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Effect of genotype on the production and quality of sweet sorghum juice [*Sorghum bicolor* (L.) Moench]

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ABSTRACT

Objectives: To evaluate the production of sweet sorghum juice (*Sorghum bicolor* (L.) Moench) and sugar, the physicochemical parameters of juice during extraction, and to quantify the differences between genotypes.

Design/Methodology/Approach: We established an experiment under irrigation with 10 sweet sorghum genotypes in southern Sonora, México, during the autumn-winter agricultural cycle. We used a randomized complete block design with four replications. The sowing dates were 03/15/2015 and 02/20/2016. The variables were: days to flowering (DF); weight of fresh biomass (t ha^{-1}): whole plant (WTo), stem (WSt), leaf (WLf), and panicle (WP). After extraction, we determined juice weight (WJ), bagasse weight (WBz), juice volume (JV), and extraction efficiency (EFx:WJ/WSt). The juice was sieved to remove impurities. Temperature (°C), pH, and soluble solids (°Brix) were determined at extraction time.

Results: The sources of variation had a significant effect on the production of biomass, juice, and sugar. The year explained 53% of variation, the genotype 36%, and the interaction (G×A) only 5%. On average, the production of fresh stem biomass was 38 t ha^{-1}, with 28% efficiency in juice extraction. The SWS686 and SWS694 genotypes exceeded both the average and the control (M81E) in juice production. Juice production in 2016 was higher (31%) than in 2015. In average, juice values were of 32 °C, 12.9 °Brix, and pH 3.8.

Study limitations/implications: The decrease in the content of soluble solids and spontaneous fermentation during juice conservation at room temperature can limit the use of sweet sorghum in areas where temperatures of >30 °C prevail during the post-harvest stage.

Findings/conclusions: The environment and the genotype affected the production and quality of sweet sorghum juice. It is necessary to make a complete life cycle analysis that indicates the challenges and opportunities to improve the efficiency of the processes to obtain sweet sorghum juice.

Keywords: Sweet sorghum, Genotype, Biomass, Sugars.

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an exotic resource in Mexico. Native grain genotypes come from Africa (Venkateswaran *et al.*, 2019), while sweet sorghum germplasm comes from the USA and India. Both are the basis for genetic improvement (Williams-Alanis *et al.*, 2020). Sucrose is the main soluble sugar obtained from sugar cane (*Saccharum officinarum* L.), with approximately 75% of the world supply (Zhao *et al.*, 2014). It is also the most abundant ingredient in sweet sorghum juice (66%), which can also have glucose (25%), fructose (13%), and minerals such as nitrogen, phosphorus, Cl^{-1}, Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+} (Olszewska-Widdrat *et al.*, 2019). Bioproducts such as ethanol are obtained by direct fermentation of the juice (Fagundes *et al.*, 2021). Sweet sorghum in semi-arid climates is used for biomass production (Mishra *et al.*, 2017). It is also a low-cost alternative non-food energy crop that can simultaneously provide juice and bagasse (Zegada and Monti, 2012). Genetic improvement of sweet sorghum has focused mainly on developing genotypes with high sugar yields. Morphological characters such as plant height and stem diameter are related to juice production (Shinde *et al.*, 2012). In Mexico, sweet sorghum has been experimentally grown to obtain green forage (Nava-Berumen *et al.*, 2017), sugars (Montes-Garcia *et al.*, 2019), and syrup as an alternative to replace corn fructose (Arvizu-Castro *et al.*, 2016). In northwestern Mexico, the adaptation of sweet sorghum crops is being evaluated, since some genotypes are photosensitive (Oliveira *et al.*, 2019) and susceptible to disease (Xavier *et al.*, 2017). The objective of the experiment was to evaluate the differences between genotypes, and to measure the effect of genotype and environment on the production of juice and sugar, as well as the physicochemical parameters of juice during extraction.

MATERIALS AND METHODS

Study area

The experiments were established in the same area in Block 2328 Valle del Yaqui, in Agua Blanca, Municipality of Villa Juárez, Sonora, Mexico (27° 7' 45.58" N, 109° 50' 14.57" W), at an altitude of 20 m. The climate is very dry and hot BW (h) hw. The mean annual temperature is 23.3 °C. The average annual rainfall is 340 mm, and July, August, and September are the rainiest months. Occasional frosts can occur from November to March.

Soil and climatic factors in the experiments

Before establishing the experiment in 2015, we conducted a soil sampling of the experimental site at a depth of 30 cm in order to determine its physical and chemical properties. We took 15 subsamples (0.5 kg) to form a composite sample of 5 kilograms. We assessed the following chemical properties: pH, measured with a potentiometer in a soil:water ratio of 1:2; electrical conductivity (CE1:5); soil organic matter (SOM), obtained with the Walkley-Black wet digestion method; Kjeldahl nitrogen, using a semi-micro method; carbon:nitrogen (C:N) ratio, calculated with SOM and Kjeldahl nitrogen results; extractable phosphorus, obtained with the Olsen method, for the analysis of available P, K, Fe, Cu, Zn, and Mn, and KCl for exchangeable acidity, Ca, and Mg. These methodologies are a standard for assessing soil properties as described by Castillo-Valdez *et al.* (2021). We recorded the climatic data during the February-August crop cycle: maximum and minimum temperature, evaporation, and rainfall, all of them taken from the Agua Blanca weather station DDR-148-EMA-26071-03.

Biological material

The genotypes used were: 1) experimental varieties of sweet sorghum (SWS) —603, 657, 658, 662, 691, 686, 694, and 817— with six selection cycles derived from the segregation of sweet sorghum Keller × Dale, both of them US public varieties (Montes *et al.*, 2013); RB-Cañero, the first Mexican variety of sweet sorghum (Montes *et al.*, 2019); and genotype M81E (control), a public domain commercial variety of sweet sorghum, released in Meridian, Mississippi, USA (Broadhead *et al.*, 1981), which was previously assessed in some towns of Sonora, Mexico (Ochoa *et al.*, 2011).

Conduction of experiment in the field

We planted ten sweet sorghum genotypes in five rows of 5 × 0.80 m for each material in a randomized complete block design with four replications. The useful plot comprised two central furrows. The irrigation frequency was based on the phenological stages of the crop. We established a surface irrigation system in the autumn-winter agricultural cycle of 2015 and 2016. The first irrigation was carried out prior to sowing, and three auxiliary irrigations were subsequently applied during the cycle. The first auxiliary irrigation was applied 40 days after sowing, the second 30 days after the first, and the third 25 days later. The sowing dates of the experiments were March 15, 2015 and February 20, 2016. After emergence, plant density was adjusted to 12 plants per meter (150,000 plants ha^{-1}).

The agronomic management of the crop followed the INIFAP technological package for this region (Ochoa *et al.*, 2011). The applied fertilization dose was 180-80-00. Half the nitrogen and all the phosphorus were applied with the pre-sowing irrigation, while the remaining nitrogen was included in the first auxiliary irrigation. As regards days to flowering (DF), a flowering of between 25 and 50% was observed from the sowing date until the appearance of 50% of plant panicles in the plot. The assessment was conducted in June and July. The plots were harvested in order according to the same phenological stage. At the dough stage of grain, about 20 days after flowering, the entire plants were manually cut at ground level. The largest accumulation of sugars in the stem occurs during this stage (Montes *et al.*, 2013).

All plants in the useful plot were cut and the following variables were observed: weight of fresh biomass per plot, and its separate components —whole plant (WTo), stem (WSt), leaf (WLf), and panicle weight (WP). The final data were reported in t ha^{-1}. The ratio to total fresh weight (WSt:WTo) was measured. Extraction was carried out with a 9HP electric artisanal roller mill. The bagasse (WBz) and juice (WJ) were weighed at the end of the extraction. Finally, extraction efficiency (EFx: WJ/WSt) was calculated and the extracted juice volume (JV) was measured in a graduated cylinder. The final data are expressed in L ha^{-1}.

Juice parameters

The juice was extracted with a mill using stems, free of leaves and panicles, of all genotypes. Then it was sieved to remove impurities. Subsequently, the total volume was divided into three subsamples and placed in 250 ml plastic bottles. At the time of extraction, the pH and temperature (°C) of each juice sample were determined using a portable potentiometric pH meter (Hanna® Instruments PHE-HI98127). Brix degrees or soluble sugars were determined with a manual refractometer (REF113/Brix/ATC 0-32).

These data were used to conduct a combined analysis of variance with a randomized complete block design with two factors: genotypes (G=10) and production years (A=2). We used Tukey's means test ($p<0.05$), with the statistical software SAS version 9.3.1.

RESULTS AND DISCUSSION

Environmental conditions

The soil analysis results showed a clay loam texture with pH 7.4, electrical conductivity of 0.25 dSm^{-1}, sodium (580 ppm), and 1.2% organic matter. The macronutrients found in soil were nitrogen (25.8 ppm), phosphorus (19.7 ppm), and potassium (400 ppm); the micronutrients found were Fe (1.92 ppm), Mg (10 ppm), Zn (4.8 ppm), Cu (0.68 ppm), and Bo (0.76 ppm). The soil is slightly alkaline, has a medium-fine texture, no salinity problems and a low organic matter content, with medium levels of nitrogen and phosphorus, a low potassium content, and is deficient in microelements Fe, Cu, and Bo, but with a sufficient level of Zn. The region's soils are characterized by a clay loam texture, are poor in organic matter (<2%), and have a pH close to neutrality (Moreno-Ramos *et al.*, 2014). Sweet sorghum is a crop that adapts to loam to light sandy soils, but grows better in loam and sandy loam soils. It can tolerate a wide range of pH (5.0-8.5) and soil drainage conditions. It can even be cultivated on marginal soils (Zegada and Monti, 2012). The most important element for the growth of sweet sorghum is nitrogen, since the latter is related to the accumulation of biomass in stem and leaf (Olson *et al.*, 2013). Montes-García *et al.* (2013) reported 60 to 62 t ha^{-1} of stem biomass with inorganic nitrogen levels between 60 and 120 kg ha^{-1}. In regions with a minimum temperature of 13.9 °C and a maximum of 36.9 °C, with rainfall levels of 600 to 700 mm, the cultivation of sweet sorghum can yield a production of 70 to 80 t ha^{-1} of fresh biomass (Rao *et al.*, 2013), even without complementary irrigation. The site where the experiments were established, which belongs to the southern region of Sonora, has a virtually constant climate (Moreno-Ramos *et al.*, 2014). During the experiments (2015 and 2016), no rain and a high evaporation were recorded (Table 1). In the arid and semi-arid regions of Arizona, USA, the cultivation of sweet sorghum requires between 900 and 1,300 mm of water per crop cycle. The M81E variety produces 39,000 liters ha^{-1} of juice, can consume up to 1190 mm, and requires 33.4 mm of water per hectare per liter of juice (Martínez-Cruz *et al.*, 2015). These experiments were conducted under irrigation conditions. Therefore, the amount of precipitation was a secondary climatic variable, since precipitation occurred at the end of the cycle. Water availability for irrigation is an important factor for sweet sorghum production in the southern region of Sonora.

Table 1. Climatic conditions of the study area.

	Month							Accumulated
Year 2015	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	
Evaporation (mm)	94.5	121.9	148.3	185.7	195.6	208.5	174.3	1128.8
Rain (mm)	11	21	0	0	3.2	3.9	79.4	118.5
Year 2016								
Evaporation (mm)	104.2	131.8	167.4	201.2	207.3	218.4	179.1	1209.4
Rain (mm)	0.6	16	0	0	4.5	30.6	57.4	109.1
Year 2015								**mean**
Tmax (°C)	27.4	30.7	30.2	33.3	35.7	37.2	36.8	33±1.8
Tmin (°C)	11.3	11.9	12.8	13.4	22.0	25.7	25.5	17.5±2.2
Year 2016								
Tmax (°C)	26.7	27.2	30.8	32.8	36.0	37.1	35.9	32.4±2.1
Tmin (°C)	8.6	9.8	11.1	13.8	21.3	25.5	24.9	16.4±2.3

Juice production according to genotypes

The analysis of variance showed a significant effect of the variation sources on the production of biomass, juice, and sugar. The year or environment explained 53% of the variation, the genotype explained 36%, and the interaction (G×A) only 5%. We observed a significant difference between genotypes regarding fresh biomass and juice production (Table 2). The genetic diversity of sweet sorghum has been widely documented from a morphological and molecular point of view, and it constitutes a genetic resource that differs from grain sorghum in its ability to accumulate biomass and sugar (Mullet *et al.*, 2014). The SWS686, SWS691, and RB-Cañero genotypes exceeded the control in total biomass production by 10 to 18 t ha^{-1}, which represents between 18 and 32%. The same genotypes exceeded the control in juice production by 36%, due to their higher extraction efficiency (Table 2). The control produced almost 50% of bagasse or fibrous fraction. Materials with low fiber percentages are more susceptible to lodging, and to attacks by pests and microorganisms (Souza *et al.*, 2016). In this experiment, we obtained an average of 53±12 t ha^{-1} of total biomass. It has been reported, for example, that the RB-Pirulí variety of sweet sorghum produces large amounts of total fresh biomass, which generate up to 121 t ha^{-1} and 26 thousand L ha^{-1} of juice (Montes-García *et al.*, 2019). The variability between sweet sorghum genotypes in the accumulation of biomass and sugars can be attributed to precocity (Viator *et al.*, 2015; Souza *et al.*, 2016). In these experiments, the genotypes with the longest growing season produced more biomass. Sweet sorghum genotypes were differentiated according to their precocity. The SWS603 genotype was the earliest with 85 days to flowering and 106 days to dough stage. There were no significant differences between the SWS materials 657, 658, 662, 694, and 817, at 98 and 120 days, respectively.

The late genotypes group (SWS686, M81E, RB-Cañero, and SWS691) took between 102 and 118 days to flower, and between 123 and 139 days to dough stage. In this experiment, the plant's leaves and panicle constituted 33% of the total biomass (Table 2)

Table 2. Biomass and juice production. Average data for years 2015 and 2016.

Genotype	Weight fresh (t ha^{-1})						VJ (L ha^{-1})	EFx	RSt:To
	WTo	WSt	WHf	WP	WJ	WBz			
M81E	52 cd	36 b	10 bc	6 bc	9 bc	27 c	7,578 b	0.25 b	0.69
SWS603	44 ef	31 cd	10 bc	7 b	8 cd	23 cd	6,381 c	0.25 b	0.70
SWS657	46 de	31 cd	10 bc	5 bc	9 bc	22 cd	8,055 bc	0.29 ab	0.67
SWS658	54 cd	35 bc	14 ab	9 a	9 bc	27 c	7,428 bc	0.24 b	0.65
SWS662	37 f	23 d	9 c	4 de	7 d	17 d	5,536 c	0.28 ab	0.62
SWS686	60 bc	42 ab	15 ab	3 e	13 a	29 bc	12,788 a	0.33 a	0.70
SWS691	75 a	52 a	18 a	5 cd	14 a	38 a	12,790 a	0.27 ab	0.69
SWS694	65 b	43 ab	15 ab	7 b	13 a	31 abc	10,983 ab	0.30 ab	0.66
RB-Cañero	71 ab	51 a	14 ab	4 de	14 a	38 a	12,827 a	0.26 b	0.72
SWS817	50 cd	35 bc	10 bc	3 e	11 ab	23 cd	9,972 ab	0.33 a	0.70
Means	**55**	**38**	**13**	**5**	**11**	**27**	**9,434**	**0.28**	**0.68**
SD	12	9.2	3.0	1.9	2.8	6.7	2,801	0.03	
CV	4.6	4.1	4.1	2.8	3.8	4.0	3.0	8.6	
Year									
2015	42 B	31 B	8 B	4 B	8 B	23 B	7,560 B	0.26 B	
2016	66 A	44 A	16 A	6 A	13 A	31 A	11,040 A	0.30 A	

Wto: weight of whole plant. Fresh weight: WSt: stem; WLf: leaf; WP: panicle; WJ: juice; and WBz: bagasse. EFx: extraction efficiency. Control (+): M81E. WSt:WTo: stem ratio:total fresh weight. SD: standard deviation. CV: coefficient of variation. Means with different letter differ significantly (Tukey, 0.05).

and were not processed during extraction because they are considered waste, since their inclusion together with the stem produces sugar loss in the juice (Viator *et al.*, 2015). Therefore, the model genotype is the one with the highest proportion of stems. The RB-Cañero and SWS817 genotypes had a stem ratio of 0.70 in relation to the total biomass, with an extraction efficiency of 0.33. On average, we obtained 28% juice extraction. This amount varied between genotypes and years (Table 2). Rao *et al.* (2013) reported differences between agricultural cycles, but not between genotypes, with 44% juice extraction from the stem, free of panicle and leaves. Genotypic differences in the fibrous fraction of the bagasse may be a factor related to grinding efficiency. Li *et al.* (2018) found that the stem's fresh biomass is a complex structure, with anatomical heterogeneity, and even in chemical composition. The average juice production was 9,400±2,800 L ha^{-1} (Table 2). Due to its capacity to adapt to changes in soil moisture in semi-arid regions, the potential of sweet sorghum as an annual crop is important for juice production (da Silva *et al.*, 2019). These data suggest that handling juice volumes on a larger scale implies a technological challenge in planning and operation, during and after juice extraction, for the efficient production of sugars and their derivatives (Aguilar-Uscanga and Montes-García, 2017).

Juice characteristics

The analysis of variance showed a significant effect of the genotype on juice parameters during extraction. These variables are important, since they affect the juice fermentation process. Brix degrees or soluble solids correlate with the amount of sucrose, the main substrate for the sweet sorghum juice fermentation (Dutra *et al*., 2013). It has been observed that sucrose decreases with time in sugar cane juice with pH=3. A temperature increase reduces the amount of sucrose in the juice. In addition, the pH level affects the concentration of sucrose, because the latter is degraded by the action of contaminating bacteria (Arvizu *et al*., 2016). The average values of the stem juice variables, taken at the time of extraction, were 32.6±2.3 (T °C), 3.8±0.7 (pH), 14.9±2.4 (°Brix), as shown in Table 3. The concentration of soluble solids and the pH of the juice can vary significantly between genotypes of sweet sorghum, as observed in this experiment. Dávila-Gómez *et al*. (2011) reported values between 10 and 13.2 °Brix and a pH between 4.43 and 4.85 in sweet sorghum juice.

In these experiments, the pH of the juice averaged 3.8. The SWS 686 genotype reached a pH value of 2.8, 26% lower than the average (Table 3). Sweet sorghum juice has low pH levels (4 to 5); in addition, these values can vary between genotypes (Holou and Stevens, 2012; Freita *et al*., 2014). Temperature and pH affect not only yeast growth, but also enzymatic activity, which is directly related to the efficiency of ethanol production (Lu *et al*., 2017). Significant differences have been reported in ethanol concentration, productivity, and yield at 37 and 40 °C, with pH values between 4 and 6, using sweet sorghum juice (Pilap *et al*., 2018). pH is important because it controls metabolism and affects the composition of microbial communities present in the juice. Even a change in percentage can affect their growth. Few native bacteria in sweet sorghum juice can survive

Table 3. Parameters of sweet sorghum juice. Average data for 2015 and 2016.

Genotype	T (°C)	°Brix	pH
M81E	29 e	14.7 c	3.4 e
SWS603	34 bc	17.7 a	3.5 de
SWS657	32 d	14.6 c	3.7 d
SWS658	33 d	11.5 d	5.2 a
SWS662	36 a	17.8 a	3.5 de
SWS686	33 d	17.0 ab	2.8 f
SWS691	33 d	14.6 c	3.5 de
SWS694	30 e	10.5 d	4.9 b
RB-Cañero	30 e	16.1 bc	4.2 c
SWS817	35 ab	14.7 c	3.4 e
Means	**32.6**	**14.9**	**3.8**
SD	2.3	2.4	0.7
CV	14.4	6.1	5.2
Year			
2015	31.7 B	14.3 B	3.5 B
2016	33.4 A	15.4 A	4.1 A

Control (M81E). SD: standard deviation and CV: coefficient of variation. Means with the same letter differ significantly (Tukey, 0.05).

even at pH 4.7 (Jin and Kirk, 2018). The optimal conditions to produce bioethanol from sweet sorghum are pH 5.5, a temperature of 28 °C, with a maximum theoretical yield efficiency of 0.75 (Ebrahimiaqda and Ogden, 2018).

CONCLUSIONS

Environmental conditions allowed us to identify efficient sweet sorghum genotypes for the production of biomass and juice. There are genotypic differences and seasonal changes in the juice quantity and quality. Bagasse percentage is a genotypic characteristic that affects the amount of juice. The SWS686 and SWS694 genotypes exceeded the average and the control M81E in juice production. Juice production was higher in 2016 (31%) than in 2015.

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