THE EFFECTS OF CYTOPLASM ON THE YIELD AND QUALITY OF SORGHUM BIOMASS HYBRIDS

A Thesis

by

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ABSTRACT

The U.S. Renewable Fuel Standard mandated increases in the amount of renewable fuel blended into transportation fuel from 9 billion gallons in 2008 to 36 billion gallons by 2022. While a portion of this renewable fuel can be derived from corn-based ethanol, the remaining balance of fuel must come from second or third generation biofuel sources. Energy sorghum was identified as one of these potential sources and improvement to produce hybrid varieties thus commenced. One tool of hybrid sorghum development involves the use of cytoplasmic male-sterility (CMS). Since there are multiple CMS systems used in the production of hybrid sorghum, it is important to understand the effects each system has on the agronomic performance and composition of biomass energy sorghum. The purpose of this study is to determine if cytoplasm affects agronomic performance and the composition of biomass sorghum. Iso-cytoplasmic hybrids were produced using four female and four male parental lines, resulting in 16 hybrid genotypes in three different cytoplasms (A1, A2, and A3) for a total of 48 hybrids. These hybrids were evaluated in three environments over two years and measured traits included biomass yield, dry stalk yield, height, stalk juice yield, juice extraction efficiency, brix, ash, lignin, glucan, and xylan. For most all traits, differences among the hybrids and environments accounted for most of the variation; cytoplasm did not significantly affect the performance of any traits. These results indicate that the different CMS systems can be used interchangeably to make sorghum

biomass energy hybrids without negative repercussions in terms of agronomic performance and composition.

DEDICATION

I dedicate this thesis to my wife and family, who have always been there to support me.

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I would like to thank my committee chair, Dr. Rooney, not only for his help with this project but also for giving me this opportunity and the lessons he has taught me. Also I would like to thank my committee members Dr. Hague and Dr. Byrne for their guidance in this research.

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NOMENCLATURE

C	Cytoplasm
CMS	Cytoplasmic male-sterility
CS	College Station
E	Environment
EE	Extraction Efficiency
F	Female
M	Male
PS	Photoperiod sensitive
YD	Yield

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The majority of energy used for transportation fuel in the United States is produced by burning of fossil fuels, which is a non-renewable resource. Scientists estimate the world will exhaust economically usable fossil fuels within the next 50 to 120 years (DOE, 2014). In addition, burning fossil fuels releases potentially harmful greenhouse gases; use of cellulosic ethanol reduces greenhouse gas emissions by 86 percent when compared to gasoline (DOE, 2014). To mitigate these concerns and facilitate a transition to more renewable fuels, the Renewable Fuel Standard called for an increase in renewable fuels from 9 billion gallons in 2008 to 36 billion by 2022 (DOE, 2014). Currently, the majority of ethanol produced in the United States is derived from corn. In 2014, the United States produced 54.4 billion liters of corn based ethanol (Renewable Fuels Association, 2014). This was approximately 11% of the 517.11 billion liters of gasoline consumed in the United States (U.S. Energy Administration, 2014). The amount of corn that can be used for ethanol production is limited due to the need for corn as a food and feed. Therefore, other options must be considered to close the gap between gasoline consumption and renewable fuels produced. Miscanthus and switchgrass also have been identified as crops to potentially bridge this gap. Both are large perennial grasses that have shown promising high biomass yields (Heaton, et al., 2008, McLaughlin, et al., 1999); however, neither of these crops have a breeding history or an established production systems, which makes their development difficult. Further,

switchgrass has been bred primarily for nutritional value as a forage crop for livestock, in which high leaf matter and nutrient content are important, not high cellulose and low ash, which are important for energy conversion (McLaughlin, et al., 1999). Also, miscanthus requires up to three years to fully establish.

Another plant species under consideration as an ethanol feedstock is sorghum (*Sorghum bicolor* L. Moench). Sorghum is unique because it has been grown as a crop for many years and has an established production system. Though it traditionally has been grown as a grain crop for food and feed (approximately 2.89 million hectares were planted to grain sorghum in the United States in 2014 and that acreage produced 433 million bushels of grain) its use as a bioenergy crop has been rising in popularity as of late (USDA, 2015). Rooney, et al. (2007) designated four factors that make sorghum a priority species for bioenergy production: i). high yield potential and variable composition; ii) high water use efficiency; iii) an established production system; and iv) the potential for rapid genetic improvement using both traditional and genomic approaches.

Sorghum originated in Northeast Africa around 5,000 years ago and then was moved through traditional trade routes throughout Africa and into Asia (India and China) (Kimber, 2000). As the Americas and Australia were colonized, sorghum moved to these regions in the 16th and 18th centuries respectively (Kimber, 2000). As a grain crop, sorghum is the fifth most widely grown cereal crop in the world, and it is grown in regions of the world where heat and drought are persistent abiotic stresses.

Specific types of sorghum have been developed for different end uses. Grain sorghum is used as both a food and feed grain, as well as for the production of industrial compounds, such as starches, and ethanol. Forage sorghums, which include grazing, hay types, and silage types are primarily for ruminant feedstock. Accurate statistics for forage sorghum production are not available, but seed sales of forage sorghum are similar to that of grain sorghum (Rooney, et al., 2007). Bioenergy sorghums are often subdivided into biomass and sweet sorghums (Rooney, et al., 2007). Sweet sorghum, as the name implies, accumulates sugar in the stem and can also be used as forage sorghum in some production systems. More commonly, juice is milled from sweet sorghum to produce syrup which is used as a food or fermented into ethanol (Rooney, et al., 2007). Bioenergy sorghum typically is photoperiod sensitive (PS) meaning it will not initiate reproductive growth until day lengths are reduced below a genetically defined length (Rooney and Aydin, 1999). Thus, plants remain in a vegetative growth phase for long periods, producing large amounts of biomass. The biomass that is harvested is primarily ligno-cellulosic and the whole biomass from these plants can then be converted to ethanol or as a combustion fuel for electricity.

PS is common in sorghum because it provided the species an evolutionary advantage in the environments where it originated. Being PS allows sorghum to grow and flower during the rainy season with the grain maturing into the dry season averting grain weathering (Morgan and Finlayson, 2000). However, PS was a highly undesirable trait for gain production in the temperate regions because these lines never flowered in time to produce grain in the fall. Consequently, photoperiod insensitive sorghum types

were selected and are predominant in these regions of the world. The relative value of PS sorghum was identified and established for both forage and bioenergy sorghum (Rooney and Aydin, 1999, Rooney, et al., 2007). Even so, to use these traits, seed production systems amenable to temperate production environments are necessary.

Four maturity genes *(Ma $_{1-4}$)* that affected PS and maturity have been described (Quinby, 1967, Quinby and Karper, 1945). Allelic variation at these four loci result in a 40 to 100 day flowering range (Quinby, 1967). Extremely late genotypes are always dominant at *Ma$_1$* and *Ma$_2$*, early genotypes were always recessive at *Ma$_1$* regardless of the allelic composition of the other three loci. In addition, *Ma$_2$* and *Ma$_4$* have temperature sensitive responses (Major, et al., 1990).

Conversion of *Ma$_1$* from a dominant to recessive genotype is the primary reason for photoperiod insensitive sorghum (Lin, et al., 1995), but there are exceptions to these observations. Rooney and Aydin (1999) described *Ma$_5$* and *Ma$_6$* loci that could explain this variation and control the PS response. These loci must both be dominant to induce the PS phenotype. In most cases, when *Ma$_5$* and *Ma$_6$* are dominant, flowering time is delayed no matter the allelic composition of the first four maturity loci. Contingent on planting date, the combination of the dominant *Ma$_5$* and *Ma$_6$* interaction and dominant alleles at the first four maturity loci can more than double the delay in flowering (Brady, 2006). Also, it is predicted that an active form of PHYB (or *Ma$_3$*) is required for *Ma$_5$*/*Ma$_6$* genotypes to express delayed flowering (Mullet, et al., 2012). Understanding of these two genes has allowed a method of producing late flowering or non-flowering hybrid sorghum plants by crossing a plant that is heterozygous dominant for at least a

Ma_5 or *Ma_6* allele. For example, a seed parent with the genotype *ma_1*, *Ma_5*, *ma_6* can be crossed to a pollinator parent with the genotype *Ma_1*, *ma_5*, *Ma_6* and the resulting progeny will be PS. Also, the same result can be achieved by crossing a plant that is homozygous dominant for at least the *Ma5* or *Ma6* allele with a second plant homozygous recessive for at least *Ma_5* or *Ma_6* (Mullet, et al., 2012).

Because sorghum has a complete flower and is self-pollinated, a cytoplasmic male-sterility (CMS) system is needed to make the production of hybrid seed economically feasible. Stephens and Holland (1954) described the first such system in crosses of Day x Kafir. Male sterility in this system was attributed to the interactions between milo cytoplasm and Kafir nuclear factors, and the degree of sterility is increased as the proportion of Kafir chromosomes in milo cytoplasm is increased. Maunder and Pickett (1959) determined that male sterility was dependent on Msc_1 being recessive. A second locus Msc_2, was later identified by (Erichsen and Ross, 1963). This locus interacted with Msc_1, so that if either was homozygous recessive, sterility in milo cytoplasm would occur.

In 1970, the Southern Corn Leaf Blight (*Helminthosporium maydis*) epidemic in maize (*Zea mays* L.) swept through the United States. That year, yield losses due to the disease were estimated at 20-30% (Ullstrup, 1972). The epidemic was caused by a then unknown susceptibility of maize with "T" cytoplasm to the disease (Tatum, 1971). At that time, this cytoplasm was used to produce about 85% of the corn hybrids in the United States. The corn industry quickly responded by reverting to normal-cytoplasm

corn and detasseling the rows of seed parent plants in seed-production fields (Ullstrup, 1972).

Similar concerns were of importance in sorghum especially because CMS was essential to hybrid seed production because detasseling is not an option. This led to the identification of multiple CMS systems. Worstell, et al. (1984) outlined the relationship among the cytoplasms of the four major systems (A1, A2, A3, and A4). Of these four systems, three are deployed to some level in the commercial sorghum industry. The A1 CMS system was the first to be used and is still the most widely used (Hoffmann Jr and Rooney, 2013). While not widely used, the A2 system works in a similar manner to the A1 and numerous maintainer and restorer lines for both grain and forage sorghum have been produced (Miller, 1986, Miller, et al., 1992, Schertz, 1977). The A3 CMS cytoplasm was introduced with the registration of A3Tx398 by (Schertz, 1984). Because the A3 system relies on gametic fertility restoration and the allele frequency of restoration is rare, it is not used in the grain sorghum industry because most hybrids made in A3 are male sterile or partially fertile (Pring, et al., 1999, Worstell, et al., 1984). Because seed set is not a high priority in the production of forage or sweet sorghums, use of the A3 CMS system is suitable for production of these hybrids and sometimes desirable in these systems (Pfeiffer, et al., 2010).

The use of different CMS systems is only viable if the agronomic productivity and crop quality of each system is similar. In grain sorghum, Maves and Atkins (1988) studied the influence of cytoplasm type upon the agronomic performance of hybrids. They crossed ten inbred lines as males to three male-sterile lines carrying A1, A2, and

A3 cytoplasms and scored the 30 hybrids for grain yield. They observed that A1 out produced A2 by 8 percent and A1 out produced A3 by seventeen percent. Moran and Rooney (2003) conducted a similar study but used iso-cytoplasmic hybrids. They used four female and three male parental lines to create 12 hybrid genotypes in three different cytoplasms (A1, A2, and A3) for a total of 36 hybrids. In this study, hybrids with A1 and A2 cytoplasms had similar yield potential, while hybrids with A3 cytoplasm were consistently lower yielding, averaging a five percent reduction in grain yield compared to some hybrids with A1 and A2 cytoplasm.

In forage sorghum, (Pedersen and Toy, 1997) compared the use of A1 and A3 cytoplasms in sorghum x sudan grass hybrids. They used bulk pollen from eight sudan grass populations to pollinate four sorghum lines that had been sterilized in A1 and A3 cytoplasm, and they reported that cytoplasm had no effect on the height, yield, or composition of the hybrids.

In biomass sorghum, Hoffmann Jr and Rooney (2013) evaluated the effects of cytoplasm on the agronomic performance and quality of biomass sorghum hybrids. In this study, three female parent lines with three cytoplasms (A1, A2, and A3) were crossed with one pollinator, for a total of nine hybrids. Their results showed that the performance of biomass hybrids is not influenced by any of the A1, A2, or A3 cytoplasms. In this study, only a single pollinator line was evaluated because at the time of that study, only one pollinator line of that type was available. Since then, additional recessive Ma_5 pollinators are now available and thus, a study is needed to assess if cytoplasm has any effect on the performance of biomass sorghum hybrids.

The objective of this project is to assess the effect A1, A2, and A3 cytoplasms have on the yield and agronomic traits of biomass sorghum hybrids.

CHAPTER II

MATERIALS AND METHODS

II.1 Plant Germplasm and Hybrid Development

Four seed parents and four pollinator parents were selected to produce hybrids for this study. The four seed parents are Tx378, Tx623, Tx626, and Tx631, and these four lines were released between 1963 and 1986 (Miller, 1986, Stephens, 1965) (Table 1). These lines were developed through conventional pedigree breeding procedures. All the seed parent lines are genetically three-dwarf (*$dw_1Dw_2dw_3dw_4$*) and photoperiod insensitive and have been used extensively in the production of both grain and forage sorghum hybrids. Lines were originally sterilized by backcrossing into A1 cytoplasm. Iso-cytoplasmic versions of these lines were developed by the Texas A&M Agrilife Research sorghum breeding program using the procedure described by Miller, et al. (1999). Inbred lines were crossed to a male sterile line of A2 and A3 cytoplasms. Male-sterile F_1 progeny rows and counterpart males were then backcrossed. A total of six backcrosses past the original cross were used to develop the A2 and A3 male-sterile lines.

Four pollinator parents R.10712, R.10717, R.10764, and R.10781 were used as pollinators onto each of the seed parents. A fifth parent, R.07007, was crossed to A1 and A2 seed parents to create check hybrids. All five lines were developed in the Texas A&M Agrilife Research sorghum breeding program for bioenergy pollinator lines (Table 1). While these lines vary in height, all five of these lines are photoperiod insensitive (*Ma_1 ma_5 Ma_6*). As aforementioned, there was only one original source of recessive

Ma_5. Therefore, the male lines used in this study were derived from this original source. The other lines in the pedigrees of the pollinator parents come from a variety of backgrounds. They include lines from the United States to Africa and original uses from grain sorghum pollinators to forage varieties. Because standard seed parents are complementary at these maturity loci (*ma_1 Ma_5 ma_6*), hybrids between these lines and the seed parents used in this study, result in PS hybrids due to interaction at *Ma_1*, *Ma_5*, and *Ma_6* (Olson, et al., 2012, Rooney and Aydin, 1999).

All seed for the hybrids was produced by hand pollination in a crossing block at College Station, Texas, in 2013. For this study, 16 hybrid combinations, each present in three different cytoplasms (A1, A2, and A3), resulted in a total of 48 hybrids, not including check hybrids. Some hybrids were limited by seed availability and thus, were not at all included in all field evaluations (Table 2).

II.2 Experimental and Field Layout

The hybrids along with the appropriate checks were evaluated in a randomized complete block design with three replications in a total of three environments. There were up to 48 hybrids in the study, but three were excluded because seed quantities were insufficient for testing (Table 2). In 2014, there were two testing environments in College Station, Texas, with different planting dates, five weeks apart. A third environment was grown in College Station in 2015. College Station is a subtropical environment located in the south central region of Texas, with a soil type of Raymondville Clay Loam. In all environments, each experimental unit was composed of two row plots with plot lengths of five meters each and row spacing of 0.76 meters

resulting in a total plot area per hybrid of 8.25 square meters. Plots were planted at a seeding rate that met a target of 148,260 plants per hectare.

The first 2014 environment was planted on 17 April and the second environment was planted on 22 May. At both locations, sorghum production followed soybeans in the previous year. Fields were cultivated four times (7 January, 3 February into hipped rows, 24 March, and 5 June rolling cultivated) and plots were fertilized twice. Fertilizer applications included 168 kilograms of 10-34-0 + 4.5 kilograms Zinc/hectare on 31 January and 148 kilograms of N^2/hectare on 12 May. For weed control, the early planting received one application of 3.5 liters of Atrazine 4L™ + 1.75 liters Brawl™ + 1.2 liters of Butracil™ per hectare applied on May 23rd. Plots were furrow irrigated once on 14 July. The first environment was harvested 19 August and the second was harvested on 02 September. The 2015 trial was planted at College Station on 21 April. The field was planted with biomass sorghum the previous year, and an application of 219 kilograms of 11-37-0 + 4.5 kilograms of Zinc/hectare was applied on 13 February. An application of 3.5 liters of Atrazine 4L™ + 1.75 pints of Brawl II™+ 2.33 liters of glyphosate/hectare was applied for weed control with a hooded sprayer.

II.3 Trait Measurement

Plant height was measured on the whole plot, by standing at the front of the plot and estimating an average for the whole plot in centimeters from the ground to the whirl (growth point) of the plant. Days to anthesis was not measured because the hybrids did not flower during this study.

Just prior to harvest, three plants per plot were randomly selected and hand harvested at the soil line in the early morning, to mitigate daily fluctuations in moisture content in the plant due to evapotranspiration. The three plant samples were weighed, and then the leaves were stripped, and the stalk weight recorded. The difference in these two weights was the fresh leaf weight. Stalks were then crushed to extract juice using a portable three-roller Ampro Sugarcane Crusher Diamond model (Ampro Exports; New Delhi, India). The extracted juice and bagasse, which is the residue left after the extraction of juice from the stalks, were weighed and used to calculate juice extraction efficiency (EE). Juice EE was calculated by dividing juice weight by stalk weight. The soluble sugar concentration (ºbrix) in the juice was measured for each juice sample using an Atago PAL-1 digital pocket refractometer (ATAGO Cc., LTD : Itabashi Japan) with a range of 0-53%. An estimate of fermentable sugars was made by multiplying brix value by 0.873, which is the average proportion of fermentable sugars in juice (Corn, 2009). A sub-sample of bagasse was collected and dried in a Grieve Corporation air-forced flux drier (The Grieve Corporation; Round Lake, IL USA) at 52º Celsius until the sample weight was stable. Bagasse was weighed before and after it was dried to determine the percent moisture of the samples. Dried bagasse was then ground using a Wiley standard model 3 knife with a 2mm sieve (Arthur H. Thomas Co.; Philadelphia, PA USA), and stored for later analysis.

Harvesting of the entire plot for yield estimation was done with a John Deere™ Silage Harvester model 5460 and weighed in a wagon equipped with Avery Weight-Tronics™. Fresh weight biomass yield was calculated by the formula *Yield=plot*

weight/percent of hectare. Dry weight yield was calculated in the same manner after multiplying fresh weight by dry matter concentration. To calculate the dry matter concentration, first juice weight was subtracted from fresh stalk weight to give fresh bagasse weight of the three plant sample. Then the equation $\frac{(JW \times °Brix)+(FBW \times DMBS)}{FSW}$ was used to calculate dry matter concentration (where JW = juice weight, FBW = fresh bagasse weight, DMBS = dry matter concentration of bagasse sample, and FSW = fresh stalk weight). The juice yield per hectare was estimated by multiplying stalk yield per hectare by juice EE.

II.4 Compositional Analysis

Compositional analysis was performed on the ground bagasse using near-infrared spectroscopy (NIRs) using a FOSS XDS MasterLab, with the XDS Rapid Content (FOSS NIR Systems Inc.; Laurel, MD USA). Each sample was scanned twice to improve accuracy, and scanned at a 2 nanometer wavelength between 600-2400. Predicted traits using the FOSS Win-ISI software and the predictive curve were developed by the Texas A&M AgriLife sorghum lab and the National Renewable Energy Laboratory (Wolfrum, et al., 2013) included ash, protein, lignin, glucan, and xylan. Ash is the total structural and nonstructural inorganics, protein is protein by combustion (nitrogen), lignin is acid insoluble residue (Klasson Lignin), glucan is C_6 sugar major (associated to hemicellulose), and xylan is C_5 sugar major (associated to hemicellulose). Each trait is predicted as a percentage of the whole, so all compositional traits combined add up to approximately 100%.

II.5 Experimental and Statistical Analysis

Statistical analysis was performed using SAS verion 9.4 (SAS Institute, 2013). The analysis was done using the Proc GLM procedure. An analysis of each environment was run separately with the model *Y= mean + replication + hybrid + cytoplasm + cytoplasm x hybrid + error* with all effects fixed. Data from all environments was fit to combine as determined by Bartlett's test for Homogeneity of Error (Bartlett, 1937). The model *Y= mean + environment + replication (environment) + range + row + hybrid + cytoplasm + cytoplasm x hybrid + environment x cytoplasm + environment x hybrid + environment x cytoplasm x hybrid + error* was used for the combined data. Replication and environment were considered random effects while hybrid and cytoplasm were fixed effects. In addition, the hybrid source of variation was split into male, female, and male*female for a separate analysis. All mean separation analyses were completed using Tukey's HSD.

CHAPTER III

RESULTS AND DISCUSSION

III.1 Effect of Sources of Variation on Performance

In all three individual environments, effects due to hybrid were significant for all traits while effects due to cytoplasm were not significant for any of the measured traits. Because there was consistency in performance within environments and the error terms among locations were not heterogeneous, individual environments were combined for further analysis.

Of the main effects in the combined analyses, hybrid and environment were significant for most traits while cytoplasm was not significant for any trait (Tables 3 and 4). In Previous studies, testing the effect of cytoplasm on sorghum performance y reported variable results. Differences among cytoplasms were detected in grain yield b several groups (Maves and Atkins, 1988, Moran and Rooney, 2003). Both studies reported lower grain yields with the A3 cytoplasm, but neither assessed total biomass yield. Pedersen and Toy (1997) compared biomass yield in sorghum x sudan grass h hybrids and found no difference between A1 and A3 cytoplasm for biomass yield, whic is consistent with the observations in the current study. In both this current study and Pedersen and Toy (1997), flowering time was either not significant or plants never flowered, while in the studies done on grain sorghum flowering time was significantly different among the cytoplasms (Maves and Atkins, 1988, Moran and Rooney, 2003). McBee, et al. (1983) reported that carbohydrate levels varied for the cultivars in the study and were highest in non-

senescent types. Thus, the different results seen between grain yield and biomass yield at least, in relation to cytoplasm, could be due to the partitioning of carbohydrates in grain hybrids.

Environment was significant for every trait, and accounted for the largest proportion of the variation in the study (Table 3 and 4). The highest fresh biomass yield and dry stalk yield occurred in 2015 College Station and the lowest in the late planting 2014 (Table 5). The later environment also produced the shortest plants (Table 5). The lower yields and reduced height in the late planted 2014 environment was primarily due to drought stress that began earlier in the growth of that crop as compared to the earlier planting. The highest juice yield was seen in the early planted 2014 (Table 5). Juice EE was in the highest statistical grouping in both environments in 2014 (Table 5). In terms of composition analysis, the highest concentrations of lignin and xylan were also in the early planting 2014 (Table 5). The correlation between lignin and xylan (0.84) was the highest correlation between traits in this study (Table 6) and this very high correlation between lignin and xylan was also reported by Stefaniak, et al. (2012). The glucan values were highest in the early planted 2014 and 2015 environments (Table 5). Multivariate analysis revealed a strong correlation (0.82) between glucan and xylan (Table 6) which was also reported by other groups (Dahlberg, et al., 2011, Hoffmann, 2012, Stefaniak, et al., 2012). The late planted 2014 and 2015 environments also had the highest juice EE and ash content, while brix concentration was the highest in environment three (Table 5). Since energy sorghum recycles nitrogen in the life cycle, it

is logical that the shortest growing season would produce the lowest yields with similar nitrogen content (Olson, et al., 2012).

In the hybrid term, significant effects were detected for every trait except height and glucan concentration (Tables 3 and 4). Upon subdivision of the hybrid term into male, female, and male x female; the male term was significant for every trait except for glucan concentration while the effect due to female was not significant for any measured trait (Tables 7 and 8). This suggests that the male parent is influencing the phenotype of these traits to a much greater extent than the female parentage. When looking back at several years of data on the males and females, the females' data for height and days to flowering show four females that hardly differ for these traits. The male data shows a different story. Differences in height reach up to around 70 centimeters between the shortest and tallest male and over a week between the earliest and latest flowering male. These differences can be the reason that the males in this study are causing the majority of the variation in the biomass sorghum hybrids. The differences in the males is a result of the development of males that would work in the crossing of two photoperiod insensitive lines to make a PS hybrid. As mentioned earlier the recessive form of Ma_5 has only been found in one original source of germplasm. Therefore, more sources of males with the recessive Ma_5 genotype were needed to create variation for breeding of PS sorghum hybrids. To develop these males, the original source of the recessive Ma_5 was crossed to a wide variety of lines to develop and find what type of R-line phenotype would work well in the production of PS sorghum hybrids by crossing two photoperiod insensitive types. For example, the male lines in this study were a result of integrating

the original source of recessive *Ma*$_5$ with lines that varied in where they originated (from Africa to the United States) and their original purposes (male lines for grain sorghum hybrids to forage varieties).

Most interaction terms that were significant involved the environment. The three notable exceptions to this trend were male*female for juice EE and hybrid*cytoplasm and female*cytoplasm for fresh biomass yield (Table 7). Among the environment interactions, hybrid*environment was significant for all traits except fresh biomass yield, stalk juice yield, brix, and glucan (Table 3 and 4). Male*environment was significant for all traits except stalk juice yield, brix, ash, glucan, and xylan (Table 7 and 8). Female*environment was only significant for xylan concentrations (Table 8). Male*female*environment was significant for stalk juice yield, ash, glucan, and xylan concentrations while, male*cytoplasm*environment was significant for fresh biomass yield and dry stalk yield (Table 7 and 8). It is important to note that no significant effects were detected for cytoplasm*environment, hybrid*cytoplasm*environment, and male*female*cytoplasm*environment (Tables 3, 4, 7, and 8). The absence of interactions with the cytoplasm term suggests that the different CMS systems, used to develop the hybrids, is not interacting with the other main effect terms to cause variation between them.

III.2 Analysis of Cytoplasm per Hybrid

Given the structure of the hybrids within this study, it was possible to assess the effect of cytoplasm not only across hybrids, but also within specific hybrid combinations. Herein, we evaluated each hybrid to determine if cytoplasm might affect

specific combinations and in some combinations differences could be detected. For every trait except height there were differences among cytoplasms detected in at least one hybrid combination (Tables 9-11). For dry stalk yield, stalk juice yield, juice EE, and brix there was one hybrid for each that showed differences across cytoplasms, but for each trait it was a different hybrid combination (Tx626/R10712, Tx378/R10764, Tx631/R10717, and Tx378/R10781 respectively) so there was no consistency (Tables 9 and 10). In the composition traits, similar trends were detectable but each involved more than one hybrid; ash content was different among cytoplasms in Tx378/R10781, and Tx623/R10717; protein content differed in Tx623/R10781and Tx626/R10764; lignin content differed in Tx378/R10764 and Tx631/R10717; and glucan content in Tx378/R10712 and Tx626/R10764 (Table 10 and 11). Four different hybrids varied among cytoplasm for fresh biomass yield and xylan content (Table 9 and 11). Differences for xylan content were detected among cytoplasms for hybrids Tx378/R10764, Tx623/R10781, Tx631/R10717, and Tx631/R07007 (Table 11). For fresh biomass yield were seen in the Tx378/R10781, Tx626/R10712, Tx631/R10717, and Tx631/R07007 hybrids (Table 9).

For the traits that had cytoplasm effects influencing at least two hybrid combinations, there was no discernable pattern as to which cytoplasm consistently produced the highest or lowest values. For example, for fresh biomass yield in the hybrid Tx378/R10781 and Tx631/R10717 the A3 cytoplasm was the highest yielding. However in hybrid Tx626/R10712, A1 was the highest yielding and A3 was in the lowest statistical group (Table 9). Similarly, in ash content in Tx378/R10781 was

highest in the A1 cytoplasm while in Tx623/R10717, the A2 cytoplasm had the highest ash content (Table 10). Comparable results were seen in protein and glucan content (Table 10 and 11). In lignin content, A1 was the highest performing for the two hybrids with differences, but A2 was the second highest performing for Tx378/R10764 and for Tx631/R10717 A3 was the second best (Table 11). For xylan content, the A1 cytoplasm was the highest performing in three out of four of the hybrids showing differences but in the fourth (Tx623/R10781) A3 was the best performing (Table 11).

There was no specific hybrid that consistently showed differences among the cytoplasms for all of the traits. For example, Tx631/R10717 showed differences across four different traits (fresh biomass yield, juice EE, lignin content, and xylan content), for lignin and xylan content the order of performance by cytoplasm was the same (A1>A3>A2) (Table 9-11). This is expected because these two traits are highly correlated, however for fresh biomass yield and juice EE the same results were not seen (Table 9 and 10). For fresh biomass yield A3 was the highest performing and A1 the lowest and for juice EE A2 was the highest and A3 the lowest (Tables 9 and 10).

It is important to note even though differences were found when the hybrids were individually analyzed, no differences were detected in the combined analysis. There are several potential reasons for this. First, these individual hybrids are only one of 16 different combinations and thus, they individually do not impact the combined analysis enough to cause differences. In many cases, the effects in individual hybrids counteracted the effect of another. For instance, glucan content in Tx378/R10712 was

different among cytoplasms (A3 > A1 > A2), but this was counteracted by Tx626/R10764 (wherein A2 > A1 > A3) (Table 11).

Results indicate that while cytoplasm per se does not influence performance, individual hybrids may differ. This hybrid by cytoplasm interaction is of importance to plant breeding programs and indicates that they must evaluate any specific combination to ensure that performance is not affected by cytoplasm within that specific hybrid combination.

III.3 Analysis of Cytoplasm per Male and Across Males

Because the effect of the male parent was consistently significant in the combined analysis, the effect of cytoplasm by individual males was analyzed for trends. Hybrids involving pollinator line R10712 differed for height (A3 > A1), and R10781 showed differences for juice yield, lignin content, and xylan content (Tables 12 and 13). In all three of these traits, the A3 cytoplasm was the highest performing (Tables 12 and 13). No other differences were detected.

Males were analyzed for differences between the pollinator lines. So, every male that was part of a hybrid combination with any A1 female was compared to look for differences. The same was done for every male that was part of a hybrid combination with any A2 or A3 females as well. In this analysis, many differences were found across the male parents. In this analysis, differences were detected across male parents within each cytoplasm. When crossed to A1 females, differences among males were detected for every trait except fresh biomass yield. When crossed to A2 females, differences among male parents were observed for every trait except for height and lignin. When

crossed to A3 females, differences among the males were not observed for fresh biomass yield, height, stalk juice yield, lignin, and xylan (Tables 12-16). Some trends were consistent. R10712 was the highest performing for juice EE no matter the cytoplasm content of the female (Table 15), and the same can be said for R10781 for brix content (Table 15). Other than these instances, there were no other males that were consistently the highest or lowest performing.

When considering the results of these two different analysis, the fact arises that many differences were found across the male lines and few were found across the cytoplasms. This further reinforces the notion that the male parent in the hybrids is more impactful on and accounts for more variation in these hybrids than does the cytoplasm. Due to these results, breeding programs should concentrate on the improvement of male parents to significantly increase productivity of sorghum biomass hybrids.

CHAPTER IV

CONCLUSION

The objective of this study is to determine if cytoplasm affects the biomass yield and composition of energy sorghum hybrids. Based on the comparison of 16 iso-cytoplasmic hybrids grown in three environments, cytoplasm did not affect any yield or composition trait. The absence of differences suggests that sorghum breeding programs can effectively use any of these three cytoplasms without concern that cytoplasm type will influence yield potential or composition of hybrids. Likewise, interactions involving cytoplasm were not significant indicating that this response was consistent across the environments tested.

The implication of these results mean that if a certain cytoplasm becomes susceptible to a disease in the future, as happened with the Southern Corn Leaf Blight epidemic, the biomass sorghum industry can use an alternate CMS and still efficiently produce biomass hybrids. Likewise, if a transgenic trait is placed into biomass sorghum, not only can PS serve as a mechanism to limit pollen flow and potential outcrossing but the use of A3 cytoplasm could be another mechanism to reduce risk of any fertile pollen from a plant that might flower early.

On a per hybrid basis, some differences were detected between the cytoplasms. While these individual differences were not strong enough to affect the significance of the combined analysis, sorghum plant breeders should be cognizant that specific hybrid combinations are subject to differences in production.

Based on the significance seen in the male term and the differences seen between male parents, the male parent has more impact than the female parent or cytoplasm on the performance of biomass hybrids. This indicates that focusing breeding efforts on improvement and testing of new male lines for hybrid combination should be the most efficient and effective way for a sorghum breeder to produce profitable biomass hybrids.

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APPENDIX

Table 1. Pedigrees of seed and pollinator parents used to make 48 sorghum biomass hybrids in study of cytoplasm in sorghum biomass hybrids.

Type	Parent	Pedigree
Seed Parent	A/BTx378	SA378
Seed Parent	A/BTx623	(BTx3197*SC170-6-4-4)-7-3-1-3-2-1
Seed Parent	A/BTx626	(BTx378*SC110-6)-2-3-4-2-3-7-3-2-1-bk
Seed Parent	A/BTx631	((BTx378*SC110-9)*BTx615)-4-3-5-2-1-2-bk
Pollinator Parent	R.10712	Macia/R07007
Pollinator Parent	R.10717	R07007/MN4466
Pollinator Parent	R.10764	SCSC242 Unconverted/R07007
Pollinator Parent	R.10781	Tx2910/DMR Sudangrass-EC40

Table 2. Sorghum biomass hybrids included and not included due to seed availability in study of cytoplasm in sorghum biomass hybrids.

	R10712	R10717	R10764	R10781
ATx378	x	x	x	x
A2Tx378	x	x	x	x
A3Tx378	x	x	x	x
ATx623	x	x	x	x
A2Tx623	x	x	x	x
A3Tx623	x	x	x	x
ATx626	x	o	x	x
A2Tx626	x	x	x	o
A3Tx626	x	x	x	x
ATx631	o	x	x	x
A2Tx631	x	x	x	x
A3Tx631	x	x	x	x

† x = hybrid included; o = hybrid not included.

Table 3. Summary of analysis (mean squares) of combined locations (CS 2014 & 2015) for the traits fresh biomass yield, dry stalk yield, height, stalk juice yield, and juice EE of 49 sorghum biomass hybrids.

Source	DF	Fresh Biomass Yield	Dry Stalk Yield	Height	Stalk Juice Yield	Juice EE
E	2	44787**	5688**	274343**	1731840594**	0.4684**
Rep[E]	2	31	80*	3302**	725301	0.0009
Range[E]	80	217	31	674	29617595	0.0033
Row[E]	17	589	24	1417**	55644467	0.0027
H	17	490**	67**	1082	102519943**	0.0245**
C	2	410	63	475	12280541	0.0029
H*C	29	361**	26	940	33370400	0.0026
C*E	4	166	15	443	19522329	0.003
H*E	34	222	39*	1483**	49685113	0.0080**
H*C*E	56	188	30	492	26043916	0.0032
Error	179	172	24	587	34222277	0.0033
Mean		86	17	252	21904	0.34
CV (%)		15.3	29.3	9.6	26.7	17

*Significant at P<0.05.

**Significant at P<0.01.

† C = Cytoplasm; E = Environment; F = Female; H = Hybrid; M = Male; Rep = Replication.

Table 4. Summary of analysis of variance (mean squares) of combined locations (CS 2014 & 2015) for the traits of brix, ash, protein, lignin, glucan, and xylan content of 49 sorghum biomass hybrids.

Source	DF	Brix	Ash	Protein	Lignin	Glucan	Xylan
E	2	497.41**	9.53**	41.05**	104.60**	585.50**	149.67**
Rep[E]	2	1.38	0.18	0	3.60*	0.76	1.48
Range[E]	80	1.32*	0.42	0.13	1.06	1.61	0.59
Row[E]	17	1.08	0.38	0.1	3.25**	5.44**	1.63**
H	17	7.73**	1.82**	0.58**	3.76**	2.61	2.05**
C	2	0.13	0.02	0.14	0.1	0.03	0.02
H*C	29	0.43	0.32	0.11	1.63	1.81	0.85
C*E	4	2.3	0.21	0.08	0.64	1.17	0.69
H*E	34	1.09	0.65**	0.45**	1.95**	1.85	0.77*
H*C*E	56	0.76	0.38	0.11	1.17	1.79	0.71
Error	179	0.91	0.33	0.16	1.04	1.61	0.51
Mean		7.64	6.37	2.28	14.42	33.23	17.47
CV (%)		12.5	9	17.4	7.1	3.8	4.1

*Significant at P<0.05

**Significant at P<0.01

† C = Cytoplasm; E = Environment; F = Female; H = Hybrid; M = Male; Rep = Replication

Table 5. Summary of differences in three environments (CS 2014 & 2015) for agronomic and compositional traits of 49 sorghum biomass hybrids. Statistical differences among environments are designated by letters and are significant at 0.05.

Environment	Fresh Biomass Yield (mt/ha)	Dry Stalk Yield (mt/ha)	Height (cm)	Stalk Juice Yield (kg/ha)	Juice EE	Brix (%)	Ash (%)	Protein (%)	Lignin (%)	Glucan (%)	Xylan (%)
CS Early 2014	85^{B}	18^{B}	307^{A}	25686^{A}	0.3770^{A}	5.5^{C}	6.59^{A}	1.94^{B}	15.40^{A}	34.51^{A}	18.49^{A}
CS Late 2014	67^{C}	9^{C}	206^{C}	18069^{C}	0.3699^{A}	8.1^{B}	6.49^{A}	3.01^{A}	13.63^{C}	30.52^{B}	16.26^{C}
CS 2015	105^{A}	22^{A}	240^{B}	21820^{B}	0.2671^{B}	9.4^{A}	6.01^{B}	1.88^{B}	14.22^{B}	34.74^{A}	17.69^{B}

Table 6. Multivariate correlations based on Residual Maximum Likelihood method for agronomic and compositional traits of 49 sorghum biomass hybrids in three environments (CS 2014 & 2015).

Trait	Fresh Biomass YD	Dry Stalk YD	Height	Stalk Juice YD	Juice EE	Brix	Ash	Protein	Lignin	Glucan	Xylan
Fresh Biomass YD	1.00	0.77	0.23	0.53	-0.45	0.21	-0.24	-0.51	0.16	0.55	0.35
Dry Stalk YD		1.00	0.41	0.40	-0.45	0.13	-0.22	-0.59	0.26	0.65	0.44
Height			1.00	0.39	0.06	-0.50	0.07	-0.49	0.50	0.54	0.60
Stalk Juice YD				1.00	0.46	-0.27	0.09	-0.35	0.33	0.37	0.44
Juice EE					1.00	-0.42	0.32	0.18	0.07	-0.23	0.03
Brix						1.00	-0.47	0.07	-0.44	-0.10	-0.47
Ash							1.00	0.22	-0.12	-0.25	0.02
Protein								1.00	-0.29	-0.79	-0.62
Lignin									1.00	0.63	0.84
Glucan										1.00	0.82
Xylan											1.00

Table 7. Summary of analysis of variance (mean squares) of combined locations (CS 2014 &2015) for the traits of fresh biomass yield, dry stalk yield, height, stalk juice yield, and juice EE of 49 sorghum biomass hybrids.

Source	DF	Fresh Biomass Yield	Dry Stalk Yield	Height	Stalk Juice Yield	Juice EE
M	4	1121*	175**	2307*	330814800**	0.0752**
F	3	166	21	1513	8373700	0.0004
M*F	10	262	37	982	30436215	0.0081*
M*C	7	369	33	689	22562604	0.002
F*C	6	589*	57	1290	30848897	0.0052
M*F*C	16	291	18	991	33089825	0.0023
M*E	8	443*	73**	4595**	24364660	0.0134**
F*E	7	73	18	667	15170583	0.0013
M*F*E	20	205	34	451	56677717*	0.0051
M*C*E	12	310*	69**	634	25216559	0.0038
F*C*E	12	202	28	455	20053933	0.0032
M*F*C*E	28	109	15	523	28842022	0.0038
Error	179	172	24	587	34222277	0.0033
Mean		86	17	252	21904	0.34
CV (%)		15.3	29.3	9.6	26.7	17

*Significant at P<0.05

**Significant at P<0.01

† C = Cytoplasm; E = Environment; F = Female; H = Hybrid; M = Male; Rep = Replication

Table 8. Summary of analysis of variance (mean squares) of combined locations (CS 2014 &2015) for the traits of brix, ash, protein, lignin, glucan, and xylan content of 49 sorghum biomass hybrids.

Source	DF	Brix	Ash	Protein	Lignin	Glucan	Xylan
M	4	22.89**	3.60**	1.70**	9.77**	5.52	5.12**
F	3	2.19	1.21	0.04	0.94	1.21	0.9
M*F	10	1.09	0.49	0.33	1.28	1.26	0.75
M*C	7	0.35	0.41	0.03	2.46	2.66	1.35
F*C	6	0.34	0.43	0.18	0.38	0.9	0.19
M*F*C	16	0.44	0.26	0.16	1.64	1.68	0.79
M*E	8	1.27	0.66	1.28**	4.06**	1.59	0.92
F*E	7	1.11	0.56	0.08	1.55	1.03	1.12*
M*F*E	20	1.06	0.55*	0.17	1.66	2.92*	1.03**
M*C*E	12	0.47	0.36	0.11	0.69	1.66	0.33
F*C*E	12	0.51	0.38	0.15	1.33	2.38	0.89
M*F*C*E	28	0.98	0.31	0.1	1.07	1.59	0.73
Error	179	0.91	0.33	0.16	1.04	1.61	0.51
Mean		7.64	6.37	2.28	14.42	33.23	17.47
CV (%)		12.5	9	17.4	7.1	3.8	4.1

*Significant at P<0.05

**Significant at P<0.01

C = Cytoplasm; E = Environment; F = Female; H = Hybrid; M = Male; Rep = Replication

Table 9. Comparison of fresh biomass yield, dry stalk yield, height, and stalk juice yield of 49 sorghum biomass hybrids evaluated in three environments (CS 2014 & 2015). Statistical differences among cytoplasms significant at 0.05 are designated by letters in each row.

	Fresh Bagasse YD (mt/ha)			Dry Stalk YD (mt/ha)			Height (cm)			Stalk Juice YD (kg/ha)		
Hybrid	A1	A2	A3	A1	A2	A3	A1	A2	A3	A1	A2	A3
ATx378/R10712	89	74	75	15	14	12	240	252	251	23703	20692	21745
ATx378/R10717	92	99	77	18	17	12	231	234	242	20902	24271	20914
ATx378/R10764	94	88	87	18	16	18	248	255	249	28830^{A}	22014^{B}	23289^{AB}
ATx378/R10781	63^{B}	81^{AB}	94^{A}	13	16	16	266	242	240	16496	16196	20815
ATx623/R10712	72	73	81	11	13	14	209	239	240	20906	20781	23607
ATx623/R10717	77	86	87	15	14	17	249	235	250	20740	20146	19771
ATx623/R10764	82	89	87	17	19	19	249	248	261	18942	21126	20628
ATx623/R10781	77	84	88	15	16	15	220	232	243	17694	18886	19561
ATx623/R07007	80	85	x	18	18	x	271	254	x	18848	20565	x
ATx626/R10712	94^{A}	84^{AB}	83^{B}	18^{A}	14^{B}	14^{B}	262	241	258	24601	26159	22863
ATx626/R10717	x	87	91	x	19	14	x	262	248	x	24250	20276
ATx626/R10764	99	98	85	21	21	17	264	276	274	25784	27792	24626
ATx626/R10781	94	x	93	22	x	20	248	x	258	22566	x	21933
ATx631/R10712	x	75	94	x	14	18	x	248	266	x	22823	27388
ATx631/R10717	87^{AB}	73^{B}	87^{A}	15	13	18	259	249	248	23908	21527	21550
ATx631/R10764	95	102	90	19	18	19	279	269	272	24887	25619	22930
ATx631/R10781	90	88	83	20	19	17	247	268	258	20235	15904	19281
ATx631/R07007	90^{A}	74^{B}	x	22	15	x	276	267	x	21463	21111	x
Combined	87	85	86	17	16	16	251	251	253	22031	21748	21897

x = missing hybrid

Table 10. Comparison of juice EE, brix, ash, and protein content of 49 sorghum biomass hybrids evaluated in three environments (CS 2014 & 2015). Statistical environments among cytoplasms significant at 0.05 are designated by letters in each row.

	Juice EE			Brix (%)			Ash (%)			Protein (%)		
Hybrid	A1	A2	A3	A1	A2	A3	A1	A2	A3	A1	A2	A3
ATx378/R10712	0.3656	0.3656	0.3816	7.20	6.50	6.90	6.40	6.80	6.56	2.32	2.37	2.39
ATx378/R10717	0.3028	0.3343	0.3570	6.96	6.81	6.72	6.47	6.37	6.65	2.30	2.27	2.20
ATx378/R10764	0.3690	0.3270	0.3465	6.70	6.78	6.49	6.72	6.59	6.58	1.95	1.96	2.01
ATx378/R10781	0.3460	0.2764	0.2885	6.52^{B}	7.92^{A}	7.70^{A}	6.71^{A}	6.08^{B}	6.39^{AB}	2.22	2.05	2.01
ATx623/R10712	0.3980	0.3788	0.4023	7.53	7.04	7.73	6.43	6.51	6.59	2.35	2.30	2.40
ATx623/R10717	0.3682	0.3206	0.3149	7.53	6.38	7.29	6.31^{B}	6.93^{A}	6.54^{AB}	2.54	2.70	2.39
ATx623/R10764	0.3002	0.3091	0.3084	6.84	7.43	6.78	6.64	6.60	6.65	2.42	2.19	2.40
ATx623/R10781	0.3099	0.3025	0.2972	8.86	8.27	8.32	5.75	5.95	5.76	2.46^{A}	2.08^{B}	2.35^{AB}
ATx623/R07007	0.3026	0.3137	x	7.84	8.00	x	6.73	6.70	x	2.57	2.31	x
ATx626/R10712	0.3546	0.4154	0.3781	8.19	8.60	8.20	6.63	6.33	6.31	2.74	2.34	2.55
ATx626/R10717	x	0.3558	0.3175	x	7.37	7.06	x	6.38	6.41	x	2.58	2.90
ATx626/R10764	0.3085	0.3569	0.3795	7.76	7.59	7.13	6.10	6.15	6.42	2.14^{AB}	1.97^{B}	2.48^{A}
ATx626/R10781	0.3043	x	0.3154	9.17	x	9.42	5.66	x	5.55	2.19	x	2.20
ATx631/R10712	x	0.4227	0.3946	x	7.66	7.74	x	6.45	6.35	x	2.15	2.24
ATx631/R10717	0.3555^{AB}	0.3890^{A}	0.3339^{B}	7.30	7.26	7.57	6.19	6.63	6.30	2.48	2.37	2.36
ATx631/R10764	0.3291	0.3177	0.3293	7.44	7.33	7.83	6.58	6.94	6.60	1.88	2.01	1.82
ATx631/R10781	0.3063	0.2890	0.3137	9.16	8.72	9.10	5.54	6.00	6.00	2.01	2.29	2.12
ATx631/R07007	0.3113	0.3647	x	8.23	8.97	x	6.24	5.91	x	2.28	2.30	x
Combined	0.3330	0.3435	0.3400	7.73	7.57	7.64	6.31	6.43	6.35	2.30	2.25	2.30

x = missing hybrid

Table 11. Comparison of lignin, glucan, and xylan content of 49 sorghum biomass hybrids evaluated in three environments (CS 2014 & 2015). Statistical differences among cytoplasms significant at 0.05 are designated by letters in each row.

	Lignin (%)			Glucan (%)			Xylan (%)		
Hybrid	A1	A2	A3	A1	A2	A3	A1	A2	A3
ATx378/R10712	14.49	13.83	14.57	33.09^{AB}	32.26^{B}	33.42^{A}	17.88	17.63	17.96
ATx378/R10717	14.07	14.47	14.34	32.92	32.97	32.97	17.37	17.47	17.66
ATx378/R10764	15.36^{A}	15.03^{AB}	14.42^{B}	34.08	33.73	32.83	18.49^{A}	18.18^{AB}	17.63^{B}
ATx378/R10781	14.37	14.58	14.71	32.23	33.67	33.6	17.46	17.68	17.81
ATx623/R10712	13.64	14.37	13.48	32.2	33.3	32.52	17	17.49	16.87
ATx623/R10717	14.95	14.76	14.4	33.59	33.4	33.43	17.73	17.79	17.29
ATx623/R10764	15.01	14.85	15.48	11.13	33.49	33.56	17.62	17.61	18.1
ATx623/R10781	13.79	13.85	15.04	33.15	33.3	33.79	16.63^{B}	16.97^{B}	17.83^{A}
ATx623/R07007	14.75	14.75	x	33.45	33.56	x	17.41	17.64	x
ATx626/R10712	13.49	13.92	14.65	32.08	32.89	33.06	16.87	17.29	17.56
ATx626/R10717	x	14.51	14.44	x	32.57	32.88	x	17.22	17.16
ATx626/R10764	14.54	15.2	14.54	32.82^{AB}	33.88^{A}	32.28^{B}	17.34	18.05	17.21
ATx626/R10781	13.73	x	14.15	33.1	x	34.29	16.69	x	17.14
ATx631/R10712	x	13.99	13.91	x	33.3	33.19	x	17.51	17.22
ATx631/R10717	15.58^{A}	14.35^{B}	14.49^{AB}	34.25	33.17	33.15	18.13^{A}	17.41^{B}	17.49^{AB}
ATx631/R10764	14.1	14.39	14.5	33.37	33.42	33.88	17.44	17.66	17.72
ATx631/R10781	13.51	13.79	14.27	33.39	32.88	33.91	16.67	16.91	17.45
ATx631/R07007	14.73	14.39	x	33.47	32.92	x	17.66^{A}	17.12^{B}	x
Combined	14.38	14.41	14.46	33.16	33.22	33.32	17.4	17.51	17.51

x = missing hybrid

Table 12. Comparison of height and stalk juice yield of male parental lines in hybrid combination in three environments (CS 2014 & 2015). Statistical differences are significant at 0.05.

	A1	A2	A3	Differences in cytoplasm	A1	A2	A3	Differences in cytoplasm
	Height (cm)				Stalk Juice YD (kg/ha)			
R10712	237 ^{B,3}	245 ^{AB}	254 ^{A}	*	23070 ^{12}	22613 ^{1}	23901	ns
R10717	247 ^{23}	245	247	ns	21892 ^{12}	22499 ^{1}	20652	ns
R10764	260 ^{12}	262	264	ns	24611 ^{1}	24224 ^{1}	23002	ns
R10781	243 ^{23}	248	249	ns	19498 ^{AB,1}	16996 ^{B,2}	20198 ^{A}	*
R07007	274 ^{1}	261	x	ns	20156 ^{2}	20822 ^{12}	x	ns
Differences among lines	*	ns	ns		*	*	ns	

Differences among cytolasms =A,B,C

Differences among lines = 1,2,3

ns = Nonsignificant

Table 13. Comparison of lignin and xylan content of male parental lines in hybrid combination in three environments (CS 2014 & 2015). Statistical differences are significant at 0.05.

	A1	A2	A3	Differences in cytoplasm	A1	A2	A3	Differences in cytoplasm
	Lignin (%)				Xylan (%)			
R10712	13.87 ^{2}	14.03	14.15	ns	17.25 ^{12}	17.48 ^{12}	17.4	ns
R10717	14.87 ^{1}	14.52	14.42	ns	17.75 ^{1}	17.48 ^{12}	17.4	ns
R10764	14.75 ^{1}	14.87	14.71	ns	17.27 ^{1}	17.87 ^{1}	17.71	ns
R10781	13.80 ^{B,2}	14.07 ^{AB}	14.59 ^{A}	*	16.81 ^{B,2}	17.19 ^{AB,2}	17.56 ^{A}	*
R07007	14.78 ^{1}	14.57	x	ns	17.53 ^{1}	17.37 ^{12}	x	ns
Differences among lines	*	ns	ns		*	*	ns	

Differences among cytoplasms = A,B,C

Differences among lines = 1,2,3

ns = Nonsignificant

Table 14. Comparison of fresh biomass yield and dry stalk yield of male parental lines in hybrid combination in three environments (CS 2014 & 2015). Statistical differences are significant at 0.05.

	A1	A2	A3	Differences in cytoplasm	A1	A2	A3	Differences in cytoplasm
	Fresh Biomass YD (mt/ha)				Dry Stalk Yield (mt/ha)			
R10712	85	77 [2]	83	ns	15 [1]	14 [2]	15 [2]	ns
R10717	86	86 [12]	85	ns	16 [12]	16 [12]	15 [2]	ns
R10764	93	94 [1]	89	ns	19 [12]	19 [1]	19 [1]	ns
R10781	83	84 [12]	88	ns	18 [12]	17 [12]	17 [12]	ns
R07007	85	80 [2]	x	ns	20 [1]	17 [12]	x	ns
Differences among lines	ns	*	ns		*	*	*	

Differences among cytoplasms =A,B,C

Differences among lines = 1,2,3

ns = Nonsignificant

Table 15. Comparison of juice EE and brix content of male parental lines in hybrid combination in three environments (CS 2014 & 2015). Statistical differences are significant at 0.05.

	A1	A2	A3	Differences in cytoplasm	A1	A2	A3	Differences in cytoplasm
	Juice EE				Brix (%)			
R10712	0.3727 [1]	0.3956 [1]	0.3892 [1]	ns	7.63 [23]	7.44 [2]	7.65 [2]	ns
R10717	0.3422 [12]	0.3500 [2]	0.3308 [2]	ns	7.26 [3]	6.97 [2]	7.16 [2]	ns
R10764	0.3267 [2]	0.3276 [23]	0.3341 [2]	ns	7.19 [3]	7.28 [2]	7.05 [2]	ns
R10781	0.3140 [2]	0.2893 [3]	0.3062 [2]	ns	8.6 [1]	8.30 [1]	8.56 [1]	ns
R07007	0.3069 [2]	0.3392 [2]	x	ns	8.04 [12]	8.48 [1]	x	ns
Differences among lines	*	*	*		*	*	*	

Differences among cytolasms = A,B,C

Differences among lines = 1,2,3

ns = Nonsignificant

Table 16. Comparison of ash and protein content of male parental lines in hybrid combination in three environments (CS 2014 & 2015). Statistical differences are significant at 0.05.

	A1	A2	A3	Differences in cytoplasm	A1	A2	A3	Differences in cytoplasm
	Ash (%)				Protein (%)			
R10712	6.49 [1]	6.53 [1]	6.45 [1]	ns	2.47 [1]	2.29 [12]	2.39 [12]	ns
R10717	6.32 [1]	6.58 [1]	6.48 [1]	ns	2.44 [1]	2.48 [1]	2.46 [1]	ns
R10764	6.51 [1]	6.57 [1]	6.62 [1]	ns	2.1 [2]	2.04 [2]	2.13 [3]	ns
R10781	5.84 [2]	6.01 [2]	5.90 [2]	ns	2.22 [12]	2.14 [2]	2.19 [23]	ns
R07007	6.49 [1]	6.31 [12]	x	ns	2.42 [1]	2.30 [12]	x	ns
Differences among lines	*	*	*		*	*	*	

Differences among cytolasms = A,B,C

Differences among lines = 1,2,3

ns = Nonsignificant