Yield Results and Stability Analysis from the Sorghum Regional Biomass Feedstock Trial

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Abstract Sorghum [Sorghum bicolor (L.) Moench] is one of four herbaceous dedicated bioenergy crops the U.S. Department of Energy identified as critical to annually produce one billion tons of dry biomass. Of these four crops, sorghum is unique as it is a drought-tolerant, annual crop established from seed that is readily tractable to genetic improvement. The purpose of this study was to assess the yield potential and stability of sorghums grown across diverse production environments in the USA. For this study, six sorghum genotypes

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(one cultivar, five hybrids) were grown in yield trials in seven locations in six states for 5 years (2008–2012). Variation in dry and fresh yield was attributable to not only genotypes, but also to the effects of year, location, and year \times location. Even with the highest yielding genotype, environmental conditions were a major factor in determining the yield in a given year. This variability affects the consistency of the biomass supply for ethanol production. In general, the southeastern USA had the highest mean yields for fresh weight and dry weight, indicating that this area may be the most reliable for biomass production. A significant variation was detected among genotypes for fresh weight, dry weight, moisture content, and brix, revealing that sufficient variation within sorghum exists for continued improvement and that certain hybrids are more tractable for biomass/bioenergy production. With dedicated bioenergy sorghum germplasm and proper production environments, sorghum will be a valuable tool in the goal of the sustainable production of one billion tons of dry biomass each year in the USA.

Keywords Sorghum . Bioenergy . Ethanol . Regional biomass feedstock trial

Introduction

Based on the Energy Independence and Security Act of 2007, the U.S. Environmental Protection Agency (EPA) has mandated that 36 billion gallons of alternative fuel must be produced by the year 2022 and 21 billion gallons of this must be from noncornstarch-based sources such as sugar or cellulose [\[6](#page-7-0)]. Current ethanol production is approximately 13 billion gallons with the majority of this supply produced from starchbased conversion of corn and/or other cereal grains [\[15\]](#page-7-0). The general consensus is that cereal grain supply for ethanol has

been maximized; consequently, additional ethanol production must be derived from other plant biomass sources.

Anticipating this need, the United States Department of Energy (DOE) identified four herbaceous bioenergy crops (switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus* \times giganteus), sugarcane (Saccharum spp.), and sorghum [Sorghum bicolor (L.) Moench]) as critical to meet potential biomass production demands. Sorghum is the fifth most widely produced cereal grain in the world with production in 2011 of 54.2 million metric tons grown on 35.5 million hectares [[8\]](#page-7-0). Although statistics are not maintained on the amount of sorghum grown as a forage crop, it is likely that acres devoted to forage sorghum are greater than those for grain [\[17](#page-7-0)].

Sorghum possesses many traits that are valuable in a bioenergy crop including high biomass yield potential, drought tolerance, established production systems, and a sequenced genome, and it is tractable to breeding and further improvement. In addition, sorghum is an annual crop established from seed that can be rotated with other crops, providing flexibility in response to fluctuating markets. Based on prior breeding history, sorghum has extensive genetic variation and is divided into end use types that can be roughly categorized as grain, forage, biomass, and sweet sorghums.

Grain sorghum is already used to produce ethanol in the USA; currently, more than 30 % of the US grain sorghum crop is used in ethanol production [[20](#page-8-0)]. Ethanol yields from sorghum grain are identical to corn, and ethanol plants are able to process both types of grain at the same facility where supply allows it [[22](#page-8-0)]. An analysis of ethanol yield from divergent sorghum genotypes revealed that ethanol production is influenced more by grain yield than the amount of starch in the genotype being processed [[22](#page-8-0)]. Hence, efforts aimed at increasing the grain yield of sorghum hybrids may be more productive than attempting to alter the composition of the grain.

Many biomass sorghums are able to accumulate large amounts of biomass in part because they are photoperiod sensitive (PS), meaning they do not flower when grown in the long-day environments of the temperate USA. Thus, they continue to accumulate vegetative biomass for a much longer growing period [[11,](#page-7-0) [17](#page-7-0)]. These types of sorghums are designed as biomass feedstock for lignocellulosic ethanol conversion programs. A unique breeding system has been devised that allows two photoperiod-insensitive (PI) parents to be crossed together to produce a PS hybrid, reducing the time and effort needed to bring new products to market [\[16](#page-7-0)]. These types of sorghum hybrids averaged 19.0 Mg/ha dry matter when grown at Bushland, Texas over 4 years [\[2](#page-7-0)]. Increased plant density had a negative effect on biomass yields of PS sorghums due to thinner stalks, while narrow row spacing resulted in increased yields [\[19\]](#page-7-0).

Sweet sorghums contain a high fermentable sugar concentration in a juicy stalk that can be extracted and fermented directly into ethanol. After juice extraction, the bagasse can be used to make ethanol from fermentation of the cellulose and hemicellulose or it can be burned to produce electrical power. Research has indicated that the greatest sugar yields are obtained when sweet sorghum is harvested at the hard dough stage of grain filling [[1\]](#page-7-0). Sweet sorghum sugar yields are greatest when late maturity cultivars or hybrids are planted early in the growing season, likely because most of these genotypes are moderately PS allowing for a longer growing season [[4](#page-7-0)]. The high biomass and sweet sorghums are particularly conducive for bioenergy production since they do not directly compete with the demand for food or feed [[7\]](#page-7-0).

The DOE has published and updated a study to estimate biomass productivity in the USA for energy conversion, and this report concluded that the USA had the capacity to produce at least one billion dry tons of biomass annually in a sustainable manner [[12](#page-7-0)]. It was estimated that this amount of biomass, if converted to biofuels, would displace approximately 30 % of the US consumption of petroleum. That study was based on yield estimates and projections; there remains a need to confirm that projected estimates and areas of production are realistic for each energy crop. Within that context, the purpose of the current research was to determine the yield and stability of six different sorghum genotypes grown at seven locations in six different states over 5 years. The results will be used to establish the maximum biomass yield potential of some of the best current biomass-producing sorghums in different production regions of the USA and their relative stability of production over the years. The data will be used to determine the role of sorghum in helping to meet the goal of producing one billion dry tons of biomass annually.

Materials and Methods

Six sorghum genotypes were evaluated in seven environments over 5 years. The six genotypes evaluated were Graze All, a PI sorghum-sudan forage hybrid; Graze N Bale, a PS sorghum-sudan forage hybrid; TX08001, a PS bioenergy hybrid; M81-E, a moderately PS sweet sorghum variety; Sugar T, a moderately PS sweet sorghum silage hybrid; and 22053, a moderately PS brown midrib (bmr) silage hybrid. TX08001 was developed by Texas A&M Agrilife Research, M81-E was developed in Mississippi by the USDA-ARS [\[3](#page-7-0)], and the remaining four hybrids are produced and marketed by Advanta, Inc. primarily as forage sorghums for silage, green chop, grazing, and hay. Given this background, it must be noted that they were not developed specifically for bioenergy. However, at the initiation of this project, numerous groups were utilizing these types for bioenergy uses due to the paucity of energy sorghum types and that is why they were included in this study.

The seven locations used for testing were: Manhattan, Kansas (KS); College Station, Texas (CS); Corpus Christi, Texas (CC); Ames, Iowa (IA); Lexington, Kentucky (KY); Raymond, Mississippi (MS); and Roper, North Carolina (NC). All yield trials were rain fed; no supplemental irrigation was used in any location. In all locations and years, trials were planted in a randomized complete block design, but plot size and number of replications varied across locations due to space availability and management capacity. Standard production practices specific to each location were observed for fertilizer, tillage, and herbicide application. Target plant densities were 125,000 plants per hectare for the sweet sorghums (Sugar T and M81-E), 150,000 plants per hectare for the bioenergy types (22053 and TX08001), and 200,000 plants per hectare for the forage sorghums (Graze All and Graze N Bale). Agronomic traits evaluated at each location were fresh weight, moisture concentration of the biomass, dry weight, and brix. Biomass samples were collected at harvest and dried in a forced air oven for a minimum of 72 h to obtain the moisture concentration and dry weights. Several environments in various years were lost due to insufficient rainfall or inconsistency in the data quality.

All analyses of variance were conducted using the mixed models procedure of SAS version 9.3 [[18\]](#page-7-0). Replications, nested within year and location, were considered a random effect; all other sources of variation were considered fixed effects. Multiple comparison procedure tests were conducted using the general linear model procedure of SAS version 9.3 [\[18](#page-7-0)]. The GGE biplot software was used in an effort to subdivide the locations into mega environments in order to make inferences about the feasibility of producing sorghum as a dedicated bioenergy crop in different areas of the USA [[23\]](#page-8-0). Rainfall data for the correlation between rainfall and yield was sourced from the PRISM Climate Group [\[14\]](#page-7-0). Rainfall is reported as the total amount received at each location for the complete calendar year (January–December).

Results and Discussion

Relative Effects of Genotypes and Environments

During the years of evaluation, the seven locations used for this study varied widely in terms of annual rainfall, seasonal temperature, and length of growing season and represented different adaptation zones. Furthermore, within the years tested, rainfall varied widely from year to year. For example, in 2009, it was too dry in CC to plant the trial, and in several other years, the rainfall was sufficient to plant but insufficient to sustain season-long growth.

In the combined analysis, the main effects of year, location, and genotype and many of the interaction terms were significant. The majority of the variation observed in the data from

this experiment was attributed to the effects of year, location, and year \times location (Table [1](#page-3-0)). The large variability due to environment may require that breeding efforts be conducted on a more regionalized basis instead of breeding germplasm adapted across the USA. This conclusion is confirmed by the significant variation observed for year \times genotype, location \times genotype, and year \times location \times genotype for each trait (Table [1](#page-3-0)). The significant effect due to genotype for each trait indicates that there is considerable variation in sorghum that can be used to breed improved varieties and hybrids for ethanol production. However, the large amount of variability caused by environmental conditions must be taken into consideration when evaluating the minimum land area required to support an ethanol production facility.

Across all environments, significant differences were detected among locations for each agronomic trait evaluated (Table [2](#page-3-0)). Across environments, mean dry yield ranged from a low of 7.1 MT/ha in CC to a high of 17.5 MT/ha in NC. The locations with the lowest average yields were in the regions traditionally associated with grain sorghum production (CC, CS, and KS). Grain and forage sorghum are common in these regions because it is drought tolerant, but the results clearly indicate that these same regions will not produce the highest yields and may not be well suited for biomass and bioenergy production due to persistent seasonal droughts unless supplemented with irrigation. Alternatively, the locations in the southeastern USA (MS, NC, KY) had greater yields for fresh weight and dry weight due to longer growing seasons, greater rainfall, and the adaptation of sorghum genotypes to warmer climates. While sorghum is quite tolerant of surviving periods of drought, the results indicate that the greatest yields occur in environments with consistently greater rainfall.

Variation from year to year demonstrates the effect of climate on productivity (Table [3\)](#page-4-0). For example, the 2011 season was dry for much of the southern USA, resulting in the lowest mean fresh and dry weights of any year in the study. In 2009, ample rainfall throughout most of the growing area produced the greatest mean fresh and dry weights of any year. However, in 2009, the CC location was not planted due to insufficient rainfall. This trend confirms the importance of consistent and timely rainfall when determining where lignocellulosic ethanol production from sorghum is potentially feasible.

Significant variation was observed among genotypes for all traits across all environments (Table [4\)](#page-4-0). Of the entries, Graze All had the lowest mean fresh and dry weights, which are partially because it is an early flowering PI sorghum sudangrass hybrid. These hybrids are designed for multiple harvests, which was not practiced in this study. The greatest average yields were produced by TX08001, which is a hybrid bred specifically for biomass production. M81-E had a similar fresh weight as TX08001, but the mean dry weight of this cultivar was significantly less because it is a sweet sorghum

Loc location, rep replication, gen genotype, ns not significant

*, significant at the 0.05 probability level; ***, significant at the 0.001 probability level

^a Percent of the total variation due to each effect

and has greater moisture content than TX08001. As expected, the highest brix values were in M81-E and Sugar T, which were the two entries known to have greater soluble sugar concentrations. Sweet sorghums have value in systems that produce both a wet and dry processing stream as fermentable sugars are found in both the juice and the biomass.

Of the entries in the test, the hybrid 22053 was the only brown midrib genotype, a mutation that results in lower lignin. Lignin can interfere with the extraction of cellulose and hemicellulose for fermentation [[10,](#page-7-0) [13](#page-7-0)]. This trait has significant value in the forage sorghum industry [\[2](#page-7-0)] and obvious potential value in the bioenergy industry [\[5](#page-7-0)], but there are potential limitations. In the current study, the yield of 22053 was low relative to the top yielding entries (Table [4](#page-4-0)), but even more problematic, the hybrid consistently lodged late in the season in most locations. Compared to a forage production system, the bioenergy growing season is longer and this time difference accounted for the problems of lodging, specifically with this hybrid. Forage sorghum breeding has effectively used both genetic selection and timely management to minimize lodging problems [[2\]](#page-7-0). It is likely that the same approaches could be used to improve biomass sorghums that possess the bmr trait.

Stability Analysis

The GGE biplot for fresh weight data divided the locations into three mega environments composed of KS, CC, and CS in one; NC, MS, and KY in another; and IA by itself (Fig. [1\)](#page-5-0). Similar results were found for GGE analysis of the dry weight data. These groupings are logical in the context of environment and they imply that adaptation and hybrid type will vary

Table 2 Means and ranges for fresh weight biomass, dry weight biomass, moisture concentration, and brix averaged over all genotypes and years for each location

Site	Fresh weight (MT/ha)		Moisture $(\%)$		Dry weight (MT/ha)		Brix $(\%)$	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Corpus Christi, TX (CC)	30.5e	$4.1 - 84.4$	70.3 _b	$39.8 - 90.7$	7.1f	$1.5 - 25.8$	9.1e	$6.0 - 13.7$
College Station, TX (CS)	40.1 _d	$5.7 - 89.0$	73.9a	$52.9 - 83.8$	10.4e	$1.8 - 25.8$	11.5 cd	$6.2 - 18.2$
Ames, IA	58.4b	$29.3 - 105.5$	73.0a	66.4–79.8	15.5c	$8.9 - 28.5$	13.5a	$7.5 - 19.3$
Manhattan, KS	41.5d	$13.9 - 79.8$	67.3c	$51.0 - 80.5$	13.3d	$4.4 - 24.6$	13.2ab	$8.4 - 16.3$
Lexington, KY	52.0c	$28.4 - 91.9$	69.6 _b	$51.7 - 89.6$	17.2ab	$4.8 - 30.8$	12.2bc	$6.0 - 17.2$
Raymond, MS	63.8a	$17.5 - 117.8$	74.0a	$53.3 - 85.0$	16.3 _{bc}	$4.1 - 34.1$	8.2e	$4.1 - 15.4$
Roper, NC	61.2ab	15.4-127.8	69.3 _b	54.4-80.8	17.5a	$5.8 - 41.1$	10.7 _d	$5.0 - 18.7$
HSD(P<0.05)	3.7		1.3		1.1		1.3	

Means within a column followed by the same letter were not significantly different at the 0.05 probability level based on Tukey's honestly significant difference [[21\]](#page-8-0)

MT/ha metric tons per hectare

Year	Fresh weight (MT/ha)			Moisture $(\%)$		Dry weight (MT/ha)		Brix $(\%)$	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
2008	42.9d	17.5-84.4	65.7e	$45.8 - 80.5$	14.1 _{bc}	$6.1 - 25.8$	14.0a	$8.4 - 18.2$	
2009	59.1a	13.9–127.8	73.3 _b	$51.0 - 82.0$	15.1ab	$4.4 - 41.1$	11.5 _b	$6.0 - 18.0$	
2010	55.6b	$20.7 - 109.2$	74.6a	54.8-90.7	13.7c	$2.8 - 34.1$	11.2 _b	$4.6 - 17.0$	
2011	41.4d	$5.7 - 101.8$	71.6c	54.4–89.6	11.5d	$1.8 - 28.8$	10.8 _{bc}	$5.0 - 19.3$	
2012	50.2c	$4.1 - 117.8$	67.8d	$39.8 - 83.8$	15.4a	$1.5 - 32.2$	10.2c	$4.1 - 18.4$	
HSD(P<0.05)	2.9		1.1		1.0		0.9		

Table 3 Means and ranges for fresh weight biomass, dry weight biomass, moisture concentration, and brix averaged over all genotypes and locations for each year

Means within a column followed by the same letter were not significantly different at the 0.05 probability level based on Tukey's honestly significant difference [[21\]](#page-8-0)

MT/ha metric tons per hectare

between these environments. Thus, genotypes developed for one mega environment are not likely to perform similarly in the other mega environments. Consequently, breeding and improvement programs will be directed at those different environments.

The adaptation of genotypes to mega environments is demonstrated in the genotype-centered biplot (Fig. [2\)](#page-5-0). For fresh weight, the cultivars M81-E and TX08001 performed best in NC, KY, and MS, and their performance in some of the other environments was slightly lower relative to other genotypes (Fig. [2](#page-5-0)). While Sugar T was not the highest yielding genotype in the study, its performance was stable compared to the other genotypes. This stability will likely be important when selecting genotypes since a steady and reliable source of feedstock is essential to any conversion facility.

Consistency of Yield in the Top Yielding Hybrid

Ultimately, biomass yield is best estimated by evaluation of the hybrid that consistently produces the greatest yield and it is

this hybrid that should be used to measure productivity of the crop in a biomass production plan. Across these tests, the hybrid TX08001 produced the greatest mean fresh and dry weights (Table 4), but the consistency of this production varied from year to year within a location (Table [5\)](#page-6-0). For example, in CS between 2009 and 2012, the dry weight yield of TX08001 ranged from 4.3 to 20.9 MT/ha. In this situation, the low yields were likely due to dry weather, which is common in the western locations. In the southeast testing sites, the variation from year to year was reduced (Table [5\)](#page-6-0).

Rainfall was not the only factor influencing productivity. Of the six genotypes, only the yield in TX08001 exhibited a consistent response to the amount of rainfall; the remaining five genotypes had a significantly lower R^2 (Fig. [3](#page-6-0)). This favorable response is likely due to TX08001 being a high biomass PS hybrid, therefore enabling it to take advantage of available moisture for a longer period of time during the growing season. Research by Olson et al. [\[11\]](#page-7-0) demonstrated that TX08001 continues to accumulate biomass throughout the season and even more so when water is not limiting. This

Genotype Fresh weight (MT/ha) Moisture (%) Dry weight (MT/ha) Brix (%) Mean Range Mean Range Mean Range Mean Range 22053 41.9d 4.2–79.9 70.7b 45.8–88.2 11.9e 1.5–25.5 10.7bc 5.0–17.7 Graze All 35.1e 4.1–113.3 68.4c 39.8–86.1 10.1f 1.8–21.0 10.0c 4.1–16.8 Graze N Bale 55.3b 7.9–116.0 73.1a 44.0–89.6 14.6c 3.3–29.4 10.1bc 4.6–18.7 M81-E 58.2ab 5.4–118.7 72.6a 52.6–87.8 15.6b 2.2–34.1 12.0a 5.2–18.2 Sugar T 51.3c 12.3–108.5 73.2a 51.7–90.7 13.3d 3.1–26.8 11.9a 4.3–19.3 TX08001 58.6a 9.1–127.8 69.5c 55.0–86.8 17.9a 2.8–41.1 10.9b 6.4–16.0 $HSD (P < 0.05)$ 3.2 1.1 1.0 0.9

Table 4 Means and ranges for fresh weight biomass, dry weight biomass, moisture concentration, and brix averaged over all environments for each genotype

Means within a column followed by the same letter were not significantly different at the 0.05 probability level based on Tukey's honestly significant difference [[21\]](#page-8-0)

MT/ha metric tons per hectare

Fig. 1 Location-centered biplot for fresh weight yield grouping the seven locations into mega environments

data suggests that high biomass PS sorghum is better suited for production in regions with ample rainfall and long growing seasons in order to take advantage of the growth potential of the germplasm. However, as noted by the modest correlation in TX08001 and the relative lack of correlation in the remaining genotypes, many other factors, including type of sorghum, specific genotype, soil type, and temperature, likely have an effect on biomass accumulation.

Unlike grain, which can be easily transported for processing, biomass conversion facilities will require locally produced biomass because transportation is cost prohibitive. Consequently, when evaluating potential production locales, high yield is important but consistency of yield is equally, if not more important. Many of the potential conversion processes assume that the biomass provided to the conversion facility arrives dry. Based on sorghum phenology and the data collected from these trials, it is our conclusion that biomass sorghum is a crop that will be harvested at high moisture concentration because it is very difficult to dry in the field. In the current study, there were differences in moisture concentration among the entries, ranging from the low 40 % range to nearly 90 % (Table [4](#page-4-0)). For TX08001, which was in the

Table 5 Agronomic per of the biomass sorghum TX08001 in College Sta Ames, IA; Raymond, M Roper, NC in four cons years (2009–2012)

Means within a column

no data

2008

lowest average moisture concentration grouping (Table [4\)](#page-4-0), the moisture concentration ranged between 65 and 76 % at harvest in four locations over 4 years (Table 5). Unlike forage

Fig. 3 Fresh weight yields of TX08001 (dashed line) versus the other five genotypes combined (solid line) in response to variation in rainfall. Rainfall values reflect the total observed rainfall at a growing site for the calendar year

120000 \Box 100000 $\overline{\bullet}$ \Box $R^2 = 0.5773$ 80000 Fresh Weight (MT/ha) \Box \Box 60000 $R^2 = 0.2311$ \bullet â 40000 С ۰ \Box 20000 $\overline{0}$ $\mathbf 0$ 20 60 80 100 120 140 160 180 40 Rainfalll (cm) \bullet Other Genotypes □ TX08001 Linear (Other Genotypes) ----- Linear (TX08001)

sorghums that are commonly dried and baled, biomass sorghums have significantly thicker stems and are harvested later in the season, and both of these factors are less conducive for

dry down. Consequently, processors who use sorghum will likely have to adopt systems that handle wet biomass. While the additional moisture increases transportation costs, the water in the sorghum genotypes tested in this trial contains substantial amounts of fermentable sugars (Tables [3,](#page-4-0) [4](#page-4-0), and [5\)](#page-6-0), so the extraction process yields two streams for industrial processing: juice and bagasse. For these reasons, it is envisioned that sorghum high in juice sugar concentration and biomass will be processed much like sugarcane. Consequently, sorghum breeding programs should select for both high biomass and sugar concentration as they maximize the productivity of both streams.

The harvest dates for the greatest yielding hybrids were typically late in the season. For northern locations, this was typically from mid-September to early October. In southern locations, the harvest season was longer, ranging from late August through early November. In all locations, harvesting earlier than optimum lowered yields and harvesting later than optimum reduced yield and quality (because the crop begins to degrade) [9]. Consequently, given that sorghum is a crop with high moisture, storage systems must be developed or the crop must be harvested as needed. If the latter, then complementary crops are essential to maintain a harvest window sufficient to justify capital costs for processing and conversion. In the southeastern USA, there is an opportunity to combine sugarcane and sorghum, which would use much of the same processing equipment [4]. Regardless of the method and conversion system, crop complementation will be essential to the productivity and economic efficiency of biomass conversion.

Conclusions

The production of biomass for a developing bioenergy industry in the USA will require the production and integration of biomass from several different crops. The results in this study confirm that sorghum can produce sufficient biomass yields to meet the needs of a developing biomass industry. The tractable genetics of sorghum coupled with established breeding systems will allow for great strides to be made in the productivity of future high biomass sorghum. Traditionally, forage and grain sorghums have been grown in the South and Central regions of the country, but this study demonstrates that sorghum for biomass is best adapted and produces the greatest and most consistent yields in the southeastern USA. Productivity in other regions can be high but is subject to years when yields are reduced due to drought or short production seasons. The large amount of variation due to the effects of environment and genotype \times environment highlights the need for and value in breeding specifically for the target area. With the proper genotypes and production environments, sorghum will be a valuable tool in the goal of the sustainable production of one billion tons of dry biomass each year in the USA.

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