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Evaluation of three cultivars of sweet sorghum as feedstocks for ethanol production in the Southeast United States

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Abstract

Sweet sorghum has become a promising alternative feedstock for biofuel production because it can be grown under reduced inputs, responds to stress more efficiently than traditional crops, and has large biomass production potential. A three-year field study was conducted to evaluate three cultivars of sweet sorghum as bioenergy crops in the Southeast United States (Fort Valley, Georgia): Dale, M81 E and Theis. Parameters evaluated were: plant density, stalk height, and diameter, number of nodes, biomass yield, juice yield, °Bx, sugar production, and theoretical ethanol yields. Yields were measured at 85, 99, and 113 days after planting. Plant fresh weight was the highest for Theis (1096 g) and the lowest for Dale (896 g). M81 E reported the highest stalk dry weight (27 Mg ha^{-1}) and Theis reported the lowest (21 Mg ha^{-1}). Theis ranked the highest °Bx (14.9), whereas M81 E was the lowest (13.2). Juice yield was the greatest for M81 E (10915 L ha^{-1}) and the lowest for Dale (6724 L ha^{-1}). Theoretical conservative sugar yield was the greatest for Theis (13 Mg ha^{-1}) and the lowest for Dale (9 Mg ha^{-1}).

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Theoretical ethanol yield was the greatest for Theis (7619 L ha^{-1}) and the lowest for Dale (5077 L ha^{-1}).

Keywords: Energy, Applied science, Environmental science

1. Introduction

The Energy Independence and Security Act of 2007 (EISA) requires the United States to produce 136.3 billion L of renewable fuels from biomass sources by 2022, with 79.5 billion L being derived from sources other than corn starch (United States Government, 2007; Cosgrove et al., 2012). Also, the U.S. Department of Energy's Energy Efficiency and Renewable Energy - Biomass Program emphasizes reduction of dependence on foreign oil; promotion of diverse, sustainable, domestic energy resources; reduction of carbon emissions; and establishment of a domestic biomass industry (EERE, 2010). At present, most of the world's ethanol production is obtained from two major crops: corn and sugarcane (Davila-Gomez et al., 2011). Meeting the renewable fuel goals of EISA 2007 while reducing dependence on foreign oil and greenhouse gas emissions, will require the production of biofuels from a large and diverse volume of sustainable feedstocks.

Sweet sorghum (*Sorghum bicolor* (L) Moench) is considered a potential bioenergy crop throughout much of the tropical and temperate zones of the world, and is a leading contender for biofuel production in the southeastern United States (Nuessly et al., 2013; Viator et al., 2015). It is a drought-tolerant C4 crop in the grass family and can be adapted to most of the temperate and tropical climates as an annual or short perennial crop (Bellmer et al., 2010; Khalil et al., 2015; Mahapatra et al., 2011). It has a 3.5 month crop cycle and can be cultivated twice a year in many regions of the U.S. (Kim and Day, 2011). The sweet sorghum plant grows to a height between 120 cm and 400 cm depending on the variety and growth conditions. It consists of about 19% leaf matter, 37% juice, 8% seed head and 36% bagasse on a wet basis and may vary from one cultivar to another. Sweet sorghum yields range from 32 to 112 Mg ha^{-1} (fresh biomass) and from 15 to 25 Mg ha^{-1} (dry biomass), depending on cultivar, climate, location and production practices (Bellmer et al., 2010). The production cost of sweet sorghum has been estimated to be \$477 ha^{-1} (Viator et al., 2009). The estimated price for fuel ethanol production from sweet sorghum is about \$200-\$300 m^{-3}, as compared to \$260 for sugarcane ethanol, \$300-\$420 for corn ethanol and \$450 for lignocellulosic ethanol (Sánchez and Cardona, 2008). The crop can be referred to as carbon neutral meaning that the CO_2 fixed during the growing cycle offsets the CO_2 produced during crop production (Jones, 2008). Sweet sorghum juice contains 12% to 20% sugars, consisting of sucrose, glucose, and fructose which can be readily converted to sugar (Khalil et al., 2015; Kim and Day, 2011), for subsequent ethanol production

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(Vasilakoglou et al., 2011). Sweet sorghum has the potential to yield up to 8,000 L ha^{-1} of ethanol or approximately twice the ethanol yield potential of corn and 30% greater than the average sugarcane productivity (Luhnow and Samor, 2006).

The major advantages of sweet sorghum over many other bioenergy feedstocks are the reducing unit operations and inputs needed for complete conversion (Veal et al., 2014). According to the USDA (2011), about 30% to 35% of total sweet sorghum crop was used for ethanol production in 2011 (Liu et al., 2013). Currently, the most important material used to produce ethanol from sweet sorghum is the stalk (Nuessly et al., 2013). As of December 2012, sorghum has been approved by the Environmental Protection Agency (EPA) as an advanced bioenergy feedstock as it is highly tolerant to drought, requires lower fertilizer inputs than corn and has lower greenhouse gas emissions on a life-cycle basis (Chen et al., 2013). There are about 4000 sweet sorghum cultivars distributed throughout the world (Rutto et al., 2013) and the evaluation of cultivars in diverse environments is one of the first steps in using sweet sorghum for biofuel production. The objective of our study was to assess the agronomic performance and yields of juice and ethanol of three sweet sorghum cultivars (Dale, M81 E, and Theis) grown in the southeastern United States (Georgia), as a potential source of biomass for ethanol production.

2. Material and methods

2.1. Experimental site

Field experiments were conducted at the Agricultural Research Station Farm, Fort Valley State University, Fort Valley, GA, USA (32°33′ N 83°53′ W) for 3 years (2012, 2013, and 2014). The soil at the planting site was a Dothan sandy loam (fine-loamy, kaolinitic, thermic, Plinthic Kandiudults) one with a pH of 6.5 to 6.7. Three common cultivars of sweet sorghum (Dale, M81 E and Theis) were planted. Seeds were purchased from Minton Lawn and Garden Center (Macon, GA). Before planting, plots were fertilized with 5/10/15 N, P, and K at a rate of 560 kg h^{-1} as a broadcast application. An experimental plot of 32 m * 17 m was used. The plot was divided into nine subplots of 17 m * 0.86 m with 0.86 m space between subplots. Each subplot had four rows. Each cultivar had four 17 m rows, spaced 0.86 m apart, with three subplots for each cultivar randomly disbursed within allocated space. Randomization of planting order was done by placing all three cultivars into a box and randomly selecting a type. Seeding was performed at a depth of 1.3 cm. Three days after planting, Gramozone and Dual Magnum Herbicide (Syngerta Crop Protection Canada, Inc., Guelph, ON) at 1.17 L ha^{-1} were used for grass and weed control. Two weeks after planting, 5.7 kg of Nitrogen was applied to side dress the plot by hand spraying the fertilizer carefully by the sides of the crop to avoid contact with the leaves.

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2.2. Plant density

Sweet sorghum populations were calculated from the number of plants in 1 m transects on both middle rows of each plot. Initial plant density and number of stalks were determined 85 days after planting (DAP).

2.3. Stalk height, diameter, and weight

Ten stalks, with panicles and leaves, from two middle rows of each sweet sorghum cultivar were randomly selected from each plot and hand harvested. Stalk height, diameter and weight were measured each year at 85, 99, and 113 DAP. Diameter was measured at three places (top, middle, and bottom) of the stalk using a digital slide gauge (Marathon Watch Company Ltd., Richmond Hill, ON, Canada). Stalks were weighed, stripped of leaves and panicles, and weighed again to determine percent stalk, leaves, and panicles. Number of leaves and nodes were counted for each stalk. Refractometer readings of stalk juice were taken at five different internodes as detailed in Section 2.4. Subsamples of chopped stalks were collected and moisture content was determined by drying three samples at 103 °C for 24 h in an oven to constant mass (ASABE Standards S358.2, 2012) and to estimate dry biomass.

2.4. Harvest and juice extraction

Ten stalks were stripped of leaves and juice was extracted using a motor operated 3-roller press (Sor-Cane Porta-Press; McClune Industries, Reynolds, GA, USA). Another set of 10 stalks was used to measure internodal °Bx. After juice extraction, the juice was weighed, and an aliquot was placed in a hand held digital refractometer (Reichert Analytical Instruments, Depew, NY, USA) to measure Brix (a measure of sugar and soluble starch in plant sap based on light refraction). The pH of extracted juice was immediately recorded using a Vital Sine Palm pH meter (Aquasonic, Wauchope, Australia). Juice extraction rate of each plant was calculated as the ratio of juice weight to stalk fresh weight, and expressed as a percentage (Sun and Yamana, 2012). Total volume of juice per hectare was estimated from an individual stalk's juice content and the plant density in a hectare. In 2014, juice of three sweet sorghum cultivars was filtered using a mesh bag and mixed before storing. To study the effect of long-term storage on ethanol yield the mixed juice was stored in sterile bottles at −20 °C for one year in the Food Engineering Laboratory. Sorghum juice was thawed at refrigeration temperature (4 °C) for High Performance Liquid Chromatography (HPLC) analysis.

2.5. Juice, sugar and theoretical ethanol yield

Juice and sugar yield were calculated using Eq. (1) (Wortmann et al., 2010):

$$CSY = (FSY - DSY) * Brix * 0.75 \qquad (1)$$

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Where, CSY is conservative sugar yield (Mg ha^{-1}), FSY is fresh stalk yield (Mg ha^{-1}), DSY is dry stalk yield (Mg ha^{-1}).

Theoretical ethanol yield was calculated using Eq. (2) (Rutto et al., 2013; Teetor et al., 2011):

$$\text{Theoretical ethanol yield (L ha}^{-1}\text{)} = \text{CSY (kg h}^{-1}\text{)} * 0.585 \quad (2)$$

The conversion factor (0.58 L ethanol per kg of sugar) was obtained from the theoretical maximum yield of ethanol produced from the complete inversion of sucrose and the complete fermentation of all resulting and native glucose (3.8 L of ethanol for every 6.7 kg of sugar) (Teetor et al., 2011).

2.6. Stem sugar content by HPLC analysis

Samples were cold-shipped (using dry ice) to the Desert Research Institute, Reno, NV (USA) for fractional sugar analysis. Concentrations of glucose, sucrose, fructose and ethanol were determined using High-Pressure Liquid Chromatography (HPLC) (Waters 2695 with 2414 RI detector, Waters Co., Milford, MA, USA). The column used was a waters sugar Pak I, 10 μm, 6.5 mm * 300 mm, water eluent and with a flow rate of 0.4 mL min^{-1}. The column and the detector temperature were 60 °C and 50 °C, respectively, with injection volume of 10 μL. Prior to analysis, samples were filtered with 0.4 μm GMF filters and run through an Alumina type A cartridge in order to remove any acids in the sample that would harm the column.

2.7. Total soluble sugar content

Total soluble sugar content was estimated using Eq. (3) (Liu et al., 2008):

$$y = 0.8111\, x - 0.37285 \quad (3)$$

Where, y = total soluble sugar content, %; x = Brix of stalk juice, °Bx

2.8. Experimental design and statistical analysis

All measurements were collected from the two middle rows of each experimental unit. Data were analyzed and discussed considering all three years (2012, 2013 and 2014). Data were subjected to the analysis of variance (ANOVA) using the General Linear Model procedures of SAS (2012). Means were generated using LS Means and separated with the PDIFF of general linear model (GLM). Comparison of the means was done using Tukey's HSD. Means were considered significant at $p < 0.05$.

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Table 1. Three-year mean stalk height and diameter as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Stalk height (cm)			Stalk diameter (cm)		
	Dale	M81 E	Theis	Dale	M81 E	Theis
85	a[*] 230 ± 13 A[**]	b 233 ± 12 A	b 245 ± 9 A	b 1.9 ± 0.07 B	a 2.2 ± 0.05 A	a 2.1 ± 0.05 A
99	a 236 ± 12 B	a,b 262 ± 11 A,B	a 281 ± 14 A	b 1.8 ± 0.05 A, B	c 1.7 ± 0.04 B	b 1.9 ± 0.04 A
113	a 259 ± 11 A	a 266 ± 9 A	a,b 270 ± 8 A	a 2.1 ± 0.06 A, B	b 1.9 ± 0.05 B	a 2.2 ± 0.07 A

[*]Within the same column, values not preceded by the same letter are significantly different ($p < 0.05$).
[**]Within the same row, values not followed by the same uppercase letter are significantly different ($p < 0.05$).

3. Results and discussion

3.1. Plant density

Dale, M81 E, and Theis showed an average of 149,843; 188,036; and 149,750 plants ha^{-1}, respectively. The average number of sweet sorghum stalks ranged from 15 to 19 stalks m^{-2} harvested.

3.2. Stalk height, diameter, and weight

Depending upon the DAP, the stalk height varied from 230 to 259 cm, 233 to 266 cm, and 245 to 281 cm for Dale, M81 E and Theis, respectively (Table 1). Theis ranked the tallest among cultivars (281 cm). A two-way analysis of variance indicated that there were significant differences in the stalk height caused by both cultivar ($p = 0.0393$) and DAP ($p = 0.0034$). However, the interaction between cultivar and DAP was not significant ($p = 0.5424$). Other studies have also reported Theis to be the tallest among similar cultivars, although their average stalk height was substantially higher than this study, likely due to environmental differences (Cole et al., 2017). Depending upon the DAP, the stalk diameter varied from 1.8 to 2.1 cm, 1.7 to 2.2 cm, and 1.9 cm to 2.2 cm for Dale, M81 E and Theis, respectively (Table 1). Of the cultivars tested stalk diameter was the largest in M81 E followed by Theis. Analysis of variance of the data showed significant differences in the stalk diameter among cultivars ($p = 0.0154$) and among DAPs ($p < 0.0001$). A significant interaction ($p < 0.0001$) was also found between cultivar and DAP. In another study, the average stalk diameters were reported as 1.47, 1.63, and 1.62 cm for Dale, M81 E and Theis, respectively (Cole et al., 2017).

Depending upon the DAP, the number of green leaves varied from 11 to 13, 13 to 14 and 15 to 16 for Dale, M81 E and Theis, respectively (Table 2). The highest number of green leaves were obtained in Theis at 113 DAP and the lowest in Dale at 85 DAP. In general, DAP had no significant effect on number of green leaves across cultivars ($p > 0.05$).

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Table 2. Three-year mean number of leaves and nodes as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Number of green leaves			Number of nodes		
	Dale	M81 E	Theis	Dale	M81 E	Theis
85	b* 11 ± 0.4C**	a 13 ± 0.4 B	a 15 ± 0.4 A	a 14 ± 0.3 B	b 14 ± 0.6 B	a 17 ± 0.7 A
99	a,b 12 ± 0.6 B	a 14 ± 0.6 A	a 15 ± 0.7 A	a 15 ± 0.2 B	b 14 ± 0.4 B	a 17 ± 0.5 A
113	a 13 ± 0.5 B	a 14 ± 0.6 A,B	a 16 ± 0.6 A	a 15 ± 0.2C	a 16 ± 0.5 B	a 18 ± 0.3 A

*Values, within the same column, not preceded by the same letter are significantly different ($p < 0.05$).
** Values, within the same row, not followed by the same uppercase letter are significantly different ($p < 0.05$).

Depending upon the DAP, number of nodes per plant varied from 14 to 15, 14 to 16 and from 17 to 18 for Dale, M81 E and Theis, respectively (Table 2). The number of nodes increased with the DAP increase. In general, DAP had no effect on number of nodes ($p > 0.05$) across cultivars.

Depending upon the DAP, total weight per plant (stalk, leaves and head) varied from 605 to 896 g, 743 to 959 g, and from 757 to 1096 g for Dale, M81 E and Theis, respectively (Table 3). The highest plant weight was obtained in Theis at 113 DAP and the lowest in Dale at 85 DAP. There were significant differences in the total plant weight among cultivars ($p = 0.0030$) and among DAPs ($p < 0.0001$). However, the interaction between cultivar and DAP was not significant ($p = 0.9176$). The Cole et al. study (2017) also reported Theis to have the highest plant weight among these cultivars.

Table 3. Three-year mean total plant weight (stalk + leaf + head) as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Plant weight (stalk, leaf, head) (g)		
	Dale	M81 E	Theis
85	b* 605 ± 82 A**	b 743 ± 70 A	b 757 ± 62 A
99	b 667 ± 65 B	a,b 795 ± 52 A,B	b 861 ± 59 A
113	a 896 ± 54 B	a 959 ± 71 A,B	a 1096 ± 65 A

*Values, within the same column, not preceded by the same letter are significantly different ($p < 0.05$).
** Values, within the same row, not followed by the same uppercase letter are significantly different ($p < 0.05$).

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3.3. Sweet sorghum leaf, head and stalk biomass at harvest

The moisture content of fresh biomass was between 72 − 87%, 75 − 84%, and 78 − 87% for Dale, M81 E and Theis, respectively. There was a significant difference in average moisture content between Dale and Theis ($p = 0.0109$) and between Theis and M81 E ($p = 0.0059$). However, there was no significant difference in moisture content between Dale and M81 E ($p = 0.8251$). The yields of stalk dry biomass were calculated as average dry weight of stalk per plant multiplied by number of plants per hectare. The average stem dry biomass was between 15–21 Mg ha^{-1}, 21–27 Mg ha^{-1}, and 18–23 Mg ha^{-1}, for Dale, M81 E and Theis, respectively (Table 4). Wortmann et al. (2010) reported a dry stalk yield of 7.51- 23.36 Mg ha^{-1} for M81 E.

Theis had the highest plant weight, likely because it had the highest moisture content; but M81E had the highest stalk dry weight. If the target product is sugar from expressed juice, then the higher moisture content cultivar would likely be the higher yielding choice.

The yields of leaf dry biomass were calculated as average dry weight of leaves per plant multiplied by number of plants per hectare. The average leaf dry biomass was between 2.5 − 3.8 Mg ha^{-1}, 4.7 − 5.2 Mg ha^{-1}, and 2.4 − 3.3 Mg ha^{-1}, for Dale, M81 E and Theis, respectively (Table 5). Rutto et al. (2013) reported a leaf dry biomass of 4.84 − 8.87 Mg ha^{-1} and 6.55 − 16.83 Mg ha^{-1} for Dale and M81 E, respectively.

It has been reported that the presence of leaves reduces sugar extraction in proportion to the weight of leaves (Guigou et al., 2011) and that extraction efficiency is reduced because of leaf absorption of juice from stalks (Cundiff and Worley, 1992; Viator et al., 2015). Having less number of leaves (low leaf biomass yield) would not be better for sweet sorghum plant, because simply it is the leaves

Table 4. Three-year mean stalk dry weight as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Stalk dry weight (Mg ha^{-1})		
	Dale	**M81 E**	**Theis**
85	b* 15 ± 1.9 B**	b 21 ± 2.0 A	b 18 ± 1.6 A,B
99	a,b 18 ± 1.7 B	a 27 ± 1.5 A	a 23 ± 2.0 A
113	a 21 ± 1.4 B	a 27 ± 1.6 A	a,b 21 ± 1.5 B

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** Values, within the same row, not followed by the same uppercase letter are significantly different ($p < 0.05$).

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Table 5. Three-year mean leaf dry weight and head dry weight as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Leaf dry weight (Mg ha^{-1})			Head dry weight (Mg ha^{-1})		
	Dale	M81 E	Theis	Dale	M81 E	Theis
85	a,b* 2.7 ± 0.3 B**	a 5.2 ± 0.5 A	a,b 3.0 ± 0.3 B	b 0.4 ± 0.06 A	b 0.1 ± 0.07 B	b 0.3 ± 0.05 A,B
99	b 2.5 ± 0.3 B	a 4.7 ± 0.5 A	a 3.3 ± 0.3 B	a,b 0.7 ± 0.08C	a 2.0 ± 0.23 A	a 1.5 ± 0.16 B
113	a 3.8 ± 0.5 A	a 4.8 ± 0.4 A	b 2.4 ± 0.3 B	a 0.9 ± 0.21 B	a 1.8 ± 0.21 A	a 1.3 ± 0.14 A,B

*Values, within the same column, not preceded by the same letter are significantly different ($p < 0.05$).
** Values, within the same row, not followed by the same uppercase letter are significantly different ($p < 0.05$).

which make the sugar. However, having a number of leaves per plant above certain number would be on the expense of plant juice content. Moreover, leaf absorption of juice from stalks would reduce sugar content, but not the extraction process itself.

The yields of head dry biomass were calculated as the average dry weight of head per plant multiplied by number of plants per hectare. The average head dry biomass was between 0.4 − 0.9 Mg ha^{-1}, 0.1 − 2.0 Mg ha^{-1}, and 0.3 − 1.5 Mg ha^{-1}, for Dale, M81 E and Theis, respectively (Table 5).

The yields of total dry biomass (leaf, stem and head) were calculated as average total dry weight per plant multiplied by number of plants per hectare (Sun and Yamana, 2012). The average total dry biomass was between 16.9–26.1 Mg ha^{-1}, 24.7 − 33.9 Mg ha^{-1}, and 20.2 − 26.5 Mg ha^{-1}, for Dale, M81 E and Theis, respectively (Table 6). In general, M81 E produced the greatest dry biomass yield ($p < 0.05$). Analysis of variance (two-way ANOVA) of the data showed significant

Table 6. Three-year mean sweet sorghum biomass (leaf, stem and head) dry weight as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Total dry biomass (leaf, head and stalk) (Mg ha^{-1})		
	Dale	M81 E	Theis
85	b* 16.9 ± 2.3 B**	b 24.7 ± 2.4 A	b 20.2 ± 1.8 A,B
99	b 20.3 ± 1.9 B	a 31.0 ± 1.8 A	a 26.5 ± 2.3 A
113	a 26.1 ± 1.8 B	a 33.9 ± 1.9 A	a,b 23.9 ± 1.5 B

*Values, within the same column, not preceded by the same letter are significantly different ($p < 0.05$).
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differences ($p < 0.0001$ in both cases) in the total dry biomass yield caused by both cultivar and DAP. However, the interaction between cultivar and DAP was not significant ($p = 0.2453$).

3.4. Composition of sweet sorghum

Sweet sorghum typically consists of approximately 75% stem, 10% leaves, 5% seeds and 10% roots by weight (Grassi et al., 2002). In our study, the ratio of stem, leaves, and seed head for Dale was 86.6%, 10.9%, and 2.5%, and for M81 E was 85.1%, 11.9%, and 2.9%, and for Theis was 88.3%, 8.8%, and 2.8%, respectively. Sipos et al. (2009) reported that the ratio of stem, leaves, and seed head was 76%, 16%, and 8%, respectively. The gross composition of sweet sorghum was reported as 37.28% juice, 36.01% bagasse, 19.14% leaves, and 7.58% seed head on wet basis (Kim et al., 2012). However, the composition will vary from one cultivar to another.

3.5. Brix

The average pH value of fresh sweet sorghum juice was 5.7, 5.7 and 5.8 for Dale, M81 E, and Theis, respectively. In another study, the average pH values were reported as 5.40, 5.33, and 5.53 for Dale, M81 E, and Theis, respectively, slightly lower than this study (Cole et al., 2017). The average °Bx values determined immediately after harvesting, ranged from 10.7 – 14.0 (107–140 g L^{-1}) for Dale, 9.4 – 13.2 (94–132 g L^{-1}) for M81 E, and 9.7 – 14.9 (97–149 g L^{-1} sugar concentration) for Theis (Table 7). °Bx represents an approximation of total solids content and has a positive correlation with the total sugar concentration in sweet sorghum juice (Davila-Gomez et al., 2011). While Theis had the highest mean °Bx at 113 DAP, it was not significantly different than Dale (14) and M81 E (13.2). There were significant differences in the °Bx value among DAPs ($p < 0.0001$) but

Table 7. Three-year mean °Bx as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	°Bx (%)		
	Dale	**M81 E**	**Theis**
85	c* 10.7 ± 0.2 A**	b 9.4 ± 0.2 B	b 9.7 ± 0.2 B
99	b 12.1 ± 0.4 A	a 12.0 ± 1.0 A	a 13.4 ± 0.9 A
113	a 14.0 ± 0.8 A	a 13.2 ± 1.0 A	a 14.9 ± 0.7 A

*Values, within the same column, not preceded by the same letter are significantly different ($p < 0.05$).
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not among cultivars ($p = 0.1108$). The interaction between cultivar and DAP was not significant ($p = 0.5344$). The average °Bx increased from 10.7 to 12.1 (13.67%) between 85 and 99 DAP and from 12.1 to 14 (15.4%) between 99 and 113 DAP for Dale. In the case of Theis, it increased from 9.7 to 13.4 (37.8%) and from 13.4 to 14.9 (10.9%). Similarly, there was an increase in °Bx of 27.6% (9.4 to 12.0) and 10.3% (12.0 to 13.2) between 85 and 99 and 99 and 113 DAP, respectively, for M81 E. A °Bx range from 10.1 to 11.5 was reported for M81 E by Vasilakoglou et al. (2011). Rutto et al. (2013) reported a °Bx of 14.3–18.7 and 13.9–17.1 for Dale and M81 E, respectively. Brix values are known to be highly dependent on temperature, environment, and agronomic practices, and thus are highly variable among different locations and planting years. Cole et al. (2017) reported a 21% difference in Brix values between two consecutive years, presumably due to environmental differences between those two years.

3.6. Juice extraction rate and yield

The average juice extraction rates were found to be in the ranges of 5.1% – 9.8%, 6.3% – 8%, and 4.7% – 6.6% for Dale, M81 E, and Theis, respectively (Table 8). In general, cultivar type did not have a significant effect on juice extraction rate at all three DAPs. Our juice extraction rates were similar to values reported in literature. Sun and Yamana (2012) reported an average juice extraction rate of 8.7% for sweet sorghum (K9041) using a gear-type extractor.

The average juice yield per stalk was between 24.5 – 44.3 g for Dale, 23.1 – 60.9 g for M81 E, and 25.0 – 61.6 g for Theis (Table 9). Sun and Yamana (2012) reported an average juice yield of 42.5 g per plant. There were significant differences in the juice yield per stalk among DAPs ($p < 0.0001$) but not among cultivars ($p = 0.0962$). The interaction between cultivar and DAP was not significant ($p = 0.0616$). The density of fresh juice ranged from 0.85 to 1.00 g

Table 8. Three-year mean juice extraction rate as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Juice extraction rate (%)		
	Dale	**M81 E**	**Theis**
85	a* 9.8 ± 1.9 A**	a 7.7 ± 3.1 A	a 5.0 ± 0.7 A
99	b 5.1 ± 0.7 A	a 6.3 ± 0.9 A	a 4.7 ± 0.9 A
113	b,c 6.0 ± 0.5 B	a 8.0 ± 0.5 A	a 6.6 ± 0.7 A,B

*Values, within the same column, not preceded by the same letter are significantly different ($p < 0.05$).
** Values, within the same row, not followed by the same uppercase letter are significantly different ($p < 0.05$).

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Table 9. Three-year mean juice yield per stalk and juice yield per hectare as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Juice yield per stalk (g)			Juice yield per hectare (L ha^{-1})		
	Dale	M81 E	Theis	Dale	M81 E	Theis
85	b* 29.7 ± 2.6 A**	b 23.1 ± 1.8 A	b 25.0 ± 2.6 A	b 4426 ± 395 A	c 4333 ± 362 A	b 3735 ± 410 A
99	b 24.5 ± 4.7 B	a 43.6 ± 8.1 A	b 28.6 ± 5.8 A,B	b 3724 ± 756 B	b 7707 ± 1293 A	b 4092 ± 849 B
113	a 44.3 ± 4.3 A	a 60.9 ± 6.8 A	a 61.6 ± 7.6 A	a 6724 ± 697 B	a 10915 ± 1040 A	a 9289 ± 1237 A,B

*Values, within the same column, not preceded by the same letter are significantly different ($p < 0.05$).
** Values, within the same row, not followed by the same uppercase letter are significantly different ($p < 0.05$).

mL^{-1}, 0.84 to 1.01 g mL^{-1}, and 0.83 to 0.96 g mL^{-1} for Dale, M81 E, and Theis, respectively. Density of fresh juice obtained from M81 E ranged from 1.04 to 1.06 g mL^{-1} as reported by Wu et al. (2015) in another study.

The juice yield per hectare was between 3724 – 6724 L ha^{-1}, 4333 – 10915 L ha^{-1}, and 3735 – 9289 L ha^{-1} for Dale, M81 E, and Theis, respectively (Table 9). M81 E ranked the highest in mean juice yield per ha with 10915 L ha^{-1} but was not a significantly higher producer of juice than Theis. Analysis of variance (two-way ANOVA) of the data showed significant differences in the juice yield per hectare among DAPs ($p = 0.0003$) but not among cultivars ($p = 0.4960$). The interaction between cultivar and DAP was significant ($p < 0.0001$). For higher juice yield, sweet sorghum should be harvested at DAP 113 since there was no significant difference in yield between DAP 85 and DAP 99. Rutto et al. (2013) reported higher juice yield for Dale (12,000 – 21,100 L ha^{-1}) and M81 E (12,900 – 23,400 L ha^{-1}). Similar findings have been reported by Cole et al. (2017), in which average juice yields were 18359 L ha^{-1} for Dale, 25646 L ha^{-1} for M81 E, and 20007 L ha^{-1} for Theis. However, Horton (2011) reported juice yields of 3817.4 L ha^{-1}, 5174.9 L ha^{-1}, and 4024.8 L ha^{-1} for Dale, M81 E and Theis, respectively. Lower juice yield in our study can be attributed to the type of press used for juice extraction. Passing whole stalks through a set of rollers have shown that usually less than 50% of the juice is collected (Veal et al., 2014).

The mean conservative sugar yield was between 5.0 – 8.7 Mg ha^{-1}, 6.6 – 10.5 Mg ha^{-1}, and 6.1 – 13.1 Mg ha^{-1} for Dale, M81 E, and Theis, respectively (Table 10). Analysis of variance (two-way ANOVA) of the data showed significant differences in the conservative sugar yield caused by both cultivar and DAP ($p < 0.0001$ in both cases). A significant interaction ($p = 0.0456$) was also found between cultivar and DAP. The mean total soluble sugar content was between 8.3% – 11%, 7.2% – 10.3%, and 7.5% – 11.7% for Dale, M81 E, and Theis, respectively (Table 10). There were significant differences in the conservative sugar yield caused by DAP

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Table 10. Three-year mean conservative sugar yield and total soluble sugar content as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Conservative sugar yield (Mg ha^{-1})			Total soluble sugar content (%)		
	Dale	M81 E	Theis	Dale	M81 E	Theis
85	b* 5.0 ± 0.6 B**	b 6.6 ± 0.6 A	c 6.1 ± 0.5 A,B	c 8.3 ± 0.1 A	b 7.2 ± 0.1 B	b 7.5 ± 0.2 B
99	b 6.4 ± 0.6 B	b 7.7 ± 0.5 A,B	b 8.4 ± 0.6 A	b 9.5 ± 0.3 A	a 9.3 ± 0.8 A	a 10.5 ± 0.7 A
113	a 8.7 ± 0.5 B	a 10.5 ± 0.8 B	a 13.1 ± 0.8 A	a 11.0 ± 0.6 A	a 10.3 ± 0.8 A	a 11.7 ± 0.6 A

*Values, within the same column, not preceded by the same letter are significantly different ($p < 0.05$).
** Values, within the same row, not followed by the same uppercase letter are significantly different ($p < 0.05$).

($p < 0.0001$) but not by cultivar ($p = 0.1108$). The interaction between cultivar and DAP was not significant ($p = 0.5344$).

3.7. Sugar profile and total sugar contents

The HPLC method provided accurate results about type of sugar found in the sweet sorghum juice. The principal sugar concentration was glucose (61.2 g L^{-1}, 61%), followed by fructose (36.9 g L^{-1}, 37%) and sucrose (2.2 g L^{-1}, 2%). Sucrose was only a minor proportion of the sugars present and the total sugar content was 100.3 g L^{-1}. The presence of small amount of ethanol (0.14 g L^{-1}) suggested that natural fermentation occurred before the samples were frozen. Crépeau et al. (2013) reported 26.8 g L^{-1} (44%) fructose, 32.7 g L^{-1} (54%) glucose, 1.4 g L^{-1} (2%) sucrose, and 60.9 g L^{-1} total sugar in the juice. Higher amount of glucose and fructose can be related to several factors such as inversion of sucrose catalyzed by a slightly acidic pH characteristic of sweet sorghum juices (Whistler and BeMiller, 1999). In contrast, Wang et al. (2012) reported 8.9% glucose, 6.5% fructose, and 84.6% sucrose.

3.8. Ethanol yield

Average theoretical ethanol yield was between 2921 – 5077 L ha^{-1}, 3847 – 6106 L ha^{-1}, and 3520 – 7619 L ha^{-1} for Dale, M81 E, and Theis, respectively (Table 11). Analysis of variance of the data showed significant differences in the theoretical ethanol yield caused by both cultivar and DAP ($p < 0.0001$ in both cases). Interaction between cultivar and DAP was also significant ($p = 0.0456$). Wortmann et al. (2010) reported an ethanol yield of 967 – 3530 L ha^{-1} for M81 E. Theoretical ethanol yields of 745 – 1331 L ha^{-1} and 893 – 1419 L ha^{-1} for Dale and M81 E, respectively, were reported by Rutto et al. (2013).

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Table 11. Theoretical ethanol yield as affected by days after planting (DAP) and sweet sorghum cultivar (mean ± SE, n = 30).

DAP	Theoretical ethanol yield (L ha^{-1})		
	Dale	M81 E	Theis
85	b* 2921 ± 352 B**	b 3847 ± 333 A	c 3520 ± 290 A,B
99	b 3711 ± 369 B	b 4468 ± 266 A,B	b 4898 ± 356 A
113	a 5077 ± 304 B	a 6106 ± 447 B	a 7619 ± 439 A

*Values, within the same column, not preceded by the same letter are significantly different ($p < 0.05$).
** Values, within the same row, not followed by the same uppercase letter are significantly different ($p < 0.05$).

4. Conclusions

The results of our 3-year study indicated that sweet sorghum produces sufficient biomass, juice, total sugar, and ethanol yields and supports other findings showing sweet sorghum's potentiality for ethanol production and this crop could play a major role in the emerging biofuel market. However, more research is needed on developing equipment for efficient harvesting and pressing of sweet sorghum plants in a single pass for in-field production of ethanol. Optimizing sweet sorghum juice production systems, combined with development of suitable harvesting and pressing equipment may help commercial-scale production of biofuel from sweet sorghum. Also, additional studies are essential to understand the relationships between rate of ethanol production and sugar concentrations. A high ratio of total sugar yield to biomass weight needs to be integrated into sweet sorghum breeding techniques to reduce cost of feedstock. Furthermore, the efficiency of using sweet sorghum as a biofuel crop can be improved by making better use of the bagasse.

Declarations

Author contribution statement

Daniel E. Ekefre: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ajit K. Mahapatra: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mark Latimore, Jr: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Danielle D. Bellmer, Archie L. Williams: Conceived and designed the experiments.

Umakanta Jena: Contributed reagents, materials, analysis tools or data.

Gerald J. Whitehead: Performed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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