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Research on the potential of some sweet sorghum genotypes as bioethanol source under Mediterranean conditions

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Abstract: Sweet sorghum (*Sorghum bicolor* var. *saccharatum* (L.) Mohlenbr.) is a variety of sorghum developed for the harvest of sweet juice, rather than grain. It has been identified as a potential ethanol feedstock crop for rainfed areas in different parts of the world. It is a promising source of bioenergy due to its high biomass, drought tolerance and low input. This study was conducted to determine the potential of bioethanol production from different sweet sorghum varieties under the Çukurova conditions with a Mediterranean climate. Experiments were conducted over two years in 2016 and 2017 on the experimental fields of the Eastern Mediterranean Agricultural Research Institute in Adana province between June and October in randomized blocks design with four replications. Harvest was performed at the dough stage of panicle grains. Based on two-year data, an average of days to harvest values of the varieties varied between 98.6 and 134.4 days, plant height between 233.2 and 429.3 cm, stalk diameters between 22.26 and 26.55 mm, stalk yields between 69.0 and 182.6 t ha⁻¹, juice yields between 22.98 and 62.74 m³ ha⁻¹, juice recoveries between 26.79% and 39.94%, brix values between 12.55% and 20.0% and theoretical bioethanol yields between 2020 and 5302 L ha⁻¹. Grass1, M81E, Roma, Theis, UNL Hybrid#3 and No91 genotypes had stalk yields over 150 t ha⁻¹ and bioethanol yields over 4500 L ha⁻¹, thus they were superior to the other varieties in stalk and bioethanol yields. Present findings revealed that the Çukurova region was quite suitable for maximum bioethanol production from sweet sorghum.

Key words: Brix, genotype, stalk and bioethanol yield, sweet sorghum

1. Introduction

The majority of the energy used in Turkey is supplied from fossil fuels. Fossil fuels release greenhouse gases to the atmosphere and these gases contribute to global warming and climate change. Therefore, today, there is a great search for alternative and renewable energy sources without any negative impacts on the environment. Ethanol-based agricultural biomass energy is among the most promising of these renewable alternative energy sources. Plant-originated ethanol (bioethanol), as a sustainable source of energy offers various advantages over fossil fuels in terms of environmental and economic outcomes. Positive attributes of sorghum bioethanol include lower sulphur content, high octane rating and potential use of up to 25% in ethanol-benzine mixtures without automobile-friendly engine modifications (Rao et al., 2013).

Mathur et al. (2017) grouped sorghums into four classes; grain sorghum, sweet sorghum, feed sorghum and energy sorghum. Sweet sorghum is able to accumulate high quantities of biomass, efficiently convert light into biomass energy, has high water use efficiency, high leaf

levels and nitrogen use efficiency. It is mostly grown in temperate and tropic climates (Dalvi et al., 2011). It can be grown on marginal lands and is highly tolerant to saline and wet conditions. Thus, it is considered an important energy crop (Taylor et al., 2010; Zhang et al., 2012; Dalla Marta et al., 2014; Jiang et al., 2019). It was reported that sweet sorghum produces biomass with high fermentable sugar content, has short vegetation period (about four months), low fertilizer demand, less water consumption per kg of DM (about 310 kg, one-third of sugarcane and half of the maize) and is quite resistant to drought and able to adapt well to different soil and climate conditions (Lima, 1998; Smith and Frederiksen, 2000; Wu et al., 2010) Sweet sorghum can be grown from the seeds, production is totally mechanized, starch is obtained both from the shoots and the grains, and the by-products of energy production are used as pulp and animal feed. Sweet sorghum has high photosynthetic activity due to its C4 photosynthesis mechanism, and it is drought tolerant. All these attributes make sweet sorghum a significant energy and feed crop (Fernandez et al., 2005; Reddy et al., 2005).

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This study was conducted to reveal the theoretical ethanol production potentials of different sweet sorghum genotypes when they were grown as the summer-sown second crop after wheat harvest under Mediterranean ecological conditions and to determine the best genotypes for bioethanol production.

2. Materials and methods

2.1. Experimental materials

Several genotypes of sweet sorghum used in the study, such as Cowley, Dale, Grass1, M81-E, Mennonita, Nebraska sugarcane, PI579753, Ramada, Roma, Rox Orange, Smith, Sugar Drip, Theis, Topper 76, Tracy, UNL-Hybrid -3 ((26297xM81 E), and Williams were supplied by UNL (University of Nebraska, Lincoln, USA). Some other genotypes, such as No2 USDA-China, No91 USDA-Taiwan and No5 USDA South Africa were obtained from Western Mediterranean Agricultural Research Institute-Antalya/Turkey (supplied from ICRISAT and USDA gene bank). Local check cultivar Gülseker was supplied by Field Crops Department of Agricultural Faculty of Uludag University, Bursa, Turkey.

Soil and climate characteristics of the experimental site

Experimental soils belong to Arikli soil series. Analyses on soil samples taken from 0–15 and 15–30 cm depths revealed that experimental soils were clay-loam (CL) in texture with pH values between 7.0 and 7.50. Total salt contents range between 0.22% and 0.27%, N contents ranges between 0.10% and 0.19%, organic carbon (OC)

contents of between 0.63% and 0.90%, phosphorus (P) contents of between 0.63 and 0.90 mg kg⁻¹, lime (CaCO₃) contents of between 32.5% and 35.0%, sand contents of between 24% and 28%, silt contents of between 41% and 43% and clay contents of between 30% and 33%.

During the experimental period (June–October), the average temperature was 25.1 °C in 2016 and 24.8 °C in 2017; relative humidity was 79.0% in 2016 and 79.6% in 2017; total precipitation was measured as 46.2 kg m⁻² in 2016 and 48.2 kg m⁻² in 2017 (Figure 1). Since the precipitation levels were not sufficient to meet the water demand of the plants, irrigations were performed as needed.

2.2 Methods

Experimental design

The experiment was conducted on the experimental fields of Eastern Mediterranean Agricultural Research Institute in Doğankent (36°51'35"N and 35°20'43"E), Adana, in randomized blocks design with four replications during the years 2016 and 2017. Sowing was performed in mid-June after wheat harvest. Before sowing, 50 kg ha⁻¹ pure nitrogen and phosphorus were applied to the experimental plots as basal fertilizers. Genotypes were sown manually in 4 rows of 5 m-long at 70 cm row spacing and 15 cm intrarow spacing. Dressing fertilizers were applied manually when the plants reached heights of 40–50 cm as to have 50 kg ha⁻¹ pure nitrogen, and irrigations were initiated. Harvests were performed at the beginning of dough stage of the grains on the panicles. Side rows

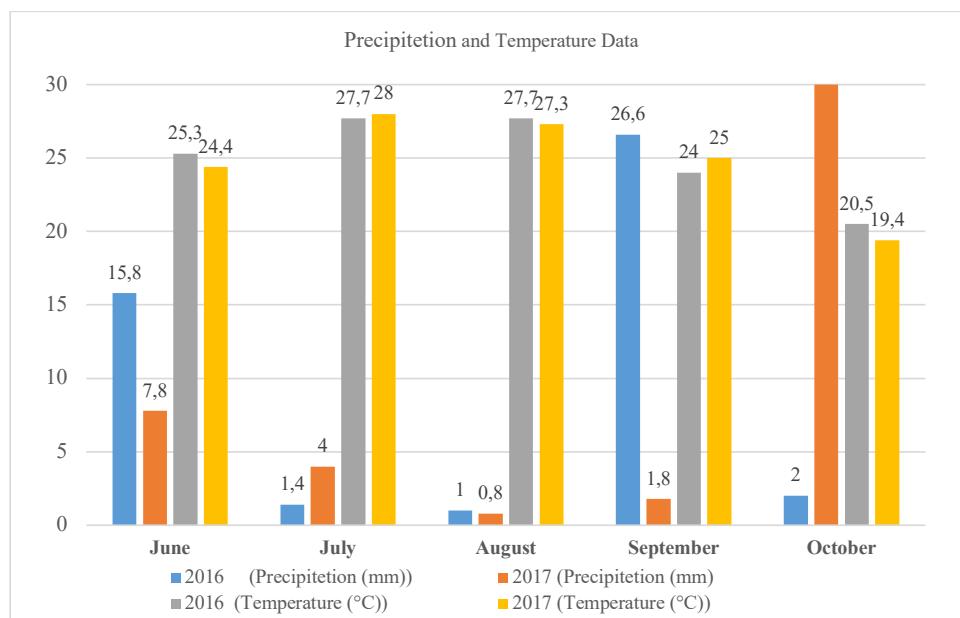


Figure 1. The monthly precipitation and average air temperature from June to October in the years of 2016 and 2017 at Adana.

and 0.5 m top and bottom of the rows in each plot were omitted as to consider side effects. Harvested plants were weighed to get plot yields and resultant values were then converted into yield per hectare. Genotypes had different harvest dates and harvests were completed in October of the first year and November of the second.

Plant Attributes and Theoretical Ethanol Potential

Harvest was performed between milk and dough stages of the grains (Hills, 1990; Prasad et al., 2007). During the harvest, randomly selected 10 panicle-forming stalks were cut at 3–5 cm above the soil surface; they were labelled and transported to a closed facility (to prevent sun-induced water loss). Plant heights were determined on 10 stalks randomly selected from each replicate of each genotype. Then, panicles and leaves were removed from the stalks and stalk weights were determined. Stalk diameter was measured between the 2nd and 3rd nodes with a caliper. To measure brix and juice values of the genotypes, stalks without panicle and leaves were subjected to the juice extraction process in a horizontal 3-roller power mill.

Theoretical ethanol yields were determined with the use of stalks without panicle and leaves. They were subjected to juice extraction in a horizontal 3-roller power mill. Amount of juice per stalk as ml was multiplied with number of stalks per unit area to get juice yield.

Juice content was calculated with the use of the equation: (weight of fresh juice / weight of fresh stalk) × 100.

Theoretical ethanol yields were determined with the use of the equation: $[(\text{total sugar} / 5.68) \times 3.78] \times 0.80$ (Anonymous, 2010; Bunphan et al., 2015).

The variance analysis was conducted on experimental data with the aid of JMP software and significant means were compared with the use of Tukey's multiple range test at 5% level.

3. Results

Days to harvest (day): There were significant differences in days to harvest values of the genotypes and the years, and genotype × year interactions were also found to be significant (Table 1). Average days to harvest was significantly greater in the second year (118.8 days) than in first year (115.6 days). Higher precipitations and lower temperatures in October of the second year delayed harvest maturity of the plants. However, significance of genotype × year interactions indicated that changes in harvest maturity of the genotypes with the years also changed with the genotypes. Thusly, while PI579753, Roma and Topper 76 genotypes reached harvest maturity quite later in the first year than in the second year, the genotypes Dale, Grass1, Mennonite N sugarcane, Rox Orange, Smith, Sugar Drip, Theis, Tracy, Williams, No2, No5 and Gulseker reached the harvest maturity later in the second year than in the first

year. On the other hand, days to harvest of 5 genotypes did not vary significantly with the years. As the average of two years, days to harvest values of the genotypes varied between 98.6 and 134.4 days. Based on these values, No2, Mennonite and Rox Orange genotypes were identified as early genotypes with days to harvest values lower than 100 days.

Plant height (cm): For plant heights (PH), genotypes, years and genotype × year interactions were found to be significant at the 1% significance level (Table 1). The average value of plant height over the genotypes was significantly greater in the second year than in the first year. However, significant genotype × year interactions indicated that effects of years on plant height significantly varied with the genotypes. Thusly, while only Smith genotype had significantly greater plant height in the second year than in the first year, plant heights of the other genotypes did not significantly change with the years. As the average of two years, plant heights of the genotypes varied between 233.2 and 429.3 cm. Grass1, M81-E and UNL-Hyb-3 genotypes had significantly higher plant heights than the other genotypes, except for Theis and No 91 genotypes. Mennonite genotype had significantly lower plant height than the other genotypes.

Stalk (cane) diameter (mm): Genotypes and genotype × year interactions were found to be significant for stalk diameters at the 1% significance level, but the effects of years on stalk diameters were not found to be significant (Table 2). However, significant year × genotype interactions indicated that effects of the years on stalk diameters varied with the genotypes. Thusly, M81-E, Ramada, Roma, Theis, Topper 76, and UNL-Hyb-3 and No91 genotypes had significantly greater stalk diameters in the first year than in the second year. On the other hand, Smith genotype had significantly greater stalk diameter in the second year than in the first year. In the other genotypes, stalk diameters did not vary significantly with the years. As the average of two years, stalk diameters of the genotypes varied between 18.53 and 28.73 mm. UNL-Hyb-3 genotype had significantly greater stalk diameter than the other genotypes, except for Grass1, Theis and No91. Mennonite genotype had significantly lower stalk diameter than the other genotypes, except for No2 genotype.

Stalk yield ($t \text{ ha}^{-1}$): Genotypes and genotype × year interactions were found to be significant for stalk yield at the 1% significance level (Table 2). As the average of genotypes, stalk yield was identified as 129.1 t ha^{-1} in the first year and as 131.4 t ha^{-1} in the second year and differences between the years were not found to be significant. However, significant year × genotype interactions revealed that effects of the years on stalk yield varied with the genotypes. Accordingly, while Smith genotype had significantly greater stalk yield in the second

Table 1. Averaged values of days to harvest and plant height for the sweet sorghum genotypes in two years.

Genotypes	Days to harvest (day)			Plant height (cm)		
	2016	2017	Mean	2016	2017	Mean
Cowley	105.5 j ¹	105.0 jk	105.3 h*	342.2 f-m ¹	338.0 h-m	340.1 cde*
Dale	113.0 h	118.0 e	115.5 ef	338.3 h-m	370.0 b-k	354.2 c
Grassl	112.5 h ₁	118.0 e	115.3 ef	408.1 a-d	450.5 a	429.3 a
M81-E	133.8 bc	132.0 c	132.9 abc	395.4 a-g	413.0 abc	404.2 a
Mennonite	96.5 m	101.0 l	98.8 i	216.1 q	250.3 pq	233.2 h
N.sugarcane	113.3 h	116.0 efg	114.6 ef	337.7 h-m	373.8 b-j	355.7 c
PI579753	116.5 ef	105.0 jk	110.8 g	349.5 e-m	323.3 i-n	336.4 cde
Ramada	133.3 bc	132.0 c	132.3 bc	340.1 g-m	360.3 b-l	350.2 cd
Roma	135.0 ab	132.0 c	133.5 ab	345.0 f-m	359.5 c-l	352.7 cd
Rox Orange	97.0 m	101.0 l	99.0 i	273.6 nop	319.8 j-n	296.7 fg
Smith	104.0 jk	118.0 e	111.0 g	328.5 i-n	401.0 a-e	364.7 bc
Sugar Drip	104.0 jk	117.0 e	110.5 g	307.5 l-o	327.8 i-n	317.6 d-g
Theis	124.5 d	133.0 bc	128.8 d	385.8 b-h	409.8 a-d	397.8 ab
Topper 76	135.8 a	133.0 bc	134.4 a	339.0 h-m	376.8 b-1	357.9 c
Tracy	110.3 i	118.0 e	114.1 f	336.2 h-m	378.3 b-1	357.2 c
UNL-Hyb-3	131.8 c	132.0 c	131.9 c	411.5 a-d	415.5 ab	413.5 a
Williams	103.0 kl	116.0efg	109.5 g	262.2 opq	316.5 k-q	289.4 g
No2	96.3 m	101.0 l	98.6 i	327.0 i-n	332.3 h-m	329.6 c-f
No91	133.0 bc	133.0 bc	133.0 abc	396.3 a-f	396.3 a-f	396.3 ab
No5	114.5 fgh	117.3 e	115.9 e	356.0 d-l	340.3 g-m	348.1 cde
Gulseker	113.8 gh	117.0 e	115.4 ef	295.5 m-p	333.0 h-m	314.2 efg
Mean	115.6 B⁺	118.8 A		337.7 B⁺	361.2 A	
CV (%)	0.73			5.6		
F Genotype (G)	**			**		
F Year (Y)	**			**		
F G × Y Int.	**			**		

¹) The means of different year-treatment combinations with the same lower case are not statistically significant different from each other according to the Tukey test at $p \leq 0.05$. *) The means with the same letter in the same column are not statistically significant different from each other according to the Tukey test at $p \leq 0.05$.

+) The means indicated with the same capital letter in the same row are not significantly different at $p \leq 0.05$.

year than in the first year, No5 genotype had significantly greater stalk yield in the first year than in the second year. Stalk yields of the other genotypes did not significantly vary with the years. As the average of two years, stalk yields of the genotypes varied between 69.0 and 182.6 t ha⁻¹. UNL-Hyb-3 genotype had significantly greater stalk yield than the other genotypes, except for Grassl, M81-E, Theis, Topper 76 and No91 genotypes. Mennonite genotype had significantly lower stalk yield than the other genotypes, except for Rox Orange and No2 genotypes.

Juice recovery (%): Similar to the results for stalk diameter and stalk yield, genotypes and genotype × year

interactions were found to be significant for juice recovery at the 1% significance level (Table 3). Year effects on juice recovery were not found to be significant. As the average of the genotypes, juice recovery was identified as 34.38% in the first year and as 33.11% in the second year (Table 3). However, significant year × genotype interactions indicated that effects of the years on juice recovery varied with the genotypes. Thusly, N. Sugarcane, Williams and No5 genotypes had significantly greater juice recovery in the first year than in the second year. On the other hand, P1579753 genotype had significantly greater juice recovery in the second year than in the first year. Juice

Table 2. Averaged values of stalk diameter and stalk yield for the sweet sorghum genotypes in two years.

Genotypes	Stalk diameter (mm)			Stalk yield (t ha ⁻¹)		
	2016	2017	Mean	2016	2017	Mean
Cowley	24.33 a-j ¹	23.78 b-j	224.05 a-e [*]	123.5 e-p ¹	123.1 e-p	1123.3 f-1*
Dale	25.80 a-f	27.30 abc	26.55 a	130.7 d-n	137.7 c-m	134.2 d-h
Grassl	26.05 a-e	26.08 a-e	26.06 ab	176.4 abc	160.4 b-f	168.4 abc
M81-E	24.93 a-i	20.33 ijk	22.63 de	163.6 a-e	147.4 b-k	155.5 a-e
Mennonite	21.30 f-k	23.23 c-j	22.26 e	58.6 r	79.4 qr	69.01
N.sugarcane	26.03 a-e	25.50 a-g	25.76 abc	119.2 f-q	126.5 e-o	122.8 f-1
PI579753	23.23 c-j	24.08 a-j	23.65 a-e	97.1 m-r	116.5 g-q	106.8 ijk
Ramada	25.80 a-f	21.03 g-k	23.41 b-e	136.0 c-m	150.3 b-j	143.1 c-g
Roma	25.03 a-h	20.83 g-k	22.93 cde	145.0 b-l	154.8 b-g	150.0 b-f
Rox Orange	22.00 e-k	23.88 b-j	22.94 cde	82.8 p-r	104.6 l-q	93.7 jkl
Smith	22.45 d-k	28.73 a	25.59 a-d	93.1 n-r	164.9 a-e	129.0 e-1
Sugar Drip	24.35 a-j	25.13 a-g	24.74 a-e	111.6 h-q	103.1 l-q	107.3 h-k
Theis	26.83 a-d	18.53 k	22.68 de	172.5 a-d	153.4 b-h	163.0 abc
Topper 76	27.98 ab	22.93 c-k	25.45 a-d	152.3 b-1	162.9 a-e	157.6 a-d
Tracy	25.43 a-g	24.63 a-j	25.03 a-e	111.5 h-q	128.6 e-n	120.0 g-j
UNL-Hyb-3	25.30 a-g	20.35 h-k	22.83 cde	203.2 a	162.0 a-e	182.6 a
Williams	24.10 a-j	24.95 a-i	24.53 a-e	107.8 k-q	108.0 j-q	107.9 h-k
No2	22.90 c-k	22.30 d-k	22.60 de	82.3 p-r	84.5 o-r	83.4 kl
No91	24.35 a-j	20.18 j-k	22.26 e	186.4 ab	16278 a-e	174.6 ab
No5	24.68 a-j	26.00 a-e	25.34 a-d	159.0 b-g	110.2 i-q	13456 d-h
Gulseker	24.75 a-j	27.93 ab	26.34 ab	105.4 k-q	119.2 f-q	112.3 hij
Mean	24.7	23.70		129.1	131.4	
CV (%)	6.84			11.49		
F Genotype (G)	**			**		
F Year (Y)	NS			NS		
F G × Y Int.	**			**		

¹) The means of different year-treatment combinations with the same lower case are not statistically significant different from each other according to the Tukey test at $p \leq 0.05$. *) The means with the same letter in the same column are not statistically significant different from each other according to the Tukey test at $p \leq 0.05$.

recovery of the other genotypes did not vary significantly with the years. As the average of two years, juice recovery of the genotypes varied between 26.79% and 39.94%. Cowley genotype had significantly greater juice recovery than the other genotypes, except for M81-E, N. Sugarcane, Sugar Drip, No2, No91, No5 and Gulseker. UNL-Hyb-3 genotype had significantly lower juice recovery than the other genotypes, except for Grassl, P1579753, Ramada, Roma, Theis and Topper 76 genotypes.

Juice yield (m³ ha⁻¹): For juice yield, genotypes and genotype x year interactions were found to be significant at the 1% significance level (Table 3). As the average of

the genotypes, juice yield was identified as 43.85 m³ ha⁻¹ in the first year and as 42.94 m³ ha⁻¹ in the second year and differences between the years were found to be not significant (Table 3). However, significant year × genotype interactions revealed that year effects on juice yield varied with the genotypes. Thusly, while Theis and No5 genotypes had significantly greater juice yields in the first year than in the second year, Smith and P159753 genotypes had significantly greater juice yields in the second year than in the first year. Juice yields of the other genotypes did not vary significantly with the years. As the average of two years, juice yields of the genotypes varied between 22.98

Table 3. Averaged values of Juice yield and juice recovery for the sweet sorghum genotypes over two years.

Genotypes	Juice yield ($m^3 ha^{-1}$)			Juice recovery (%)		
	2016	2017	Mean	2016	2017	Mean
Cowley	48.64 c-1 ¹	48.47 c-1	48.55 cde [*]	39.95 a-d ¹	39.94 a-d	39.94 a*
Dale	43.73 f-l	47.43 d-1	45.58 c-g	31.69 d-k	34.40 b-j	33.04 c-g
Grassl	53.97 b-g	48.58 d-1	50.77 cd	30.68 e-k	29.63 f-k	30.15 e-h
M81-E	58.68 a-d	59.16 a-d	58.92 ab	35.95 b-h	40.99 abc	38.47 abc
Mennonite	22.02 q	23.94 o-q	22.98 l	37.71 a-g	30.21 e-k	33.96 b-f
N.sugarcane	44.91 f-k	39.66 i-n	42.29 e-h	45.32 a	31.32 d-k	38.32 abc
P1579753	23.52 p-q	44.14 f-k	33.83 ijk	23.17 k	38.41 a-f	30.79 d-h
Ramada	38.29 i-n	41.86 h-m	40.08 f-i	28.20 h-k	27.90 h-k	28.05 gh
Roma	43.09 f-l	44.39 f-k	43.74 d-h	29.03 g-k	29.05 g-k	29.04 fgh
Rox Orange	28.11 n-q	34.52 k-p	31.32 j-k	33.97 b-j	33.00 b-j	33.48 c-g
Smith	34.92 j-p	52.01 b-h	43.47 d-h	35.85 b-h	31.61 d-k	33.78 c-f
Sugar Drip	42.88 f-m	34.45 k-p	38.67 g-j	38.51 a-e	33.48 b-j	35.99 a-d
Theis	59.62 abc	46.36 e-j	52.99 bc	34.54 b-j	30.27 e-k	32.41 d-h
Topper 76	47.49 d-1	45.73 f-k	46.61 c-f	31.26 d-k	28.49 h-k	29.88 fgh
Tracy	37.32 i-n	42.29 g-m	39.81 f-i	33.45 b-j	33.16 b-j	33.31 c-g
UNL-Hyb-3	54.39 b-f	43.72 f-l	49.06 cde	26.73 j-k	26.85 ijk	26.79 h
Williams	41.45 h-m	31.18 m-q	36.32 h-k	38.44 a-f	28.99 g-k	33.71 c-g
No2	28.29 n-q	32.15 l-q	30.22 kl	34.41 b-j	38.25 a-f	36.33 a-d
No91	67.37 a	58.10 a-e	62.74 a	35.48 b-j	35.67 b-i	35.58 a-e
No5	62.77 ab	35.57 j-o	49.17 cde	40.00 a-d	32.22 e-j	36.11 a-d
Gulseker	39.29 i-n	49.10 c-i	44.19 c-g	37.73 a-g	41.40 ab	39.56 ab
Mean	43.85	42.94		34.38	33.11	
CV (%)	9.56			11.21		
F Genotype (G)	**			**		
F Year (Y)	NS			NS		
F G × Y Int.	**			**		

¹) The means of different year-treatment combinations with the same lower case are not statistically significant different from each other according to the Tukey test at $p \leq 0.05$. *) The means with the same letter in the same column are not statistically significant different from each other according to the Tukey test at $p \leq 0.05$.

and $62.74 m^3 ha^{-1}$. No91 genotype had significantly greater juice yield than the other genotypes with the exception of M81-E genotype. Mennonite genotype had significantly lower juice yield than the other genotypes, except for No2 genotype.

Brix (%): Years, genotypes and year × genotype interactions were all found to be significant for brix value. Average brix value was significantly greater in the second year (17.33%) than in the first year (16.93%) (Table 4). However, significant year × genotype interactions revealed that effects of the years on brix value varied with the genotypes. Thusly, while Cowley, Mennonite and Gulseker genotypes had significantly greater brix values in

the second year than in the first year, M81-E and No91 genotypes had significantly greater brix values in the first year than in the second year. Brix values of the other genotypes did not vary significantly with the years. As the average of two years, brix values of the genotypes varied between 12.25% and 20.00%. Roma genotype with a brix value of 20% had significantly greater value than the other genotypes, except for Ramada, Tracy and UNL-Hyb-3 genotypes. Gulseker genotype had significantly lower brix value than the other genotypes.

Theoretical ethanol yield (L ha^{-1}): There were significant differences in theoretical ethanol yields of the genotypes. Effects of the years on theoretical ethanol

Table 4. Averaged values of brix and theoretical ethanol yield for the sweet sorghum genotypes over two years.

Genotypes	Brix value (%)			Theoretical ethanol yield (L ha ⁻¹)		
	2016	2017	Mean	2016	2017	Mean
Cowley	16.00 f-k ¹	18.50 a-e	17.25 c-h [*]	4106 d-j ¹	4771 b-g	4439 bcd*
Dale	16.50 e-j	17.25 c-1	16.88 d-1	3642 g-k	4322 c-h	3982 def
Grassl	16.75 d-j	17.50 c-h	17.13 d-h	4784 b-g	4394 c-h	4589 a-d
M81-E	17.50 c-h	13.50 l	15.50 i	5474 abc	4244 c-1	4859 abc
Mennonite	15.00 i-l	18.00 b-g	16.50 e-i	1759 o	2281 mno	2020 i
N.sugarcane	18.50 a-e	17.75 b-g	18.13 bcd	5189 a-e	3696 g-k	4422 bcd
P1579753	16.75 d-j	18.25 b-f	17.50 c-g	1974 no	4171 d-j	3073 gh
Ramada	19.50 abc	19.50 abc	19.50 ab	3977 e-j	4354 c-h	4165 cde
Roma	20.75 a	19.25 abc	20.00 a	4640 b-g	4547 c-g	4594 a-d
Rox Orange	15.25 h-l	16.75 d-j	16.00 ghi	2286 mno	3022 i-n	2659 gh1
Smith	17.25 c-1	18.50 a-e	17.88 cde	2987 j-o	5118 a	4052 def
Sugar Drip	16.50 e-j	17.75 b-g	17.13 d-h	3709 g-k	3208 h-n	3459 efg
Theis	16.50 e-j	15.75 g-l	16.13 f-i	5253 a-d	3873 f-k	4563 a-d
Topper 76	17.25 c-1	17.50 c-h	17.38 c-h	4366 c-h	4260 c-1	4313 bcd
Tracy	18.50 a-e	19.00 a-d	18.75 abc	3654 g-k	4191 d-j	3923 def
UNL-Hyb-3	20.00 ab	19.25 abc	19.63 ab	5800 ab	4395 c-h	5097 ab
Williams	17.25 c-1	18.00 b-g	17.63 c-f	3730 g-k	2988 j-o	3359 fg
No2	15.75 g-l	16.00 f-k	15.88 hi	2363 l-o	2718 k-o	2541 hi
No91	17.50 c-h	14.75 jkl	16.13 f-i	6065 a	4540 c-g	5302 a
No5	15.75 g-l	17.50 c-h	16.63 d-i	5014 a-f	3284 h-m	4149 c-f
Gulseker	10.75 m	13.75 kl	12.25 j	2249 mno	3586 g-l	2918 gh
Mean	16.93 B⁺	17.33 A		3953	3903	
CV (%)	4.83			11.21		
F Genotype (G)	**			**		
F Year (Y)	**			NS		
F G × Y Int.	**			**		

¹) The means of different year-treatment combinations with the same lower case are not statistically significant different from each other according to the Tukey test at $p \leq 0.05$.

*) The means with the same letter in the same column are not statistically significant different from each other according to the Tukey test at $p \leq 0.05$.

+) The means indicated with the same capital letter in the same row are not significantly different at $p \leq 0.05$.

yields were found to be not significant. Genotype × year interactions were found to be significant for theoretical ethanol yield (Table 4). As the average of the genotypes, the theoretical ethanol yield was measured as 3953 L ha⁻¹ in the first year and as 3903 L ha⁻¹ in the second year. Significant year × genotype interactions revealed that the effects of years on theoretical ethanol yields varied with the genotypes. Thusly, while N sugarcane, Theis, UNL-Hyb-3, No91 and No5 genotypes had significantly higher theoretical ethanol yields in the first year than in

the second year, P1579753, Smith and Gulseker genotypes had significantly greater theoretical ethanol yields in the second year than in the first year. Theoretical ethanol yields of the other genotypes did not vary significantly with the years. As the average of two years, theoretical ethanol yields of the genotypes varied between 2020 and 5302 L ha⁻¹. No91 genotype (5302 L ha⁻¹) had significantly greater theoretical ethanol yield than the other genotypes, except for Grassl, M81-E, Roma, Theis and UNL-Hyb-3 genotypes. Mennonite genotype had significantly lower

theoretical ethanol yield than the other genotypes, except for Rox orange and No2 genotypes.

4. Discussion

Days to harvest: As the average of two years, days to harvest values of present genotypes varied between 98.6 and 134.4 days. With these values, genotypes were classified as early, mid-early and late genotypes. The early genotypes of the present study (No2, Mennonita and Rox Orange) had short plant heights and the lowest stalk and ethanol yields. On the other hand, later genotypes (Grass1, M81E, Roma, Theis, UNL Hybrid-3 and No91) had the greatest stalk and ethanol yields (Tables 2 and 4). Significant positive correlations of days to flowering with stalk diameter, plant height and stalk yield of sorghum were reported (Vendruscolo et al., 2016). Vijendra (2005) indicated that genotype performance varied with the environmental conditions, and sorghum genotypes should be harvested in the IV and Vth stage of growth (104 to 117 days after planting) for high grain and ethanol yield. Ratnavati et al. (2010) indicated that sugar accumulation in stalks started with the flowering stage and reached maximum levels with physiological maturity. Days to harvest value was greater in the second year because of lower average temperatures throughout the growing season (Figure 1).

Plant height: As the average of two years, plant heights of the genotypes varied between 233.2 and 429.3 cm. The genotypes with taller plant heights had larger stalk diameters and were mostly late genotypes with greater unit area yields. Significant positive correlations were reported between plant height and biomass (Audilakshmi et al., 2010; Iyanar et al., 2010), between plant height and stalk diameter; between ethanol yield and biomass, stalk yield, juice recovery and total sugar content (Prasad et al., 2013). Sweet sorghum may reach a height of 4.5 m in 4–5 month growing season under proper conditions (Dweikat, 2014). Plant heights of different sorghum genotypes under different ecological conditions were reported as between 93 and 480 cm (Subramanian, 2013; Prasad et al., 2013; Udoh et al., 2018). Present findings on plant heights comply with those earlier reports. Plant heights were greater in the second year of the present study because of precipitation at the harvest period and delayed harvests. Vendruscolo et al. (2016) reported significant positive correlations between days to flowering and plant height. It was reported that plant heights varied with the locations and the environment had significant effects on plant heights (Udoh et al., 2018).

Stalk diameter: Since stalk diameter directly influences stalk yield, a significant parameter in ethanol production from sorghum through extracting stalk juice, thick stalks are desired for higher stalk and ethanol yields. As the average of two years, stalk diameters of the genotypes

varied between 22.26 and 26.55 mm. Stalk diameters of sweet sorghum genotypes were reported as between 8 and 27 mm with an average value of 17 mm (Subramanian, 2013). Taller genotypes also had larger stalk diameters. Previous research also reported significant positive correlations between plant height and stalk diameter (Ali et al., 2008; Murray et al., 2009; Audilakshmi et al., 2010).

Stalk yield: Leaves play a significant role in photosynthesis, but are less important in ethanol production (less than 2% sugar). Therefore, leaves were removed during the harvest to prevent juice suction from the stalks and yield losses. Stalks are used in plain fashion without panicles and leaves in ethanol production. Sweet sorghum at harvest maturity is composed of 75% stalk, 10% leaf, 5% grain and 10% roots (Grassi et al., 2002). Stalk yields of the genotypes tested in this study varied between 69.0 and 182.6 t ha⁻¹. Taller and thicker stalks also had greater fresh stalk yields. Therefore, late genotypes with taller and thicker stalks should be preferred for high stalk yield per unit area. Significant positive correlations of stalk yield with plant height and stalk diameter were reported by Audilakshmi et al. (2010). Stalk yields of different sweet sorghum genotypes in different locations were reported as between 54 and 209 t ha⁻¹ (Almodares et al., 2008; Rutto et al., 2013; Junior et al., 2015; Mahdy et al., 2018).

Juice yield: Juice was extracted from the stalks without panicles and leaves with the use of specially designed machines. Juice yields of the genotypes varied between 22.98 and 62.74 m³ ha⁻¹. The genotypes with a high stalk yield also had high juice yields. Significant positive correlations were reported between stalk yield and juice yield (Murray et al., 2008). In sweet sorghum, fresh stalk weight, juice yield, brix and sugar content are significant characteristics for biofuel production (Murray et al., 2008; Pfeiffer et al., 2010). Subramanian (2013) reported juice yields of sweet sorghum genotypes as between 124.7 and 914.2 (g/plant). Also, Mahdy et al. (2018) reported juice yields as between 16.9 and 24.5 t ha⁻¹. Dalvi et al. (2011), Prasad et al. (2013) and Erdurmuş et al. (2018) reported juice yields of the sweet sorghum genotypes as between 3940 and 35143 L ha⁻¹. Rutto et al. (2013) reported juice yields as between 7.6 and 18.9 m³ ha⁻¹.

Juice recovery: The juice quantity obtained through pressing the stalks was proportioned to stalk weight to get juice recovery (JR) values. As the average of two years, juice recovery of the genotypes varied between 26.79% and 39.94%. Prasad et al. (2013) reported the juice recovery of different genotypes as between 27.3% and 40.1%. They also reported significant positive correlations between plant height and stalk diameter, between ethanol yield and biomass and stalk yields and between juice yield and total sugar.

Brix: Sugar concentration of juice from sweet sorghum stalks is expressed in brix units representing soluble sugar percentages. A brix unit is equal to 1 g sugar per 100 g juice (Qazi et al., 2012). Harvest season directly influences juice yields and brix values. At physiological maturity stage, juice sugar contents (brix) vary between 10% and 25% (Reddy et al., 2007). Hills (1990) indicated that sugar content of juice from sweet sorghum stalks increased between milk and dough stages, then decreased toward to physiological maturity. Juice sugar content was reported as about 12.5% at the beginning of the harvest and the value increased up to 17% at maturity (Prasad et al., 2007). Almodares et al. (2007) reported low sugar content at flowering period mostly because of high quantities of acid invertase enzyme. Hunter and Anderson (1997) reported about twice as much sugar content at dough stage as compared to milk stage. As the average of two years, brix values of the genotypes tested in this research, except for control cultivar (Gulşeker), varied between 15% and 20%. Brix values of sorghum genotypes grown in different ecologies were reported as between 6.2% and 20.7% (Rutto et al., 2013; Subramanian, 2013; Erdurmuş et al., 2018; Udoth et al., 2018). Subramanian (2013) reported significant correlations between sugar yield and juice yield and fresh stalk weight.

Theoretical ethanol yield: As the average of two years, theoretical ethanol yields of the genotypes varied between 2020 and 5303 L ha⁻¹. The Grass1, M81E, Roma, Theis, UNL Hybrid-3 and No91 genotypes had bioethanol yields over 4500 L ha⁻¹ and these genotypes had greater stalk bioethanol yields than the other genotypes. The genotypes with a high theoretical ethanol yield also had high juice yields and brix values. Juice yield and brix values directly contribute to ethanol yields. Juice composition also significantly influence ethanol yields (Widianto et al.,

2010) and juice composition is influenced by genotype, environment and harvest date (Almodares and Hadi, 2009). Rono et al. (2018) indicated that the genotypes with a high stalk yield, juice yield and plant height had greater ethanol yields. For maximum ethanol yields, taller sweet sorghum genotypes with high brix, total sugar, nonreducing sugar, biomass, stalk and juice yields should be selected since these parameters have positive correlations with ethanol yield (Rani and Umakanth, 2012). Vijendra (2005) indicated that sweet sorghum genotypes exhibited different performances under different environmental conditions. Ethanol yields of the sorghum genotypes grown under different ecologies were reported as between 298 and 8390 L ha⁻¹ (Sakellariou Makrantonaki et al., 2007; Murray et al., 2009; Dalvi et al., 2011; Teetor et al., 2011; Rutto et al., 2013; Erdurmuş et al., 2018).

5. Conclusion

In the present study, different sorghum genotypes were tested for theoretical ethanol yield under Eastern Mediterranean (Adana) conditions during the two years as the second crop after wheat harvest. Grass1, M81-E, Roma, Theis, UNL hybrid-3 and No91 genotypes had theoretical ethanol yields over 4500 L ha⁻¹, stalk yields over 150 t ha⁻¹ and brix values over 15.5%. It was concluded that these genotypes could successfully be grown as the second crop (June–October) for high stalk and theoretical ethanol yields in the southern regions of Turkey with a Mediterranean climate.

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References

Ali ML, Rajewski JF, Baenziger PS, Gill KS, Eskridge KM et al. (2008). Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. *Molecular Breeding* 21: 497-509. doi: 10.1007/s11032-007-9149-z

Almodares A, Taheri R, Adeli S (2007). Inter-relationship between growth analysis and carbohydrate contents of sweet sorghum cultivars and lines. *Journal of Environmental Biology* 28:527-531.

Almodares A, Taheri R, Adeli S (2008). Stalk yield and carbohydrate composition of sweet sorghum [*Sorghum bicolor* (L.) Moench] cultivars and lines at different growth stages. *Malaysian Applied Biology* 37: 31-36.

Almodares A, Hadi MR (2009). Production of bioethanol from sweet sorghum: A review. *African Journal of Agricultural Research* 4: 72-780.

Anonymous (2010). Technical directive for the experiments of measuring agricultural properties, Sorghum (Sorghum spp) (in Turkish). Central Directorate of Seed Control and Certification. Website <http://www.ttsm.gov.tr/TR/belge/1-296/teknik-talimatlar.html>, Ankara (accessed 20 June 2019).

Audilakshmi S, Mall AK, Swarnalatha M, Seetharama N (2010). Inheritance of sugar concentration in stalk (brix), sucrose content, stalk and juice yield in sorghum. *Biomass & Bioenergy* 34: 813-820.

Bunphan D, Jaisil P, Sanitchon J, Knoll JE, Anderson WF (2015). Estimation methods and parameter assessment for ethanol yields from total soluble solids of sweet sorghum. *Industrial Crop and Products* 63: 349-356.

Dalla Marta A, Mancini M, Orlando F et al. (2014). Sweet sorghum for bioethanol production: crop responses to different water stress levels. *Biomass & Bioenergy* 64: 211-219.

Dalvi US, Chavan UD, Shinde MS, Gadakh SR (2011). Assessment of sweet sorghum cultivars for efficient ethanol production. *Sugar Technology* 13 (3): 186-190. doi: 10.1007/s12355-011-0094-0

Dweikat I (2014). Sorghum diversity paper, sweet energy crop article (online). Website <http://agronomy.unl.edu/sweetssorghum> (accessed 07 July 2014).

Erdurmus C, Yucel C, Cinar C, Yegin AB, Öten M (2018). Bioethanol and sugar yields of sweet sorghum. *The International Journal of Engineering and Science* 7 (11): 21-26.

Fernandez J, Curt MD (2005). New energy crops for bioethanol production in the Mediterranean region. *International Sugar Journal* 107 (1283): 622-627.

Grassi G, Qiong Z, Grassi A, Fjallstrom T, Helm P (2002). Small-scale modern autonomous bioenergy complexes: development instrument for fighting poverty and social exclusion in rural villages. *Proceedings of 12th European Conference on Biomass for Energy, Industry and Climate Change; 2002 June 17th-21st; Amsterdam, Netherlands.*

Hills FJ, Lewellen RT, Skoyen IO (1990). Sweet sorghum cultivars for alcohol production. *California Agriculture* 44: 14-16.

Hunter EL, Anderson IC (1997). Sweet Sorghum, In J. Janick, Ed., *Horticultural Reviews*, John Wiley & Sons, Inc., Oxford, 21:73-104.

Iyanar K, Vijayakumar G, Fazlullah Khan AK (2010). Correlation and path analysis in multicut fodder sorghum. *Electronic Journal of Plant Breeding* 1 (4): 1006-1009.

Jiang D, Hao M, Fu J, Liu K, Yan X (2019). Potential bioethanol production from sweet sorghum on marginal land in China. *Journal of Cleaner Production* 220: 225-234. doi: 10.1016/j.jclepro.2019.01.294

Junior MAPO, Retore M, Manarelli DM, de Souza FB, Ledesma LLM et al. (2015). Forage potential and silage quality of four varieties of saccharine sorghum. *Pesquisa Agropecuaria Brasileira* Brasília 50 (12): 1201-1207. doi: 10.1590/S0100-204X2015001200010

Lima GS (1998). Estudo comparativo da resistencia à seca no sorgo forrageiro (*Sorghum bicolor* L Moench) em diferentes estadios de desenvolvimento. Recife: UFRPE. 128 f.Dissertação (Mestrado)-Universidade Federal Rural de Pernambuco, Recife.

Mahdy EME, Emara HA, Ramadan MA (2018). Suitability of some sweet sorghum varieties for bioethanol production. *Journal of Agricultural Chemistry and Biotechnology*, Mansoura University 9 (9): 205-210.

Mathur S, Umakanth AV, Tonapi VA, Sharma R, Sharma MK (2017). Sweet sorghum as biofuel feedstock: Recent advances and available resources. *Biotechnology for Biofuels* 10,146:1-19. doi: 10.1186/s13068-017-0834-9

Murray SC, Sharma A, Rooney WL, Klein PE, Mullet JE et al. (2008). Genetic improvement of sorghum as a biofuel feedstock I: Quantitative loci for stem sugar and grain nonstructural carbohydrates. *Crop Science* 48: 2165-2179. doi: 10.2135/cropsci2008.01.0016

Murray SC, Rooney WL, Hamblin MT, Mitchell SE, Kresovich S (2009). Sweet sorghum genetic diversity and association mapping for Brix and height. *The Plant Genome* 2: 48-62. doi: 10.3835/plantgenome2008.10.0011

Pfeiffer TW, Bitzer MJ, Toy JJ, Pedersen JF (2010). Heterosis in sweet sorghum and selection of a new sweet sorghum hybrid for use in syrup production in Appalachia. *Crop Science* 50: 1788-1794. doi: 10.2135/cropsci2009.09.0475

Prasad S, Singh A, Jain N, Joshi HC (2007). Ethanol production from sweet sorghum syrup for utilization as automotive fuels in India. *Energy Fuels* 21: 2415-20. doi: 10.1021/ef060328z

Prasad S, Kumar A, Muralikrishna KS (2013). Assessment of ethanol yield associated characters in sweet sorghum. *Maydica Electronic Publication* 2013: 299-303 <https://www.researchgate.net/publication/259964589>.

Qazi HA, Paranjpe S, Bhargava S (2012) Stem sugar accumulation in sweet sorghum activity and expression of sucrose metabolizing enzymes and sucrose transporters. *Journal of Plant Physiology* 169 (6): 605-613. doi: 10.1016/j.jplph.2012.01.005

Rani C, Umakanth AV (2012). Genetic variation and trait inter-relationship in F1 hybrids of sweet sorghum (*Sorghum bicolor* L. Moench). *Journal of Tropical Agriculture* 50: 80-83.

Rao SS, Patil JV, Prasad PV, Reddy DS, Mishra JS et al. (2013). Sweet sorghum planting effects on stalk yield and sugar quality in semi-arid tropical environments. *Agronomy Journal* 105: 1458-1465. doi: 10.2134/agronj2013.0156

Ratnavati CV, Suresh K, Vijay Kumar BS, Pallavi M, Komala VV et al. (2010). Study on genotypic variation for ethanol production from sweet sorghum juice. *Biomass and Bioenergy* 34 (7): 947-952. doi: 10.1016/j.biombioe.2010.02.002

Reddy BVS, Ramesh S, Reddy PS, Ramaiah B, Salimath PM et al. (2005). Sweet sorghum a potential alternate raw material for bio-ethanol and bioenergy. *International Sorghum and Millets Newsletter* 46: 79-86. http://oar.icrisat.org/1234/1/ISMN-46_79-86_2005.pdf

Reddy B, Kumar A, Ramesh S (2007). Sweet sorghum: a water saving bioenergy crop. ICRISAT, International Conference on Linkages Between Energy and Water Management for Agriculture in Developing Countries, January 29-30, 2007, IWMI, ICRISAT Campus, Hyderabad, India.

Rono JK, Cheruiyot EK, Othira JO, Njuguna VW (2018). Cane yield and juice volume determine ethanol yield in sweet sorghum (*Sorghum bicolor* L. Moench). *International Journal of Applied Science* 1 (2): 29-36. doi: 10.30560/ijas.v1n2p29

Rutto LK, Xu Y, Brandt M, Ren S, Kering MK (2013). Juice, ethanol, and grain yield potential of five sweet sorghum (*Sorghum bicolor* [L.] Moench) cultivars. *Journal of Sustainable Bioenergy Systems* 3: 113-118. doi: 10.4236/j.sbs.2013.32016

Sakellariou-Makrantonaki M, Papalexis D, Nakos N, Kalavrouzioti IK (2007). Effect of modern irrigation methods on growth and energy production of sweet sorghum (var. Keller) on a dry year in ventral Greece. *Agricultural Water Management* 90 (3): 181-189. doi: 10.1016/j.agwat.2007.03.004

Smith CW, Frederiksen RA (2000). Sorghum: origin, history, technology, and production. New York: John Wiley & Sons. 824 pp.

Subramanian SK (2013). Agronomical, physiological and biochemical approaches to characterize sweet sorghum genotypes for biofuel production. M.S.,Tamil Nadu Agricultural University, India, 2003. An abstract of a dissertation submitted in partial fulfillment of the requirements for the degree, Doctor of Philosophy. Department of Agronomy College of Agriculture. Kansas State University, Manhattan, Kansas.

Taylor SH, Hulme SP, Rees M, Ripley BS, Woodward FI et al. (2010). Ecophysiological traits in C3 and C4 grasses: a phylogenetically controlled screening experiment. *New Phytologist* 185 (3): 780-791. doi: 10.1111/j.1469-8137.2009.03102.x

Teeter VH, Duclos DV, Wittenberg ET, Young KM, Chawhuaymak J et al. (2011). Effects of planting date on sugar and ethanol yield of sweet sorghum grown in Arizona. *Industrial Crops Products* 34 (2): 1293-1300. doi: 10.1016/j.indcrop.2010.09.010

Udoh DA, Rasmussen SK, Jacobsen SE, Iwo GA, Milliano W de (2018). Yield stability of sweet sorghum genotypes for bioenergy production under contrasting temperate and tropical environments. *Journal of Agricultural Science* 10 (12): 42-53. doi: 10.5539/jas.v10n12pxx

Vendruscolo TPS, Barelli MAA, Castrillon MAS, da Silva RS, de Oliveira FT et al. (2016). Correlation and path analysis of biomass sorghum production. *Genetics and Molecular Research* 15 (4): 1-10. doi: 10.4238/gmr15049086

Vijendra LD das (2005). Genetic and plant breeding (pp. 76-79). New Delhi: New Age International Limited Publishers.

Widianto D, Arofatullah A, Yuwono T, Prijambada ID (2010). Ethanol production by fermentation of various sweet-stalk sorghum juices using various yeast strains. *Indonesian Journal of Biotechnology* 15 (2): 87-93.

Wu X, Staggenborg S, Propheter JL, Rooney WL, Yu J et al. (2010). Features of sweet sorghum juice and their performance in ethanol fermentation. *Industrial Crops and Products* 31 (1): 164-170. doi: 10.1016/j.indcrop.2009.10.006

Zhang Q, Ma J, Qiu G, Li L, Geng S et al (2012). Potential energy production from algae on marginal land in China. *Bioresource Technology* 109: 252-260.