*) epidemic in corn (*Zea mays* L.) devastated corn hybrids possessing the single 'T' cytoplasm system and it had serious consequences for hybrid crop production (Tatum, 1971). Since then, multiple different CMS systems were identified, characterized and made available for

deployment in sorghum breeding programs (Rao, 1962; Hussaini and Rao, 1964; Webster and Singh, 1964; Ross and Hackerott, 1972; Schertz, 1977; Schertz and Ritchey, 1978; Worstell et al., 1984). Based on fertility reactions, these sources represent at least six different unique cytoplasm sources. Further characterization of these sources utilizing mitochondrial DNA sequences identified four distinct groups with the possibility that at least three more are present (Xu et al., 1995). Thus, it is apparent that there is sufficient variation for CMS in sorghum to diversify if needed.

These sources have been used to develop three distinct CMS systems that can be and are used in sorghum seed production. The original A1 CMS remains the most commonly used system in sorghum. Schertz (1977) documented and released A2 CMS in which the cytoplasm source was derived from a non-milo parent. Miller (1986) and Miller et al. (1992) released the seed parents with this CMS system A2Tx632, and A2Tx636 and A2Tx637, respectively. For restoration of fertility in A2 CMS in grain sorghum hybrids, RTx432 was released (Miller, 1984). The A3 cytoplasm system was introduced with the release of A3Tx398 (Schertz, 1984). Since that time, several groups have released seed parents with A3 CMS (Pedersen and Toy, 1997b; Pedersen et al., 1997; Miller et al., 1999). These have not been used for grain sorghum hybrids because the restoration of the A3 system is gametic (Tang and Pring, 2003) and the frequency of restoration alleles in sorghum populations for A3 is rare (Tang et al., 2007). The A3 CMS has been used for the production of forage or sweet hybrids where grain is not important or desired in the commercial product.

The development of hybrid biomass sorghums for the biofuel industry makes it important to re-evaluate which CMS is the most appropriate to use for seed and commercial crop production. First, biomass sorghum hybrids are not grown for grain production; they are photoperiod sensitive and they typically do not flower prior to harvest. Thus, the restoration of fertility in a hybrid system is not relevant for production. Furthermore, if transgenes were to be introduced into biomass sorghums, the presence of male sterility in the photoperiod-sensitive hybrid provides a second level of protection against transgene transfer to other plant species.

In addition to these considerations, the selected CMS system must not reduce the yield or agronomic adaptability of the hybrid. In grain sorghum hybrids the effect of cytoplasm on performance of grain sorghum hybrids has varied, depending on study. Maves and Atkins (1988) reported a reduction in grain yield in A2 hybrids compared to A1 hybrids while Kishan and Borikar (1989) reported that A2 was superior to A1 for grain size and yield. Secrist and Atkins (1989) found no significant differences ($P > 0.05$) in grain yield between A1 and A2 hybrid, but they reported a 6% reduction in grain yield in A3 hybrids compared to A1 hybrids. Moran and Rooney (2003), evaluating iso-cytoplasmic hybrids also reported reduced grain yield in A3 hybrids compared to both A1 and A2 hybrids. In forage sorghum, Pedersen and Toy (1997a) tested the effect of A1 and A3 cytoplasm in forage hybrids of sorghum x sudan grass, and they found no differences associated to cytoplasm alone for maturity, height, dry yield, total yield, crude protein and in vitro dry matter disappearance.

As with grain and forage sorghum, it is important to determine if different CMS will affect agronomic performance and biomass yield. Within this context the objective of this study was to evaluate the effect of the A1, A2 and A3 CMS on agronomic performance and the composition of nine iso-cytoplasmic biomass sorghum hybrids.

Materials and Methods

Three seed parent lines and one pollinator parent were selected to produce the hybrids used in this study. The three seed parents were Tx378, Tx623 and Tx631 and all of these lines were originally developed and released with A1 CMS (Stephens and Karper, 1965; Miller, 1986). For all three, iso-cytoplasmic versions were developed in the Texas AgriLife Research sorghum breeding program (Miller et al., 1999). These same lines were also used by Moran and Rooney (2003). Each seed parent was hybridized using the pollinator line R.07007. R.07007 is a photoperiod insensitive breeding line in the Texas AgriLife Research program that when hybridized to standard seed parents (such as Tx378, Tx623 and Tx631) produces a photoperiod sensitive hybrid based on epistatic genetic interactions at specific maturity loci (Rooney and Aydin, 1999; Olson et al., 2012). Thus a total of nine hybrids, representing three genetically distinct inbreds and three distinct CMS were included in the agronomic analyses. All hybrid seed production was completed in a crossing block in College Station in 2008.

The nine hybrids were evaluated in a randomized complete block design (RCBD) with five replications at three different locations in Texas in 2010. At each location, the experimental unit was composed of two rows of 7.92 meters in length and spaced 0.762

meters totaling 6.03 square meters per plot. Plots were planted and thinned to a plant density of 160,000 plants ha^{-1}. Trials were planted in College Station (CS) on April 7th, in Halfway (HW) on June 1st and Weslaco (WE) on August 16th. These locations represent a range of different of sorghum production regions in Texas. College Station is located in the South Central region and is a subtropical environment. Halfway is in the Texas High Plains and has dry, temperate climate. Weslaco is in the Rio Grande Valley in a semi-arid but humid tropical climate. Agronomic production practices common to each region for forage sorghum were used in these studies. At WE, supplemental furrow irrigation was supplied to ensure uniform germination and seedling growth and at HW, plots were irrigated using a center pivot systems. The CS trial was rainfed.

In each location, plant height, stem diameter, and biomass yield were measured immediately prior to or at harvest. Days-to-anthesis was not recorded because all of these hybrids were photoperiod sensitive and flowering did not occur in either CS or HW. Because the WE location was grown in the fall, the hybrids were just at anthesis at harvest but all flowered within 2 days so there was no variation among hybrids. At CS, HW, and WE, plots were harvested on 25 Aug, 16 Sept and 12 Nov, respectively. Plant height (cm) was measured from the base of plants (soil level) to the growing point of the plants. Plant stem diameter (Stem dia.) was measured in mm at the second fully extended internode from the base of three random plants prior to harvest. Plots were then harvested in CS and WE with a John Deere Silage Harvester model 5460 with three-row silage header and weighed in a wagon equipped with the Avery Weight-

Tronics, model 640 electronic. In Halfway harvest was performed with a one-row New Holland model 707 forage harvester, and the plot biomass was collected and weighed in an attached bin equipped with an Avery Weight-Tronics scale system model 640 with increments of 500g. From each plot a fresh sample was collected. Fresh samples were weighed and dried in an air-forced flux drier at 52^{o}C +- 1^{o}C until the weight stabilized. The dry samples were then weighed and moisture content was calculated as the difference in weight between the fresh and dry sample. The dried samples were ground in a Wiley Mill (Thomas Scientifics, Inc.) until the tissue passed through a 2 mm mesh screen and was then stored in air-tight plastic bags.

The composition analysis was predicted by near infrared spectroscopy using a FOSS XDS near infrared spectroscopy instrument (rapid solid analyzer). Spectrum data were converted into composition prediction data using a calibration curve which was developed through cooperation between the sorghum research team at Texas A&M University sorghum quality lab and National Renewable Energy Laboratory (Wolfrum et al. in review). Prediction parameters were ash, lignin, xylan (hemicellulose) and glucan (cellulose and starch).

Statistical analysis

Data from each environment were analyzed independently using ANOVA in Statistics Software JMP version 9 (SAS Institute, 2010). For each location the statistical model was $Y = mean + replication + cytoplasm + female + cytoplasm*female + error$ with all effect fixed. Prior to combining the data from environments, Bartlett's test for Homogeneity of Error was run to determine if combining the data was statistically valid

(Bartlett, 1937). No evidence of error heterogeneity was detected; hence the data from across environments was combined and analyzed using the statistical model $Y = mean + environment + replication(environment) + cytoplasm + cytoplasm*environment + female + female*environment + female*cytoplasm + female *cytoplasm*environment + error$ with all sources of variation considered fixed. If a source of variation was significant, then mean separation was completed using the Student's T method.

Results

Few significant effects were detected within either environment; the cytoplasm effect was significant for dry biomass yield in CS (A3 > A2 and A1) and for ash and lignin content in HW. Effects due to female were significant for lignin and xylan concentration and fresh and dry biomass yield in WE, stem diameter in HW and xylan in CS. Differences due to female were expected as these three seed parents are quite diverse in phenotype and adaptation. The paucity of significant effects within each location indicates that cytoplasm *per se* does not affect biomass yield or composition. The interaction term (cytoplasm x female) was not significant for any measured trait.

Table 4.1. Summary of analysis of variance (mean squares) of combined locations of agronomic and compositional traits of three photoperiod sensitive biomass sorghum hybrids with the same male parent and three different female parents in different cytoplasm systems (A1, A2 and A3) in three locations, College Station (CS), Halfway (HW) and Weslaco (WE), in Texas, 2010.

Source	DF	Fresh yield	Dry yield	Stem dia.	Height	Dry Matter	Ash	Lignin	Glucan	Xylan
Rep[Environment]	**12**	207.45	15.15	10.65	813.73*	3.93	0.32	0.59	3.85	0.70*
Cytoplasm	**2**	29.78	5.54	6.31	375.24	6.54	0.47	0.22	0.52	0.07
Environment	**2**	21425.20*	1508.51*	187.47*	59146.00*	484.02*	60.88*	122.12*	103.58*	76.20*
Female	**2**	657.76*	9.91	28.63	499.37	67.87 *	0.29	0.72	3.50*	2.90*
Cyto x Env	**4**	354.96	22.25	6.00	635.82	1.96	0.80*	0.83	0.13	0.35
Cyto x Fem	**4**	237.47	10.99	11.62	128.09	11.02 *	0.42	0.53	3.24*	0.37
Env x Fem	**4**	155.06	12.52	3.90	649.70	1.76	0.35	0.65	1.72	0.29
Cyto x Env x Fem	**8**	372.57*	13.58	7.96	451.86	8.16*	0.24	0.59	0.91	0.36
Error	**93**	166.68	11.63	8.82	370.71	3.32	0.29	0.38	0.87	0.25
Mean of Response		53.70	13.70	20.27	306.89	25.30	7.88	14.06	30.75	16.95
CV%		24.04	24.90	14.65	6.27	7.20	6.79	4.37	3.04	2.93

* Significant at level of 0.05 of probability.

In the combined analysis of variance, the environment was a significant source of variation for every trait measured and cytoplasm was not significant for any agronomic or compositional traits (Table 4.1). The effect due to female (seed parent) was significant for fresh biomass yield, dry matter, glucan and xylan, indicating that hybrids differed for these three traits. The interactions were not significant with the following exceptions: cytoplasm X environment (ash), cytoplasm X female (glucan and dry matter content), and cytoplasm X location X female (fresh biomass yield and dry matter content).

Environmental effects accounted for the majority of variation in the test. Biomass yields were highest in HW and lowest in WE (Table 4.2). The lower yields in WE were the result of the late summer planting date resulting in a crop that developed into the fall season where active growth and biomass accumulation was slowed or stopped due to cool/cold weather, short daylengths and reduced light intensity. Plants were tallest in HW and shortest in WE, again likely due to the shorter growing season and daylengths in WE. Stem diameter was greater in WE and CS than in HW (Table 4.2). Concentrations of ash, xylan, and glucan in HW were consistently and often significantly higher than the other two environments (Table 4.2). There is not an obvious reason as to why this is observed, but because these are concentrations, other compounds not evaluated in this study (protein, soluble sugar) must be higher in the CS and WE environments to offset these differences. Protein and soluble sugar content are known to vary in the sorghum by genotype, maturity and environment (Packer, 2011). Thus, differences in performance due to environment were expected as these three environments are distinct in climate, soils and production season.

Since a single pollinator parent was used in this study, the effect of the female (seed) parent reflects any differential performance among the hybrids in this test. Among these genotypes, ATx378/R07007 produced the highest fresh biomass yields (Table 4.2). Differences in composition were also detected among the hybrids, specifically for glucan and xylan concentrations (Table 4.2). Tx378 hybrids were higher in glucan and xylan than either Tx631 or Tx623 hybrids. Both Tx631 and Tx623 tend to have higher soluble carbohydrate levels than Tx378 (data not shown) and this is reflected in the lower glucan and xylan concentrations. Overall, there were fewer differences in hybrid performance in this study than in previous cytoplasm studies in grain sorghum (Maves and Atkins, 1988; Secrist and Atkins, 1989; Pedersen and Toy, 1997a; Moran and Rooney, 2003).

Table 4.2. Mean separation test for three locations College Station (CS), Halfway (HW) and Weslaco (WE) and for three biomass sorghum females and three cytoplasm types, in Texas, 2010.

	Fresh Yield	**Dry Yield**	**Stem diam.**	**Height**	**Dry Matter**	**Ash**	**Lignin**	**Glucan**	**Xylan**
	$Mg*ha^{-1}$	$Mg*ha^{-1}$	mm	cm	%	%	%	%	%
CS	53.41 b	15.46 b	20.98 a	301.41 b	28.88 a	6.77 c	12.22 c	29.21 c	15.67 c
HW	76.80 a	18.69 a	17.85 b	348.17 a	24.57 b	9.15 a	14.66 b	32.31 a	18.34 a
WE	32.01 c	7.27 c	21.86 a	274.53 c	22.44 c	7.80 b	15.36 a	30.86 b	16.96 b
ATx631	52.86 b	13.33 ns	21.10 a	305.01 ns	25.07 b	7.86 ns	14.04 ns	30.59 b	16.99 b
ATx378	58.42 a	14.28 ns	20.10 ab	307.40 ns	24.20 c	8. 00 ns	14.22 ns	31.12 a	17.24 a
ATx623	51.02 b	13.78 ns	19.48 b	311.71 ns	26.62 a	7.86 ns	13.97 ns	30.68 b	16.73 c
A1	53.20 ns	13.38 ns	20.64 ns	305.00 ns	25.00 ns	7.81 ns	14.08 ns	30.90 ns	17.00 ns
A2	54.83 ns	13.94 ns	19.89 ns	306.83 ns	25.16 ns	8.02 ns	14.01 ns	30.68 ns	16.94 ns
A3	54.27 ns	14.06 ns	20.15 ns	311.39 ns	25.73 ns	7.88 ns	14.15 ns	30.80 ns	17.02 ns

*Means followed by different letters are different at the level of 0.05 of probability by Student's T test.

Biplot analysis of all the agronomic traits and composition visualizes the variability among environments for the measured traits (Fig. 4.1). Two principal components explained 73.2% of total variation of the experiment. Clear segregation patterns were evident based on environment and the differences in these environments were confirmed. Among the traits, positive associations were detected between fresh biomass yield, dry biomass yield and plant height (Fig. 4.1). Another positive relationship was detected between glucan and xylan, meaning that hybrids with high amounts of xylan usually have high levels of glucan as well. This observation has been made in other studies of sorghum biomass (Dahlberg et al., 2012; Stefaniak et al., 2012). The relationship of dry matter and lignin shown in the biplot indicates that low moisture contents are correlated with higher lignin concentrations. The biplot also associates traits with environments wherein CS had the highest dry matter percentage while Weslaco (W) had hybrids with larger stem diameters (Fig. 4.1). Most other traits tended to be more favorably associated with HW (Fig. 4.1).

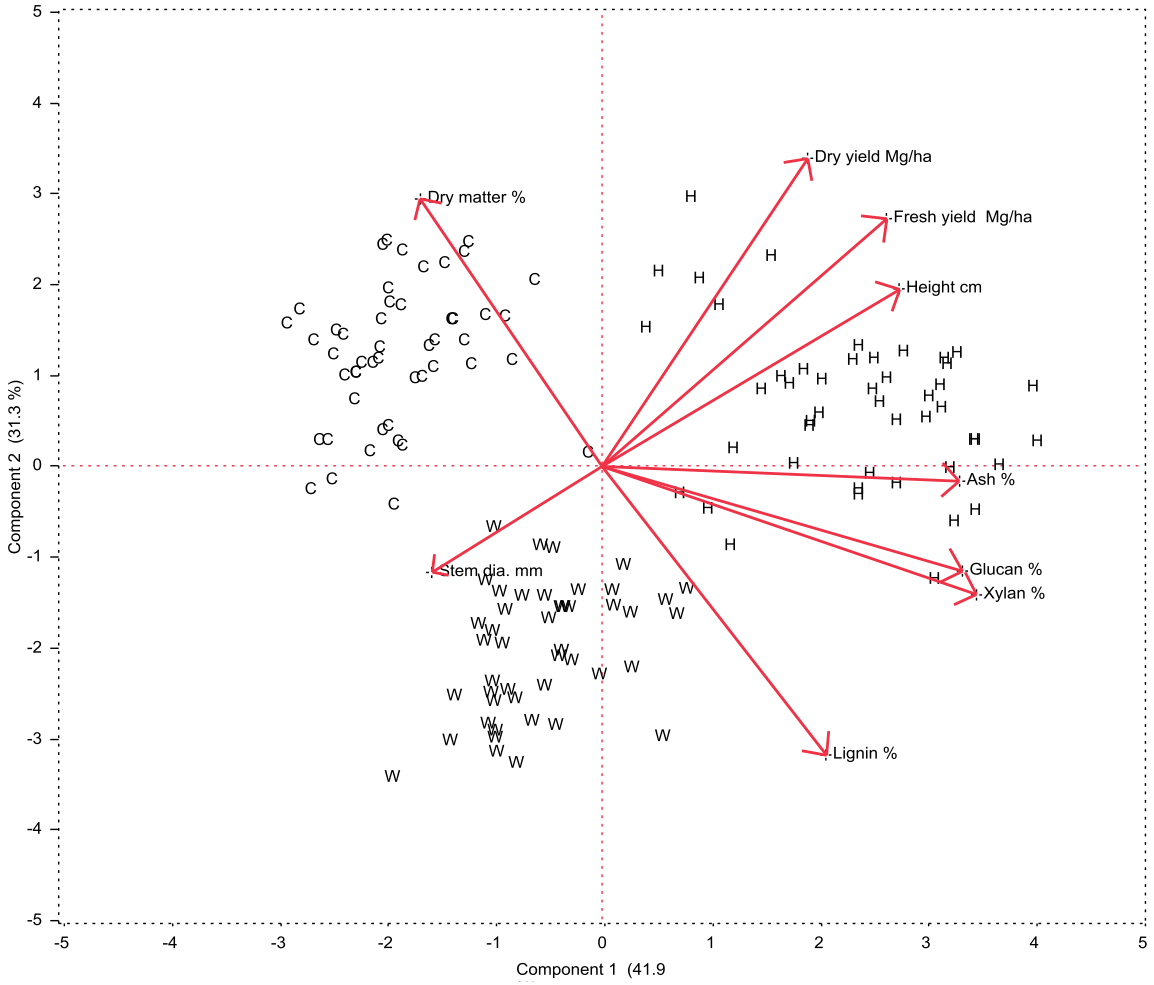

Figure 4.1. Biplot of agronomic and compositional traits of three photoperiod sensitive biomass sorghum hybrids with the same male (R.07007) and three different females (ATx378, ATx623 and ATx631) in three different cytoplasm systems (A1, A2 and A3). Tests were conducted in three different locations College Station (C), Halfway (H) and Weslaco (W), in Texas, 2010.

Discussion

The results of this study indicate that the performance of biomass sorghum hybrids is not influenced by any of the three cytoplasm systems (A1, A2 and A3) tested. These results are slightly different from other cytoplasm studies in sorghum wherein differences in grain yield were detected between different cytoplasms (Kishan and Borikar, 1989; Moran and Rooney, 2003). In these studies, the A3 cytoplasm was the poorest performing cytoplasm (Secrist and Atkins, 1989; Lee et al., 1992). However, all previous studies considered the grain yield and not total biomass yield. In a study done of forage sorghum x sudan hybrids, no differences were detected between A1 and A3 forage sorghum hybrids (Pedersen and Toy, 1997a). In the current study, grain yield was irrelevant; these hybrids did not flower or were flowering at harvest. Given that variation in total biomass yield is typically lower than for grain yield, which is a subset of total yield, it suggests that grain yield differences due to cytoplasm are affected by partitioning of carbohydrate in grain hybrids.

Numerous other reports have documented the importance of environment on performance (Murray et al., 2008a; Corn, 2009; Packer, 2011) and while it is not the focus of this work the results herein confirm that environment is a critical factor in biomass sorghum and composition. The biplot data (Fig. 4.1) illustrates how each of the environments is distinct.

High biomass sorghum hybrids have a promising future as a source for feedstock for biofuel production. Given that cytoplasm *per se* had no effect on hybrid performance, any of the tested cytoplasms (A1 A2 and A3) can be deployed in hybrid

biomass sorghums. The use of A3 cytoplasm to produce male sterile hybrids will provide breeding programs the best secondary containment mechanism to avoid any potential gene flow from future transgenic energy sorghums.

CHAPTER V

CONCLUSIONS

In the course of this study, thousands of biomass dry samples were used to achieve the compositional results here presented. For that, the use of near infrared (NIR) spectroscopy technology was a key element of our research approach. Although testing the efficiency of NIR techniques was not the purpose of our study, given the relevance of the results achieved in this study, it is possible to say that the use of NIR was a fast and economical form of phenotyping large numbers of entries, making it an important tool for the breeder interested in compositional traits.

The efficiency of the pre-classification phase of this project was tested. Although contrast analysis did separate the high lignin and low lignin genotype groups, the comparison on entries revealed significant overlap between the two groups. This means that the pre-classification was useful in a general sense, but further testing is essential to confirm initial assignments. Also, the significant effect on the environment and the interaction to genotype and environment effect was present. Sufficient genotypic variation was observed for most traits to support the use of entry for breeding and research purposes in the biomass sorghum. Hence large effects from environment were observed on the traits measured and because of that the importance of locations of growth and agronomic management was very important step on biomass sorghum establishment. In addition, it was possible to identify genotypes with high and low lignin content in the biomass and stable high biomass yield performance.

The observation of biomass growth and changes in composition over the growth season revealed that the environment, stage of maturity and genotype influence and change these traits. Based on the observations, biomass sorghum can be harvested between 120 and 210 days after planting for the best potential. With some variation on genotype response and variation on compositional quality, agronomics and management can explore the biomass sorghum samples evaluated in this study for optimum biomass processing and biofuel conversion.

The evaluation of a set of iso-cytoplasmic hybrids revealed that different CMS (A1, A2 and A3) do not affect biomass yield or composition. These results mean that any of the three CMS systems can be used to produce biomass sorghum hybrids without any effect on productivity. Access to different CMS may be important when and if transgenic sorghums are developed; the CMS could serve as a pollen control mechanism.

Finally, the information gathered here regarding biomass yield potential, biomass compositional variability, period of time to have a harvestable crop, genetic variability and environmental effect on the biomass sorghum crop, it is possible to say that biomass sorghum can be a competitive and reliable lignocellulosic feedstock for second generation biofuel industry.

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APPENDIX

Table A.1. List of genotypes, their pedigree, composition classification and objectives where they will be used in different objectives in this current study.

Entry #	Pedigree	Classification	Used in Objective	
1	R.09072	Agronomic fit		3
2	R.09075	High Lignin	2	
3	R.09076	High Lignin	2	
4	R.09077	High Lignin	2	
5	R.09078	High Lignin	2	
6	R.09079	High Lignin	2	
7	R.09080	High Lignin	2	
8	R.09081	High Lignin	2	
9	R.09083	Low Lignin	2	
10	R.09084	Low Lignin	2	3
11	R.09085	Low Lignin	2	
12	R.09087	Low Lignin	2	
13	R.09088	Low Lignin	2	
14	R.09089	Low Lignin	2	
15	R.09090	Low Lignin	2	
16	R.09091	High Lignin	2	
17	R.09092	High Lignin	2	
18	R.09093	High Lignin	2	3
19	R.09094	High Lignin	2	
20	R.09095	High Lignin	2	
21	R.09096	High Lignin	2	
22	R.09098	Low Lignin	2	3
23	R.09099	Low Lignin	2	
24	R.09101	Low Lignin	2	
25	R.09102	Low Lignin	2	
26	R.09103	Low Lignin	2	
27	R.09104	Low Lignin	2	
28	R.09105	High Lignin	2	
29	R.09106	High Lignin	2	3
30	R.09108	High Lignin	2	
31	R.09109	High Lignin	2	
32	R.09110	High Lignin	2	3
33	R.09112	Low Lignin	2	
34	R.09114	Low Lignin	2	
35	R.09115	Low Lignin	2	
36	R.09116	Low Lignin	2	
37	R.09117	Low Lignin	2	
38	ATx2928/BTx2752//R07020	Hybrid	2	
39	R.08028	Agronomic fit	2	
40	R.07020	Agronomic fit	2	

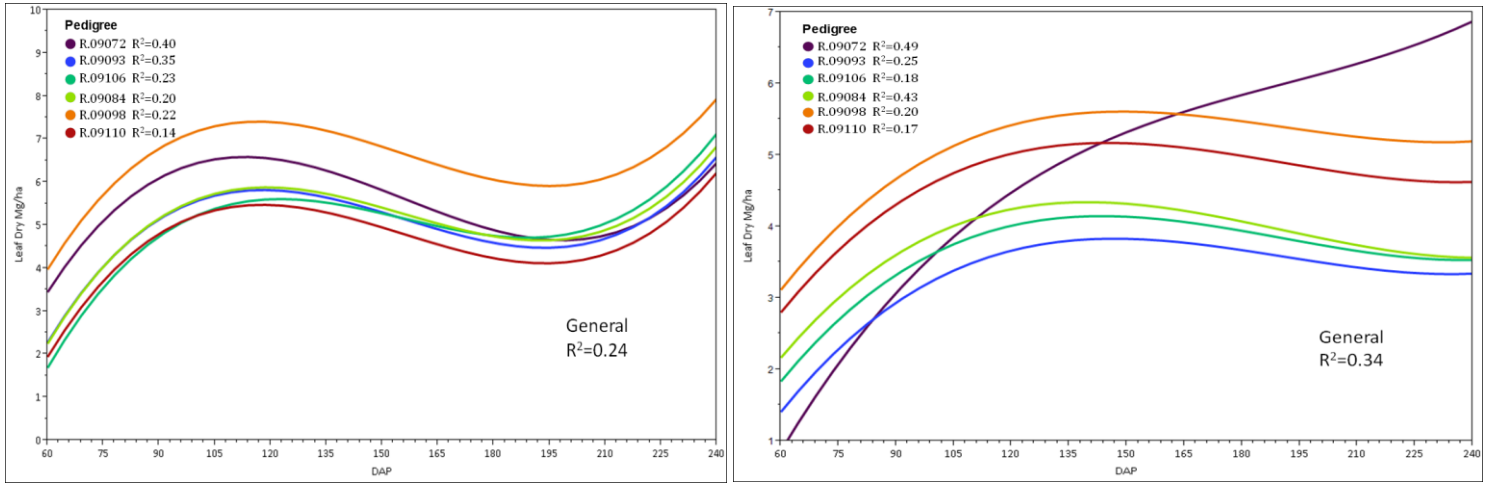

Figure A.1. Cubic regression for leaf dry biomass yield of six genotypes of biomass PS sorghum in CC (left) and CS (right), in Texas, 2010.

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