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RESEARCH ARTICLE

Tapping the US Sweet Sorghum Collection to Identify Biofuel Germplasm

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Abstract The narrow genetic base in sweet sorghum [*Sorghum bicolor* (L.) Moench] breeding programs is limiting the development of new varieties for biofuel production. Therefore, the identification of genetically diverse sweet sorghum germplasm in the U.S. National Plant Germplasm System (NPGS) collection is imperative for biofuel breeding programs as biofuel production expands to new regions. Nine-hundred twenty-five sweet sorghum accessions from the NPGS collection were agronomically evaluated and a subset of 56 accessions selected for further evaluation. A 2 year replicated trial of this subset together with 17 U.S. sweet sorghum varieties were evaluated for agronomic and biofuel traits flowering time, plant height, fresh and dry weight, brix, juice volume, percent of moisture, and fermentable sugars [dinitrosalicylic (DNS) method] and disease response [anthracnose (*Colletotricum sublineolum*) and rust (*Purcina purpurea*)]. Nine accessions from the NPGS collection originally from South Africa, Ethiopia, Sudan, Zimbabwe, and the U.S. showed brix values ranging from 10 to 14, with five accessions having a higher amount of fermentable sugars than U.S. references accessions (DNS = 9.86–11.42). Likewise, the total dry matter content of three accessions originally from Ethiopia and U.S. were higher than the U.S. reference accessions (>156.87 g/plant). Multiple new sources of anthracnose and rust resistance were identified; being PI 156424 from Tanzania resistant to both diseases. The results demonstrated that accessions in the NPGS sorghum collection enclose valuable genes/alleles for biofuel traits that are not being used in U.S. biofuel breeding programs. Thus, the integration of these accessions into these programs will aid to increase genetic diversity and development of new biofuel varieties.

Keywords Biofuel · Biomass · Brix · Exotic germplasm · Sorghum bicolor · Sweet sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a C_4 tropical grass with the ability to accumulate large amount of biomass. Sweet sorghum is a subgroup of sorghum germplasm with juicy stalks that accumulate higher concentration of sugars, which can be extracted and utilized in bioenergy production (Vermerris 2008). Compared to other bioenergy crops such as corn, wheat, sugarcane, sugar beet, cassava, sweet potato, etc. (Drapcho et al. 2008), sweet sorghum is drought tolerant, can be cultivated in temperate, subtropical and tropical climates, require low quantity of water (e.g. 1/3 of sugarcane) and has tolerance to salinity (i.e. can be grown in marginal regions that are not commonly used for crop production) (Almodores and Hadi 2009). Presently, sweet sorghum is the most suitable crop for biofuel production worldwide (Rooney et al. 2007).

The potential use of sweet sorghum as a feedstock for biofuel production dates back to approximately 40 years in the United Sates (Jackson et al. 1980), Europe (Dalianis

1997) and Japan (Hoshikawa et al. 1988) after the oil embargo of the 1970s. Later, India explored the potential of sweet sorghum from cultivation to ethanol in tropical dry land (Rajavanshi and Nimbkar 1997). Presently, India and other Asian countries are using sweet sorghum as a source of ethanol, while research is still ongoing in other developed countries to incorporate it as bioenergy feedstock. The goal of deriving up to 30 % of transportation fuels from renewable sources by 2030 by the European Union, U.S., and China has catapulted the evaluation of sweet sorghum as a promising source of ethanol. Therefore, understanding the physiological process involved in sugar accumulation and increase dry matter content, improve the means of stabilizing the juice to minimize sugar loss during storage, and the development of new, high sugar varieties particularly for bioenergy production are some of the priority research areas to make sweet sorghum a viable biofuel source (Regassa and Wortmann 2014).

The sweet sorghum collection of the US National Plant Germplam System (NPGS) is the primary source of genetic diversity for the development of new sweet sorghum varieties for biofuel production with approximately 2,180 accessions in the collection (Pederson and Spinks 2006). Nevertheless, few sweet sorghum varieties have been evaluated for biofuel in recent years. Ali et al. (2008) studied the genetic diversity and relationship among 68 sweet sorghum cultivars and breeding lines cultivated in the U.S. employing 41 simple sequence repeat markers (SSRs), and phenotypic traits such as plant height (PH), flowering and brix. Cluster analysis grouped these accessions into ten distinct groups and indicated that varieties with high sugar content were genetically similar. An assessment of the population structure and genetic diversity present in NPGS sweet sorghum collection based on 96 accessions and 95 SSRs markers indicated the presence of four main clusters which resemble the pattern of African sorghum dispersion (Wang et al. 2009), indicating narrow genetic diversity. Recently, a sweet sorghum panel of 125 diverse accessions was separated into three main groups: (1) amber types, (2) historical and modern syrup types and (3) modern sugar and energy types (Murray et al. 2009). Moreover, genetic analysis of sweet sorghum cultivars developed in U.S. determined that all cultivars were derived from six genetically similar African landraces (MN960, MN1048, MN1054, MN1056, MN1060, and MN1500) (Murray et al. 2009; Ritter et al. 2007). These studies highlight the narrow genetic diversity present among improved sweet sorghum varieties, and the necessity of identifying and evaluating additional germplasm for the development of new bioenergy sorghum cultivars. In this regard, there may be additional sweet sorghum accessions in the NPGS collection that have not yet been identified and evaluated.

The development of new bioenergy sorghum cultivars involve the modification of many factors such as biomass yield, cell wall composition, type of sugar, lignin content, disease resistance, etc. (Saballos 2008). For instance, there are number of foliar diseases that affect grain sorghum with small yield losses, but will become more prominent in new sorghum energy types. For instance, foliar diseases such as rust (*P. purpurea* Cooke, and *P. prunicolor*) and anthracnose (*Colletotrichum sublineolum*) can cause losses of seed yield of up to 50 % in severely affected fields (Thakur and Mathur 2000) but their losses are expected to be larger in accessions grow for biomass and biofuel production. Moreover, these diseases can be observed on all above ground tissues of the sorghum plant (Thakur and Mathur 2000; Frederiksen and Odvody 2000), and can alter sorghum juiciness, reduce the amount of sugar and contribute to lodging. The use of host plant resistance is the most practical and reliable form to manage these diseases, in this regard, the resistance genes found in sweet sorghum germplasm are the primary resistance source to be used in biofuel breeding programs. In this study, 925 sweet sorghum accessions from the NPGS collection were visually evaluated for agronomic traits in order to identify a subset of accessions with biofuel traits. Fifty five accessions were selected and its biofuel performance compared with 17 sweet sorghum varieties used in U.S. sweet sorghum breeding programs and commercial fields (Wang et al. 2009).

Materials and Methods

Germplasm Material

Nine-hundred twenty five sorghum accessions from the U.S. NPGS collection were selected based on available information that these accessions were sweet sorghum germplasm. The accessions were grown at the USDA-ARS Tropical Agriculture Research Station experimental farm at Isabela, Puerto Rico during the wet season (September–December) of 2009 using standard management practices. These accessions were visually inspected at maturity for bioenergy associated traits such as height, disease resistance, lodging, and stalk juiciness and sweetness [organoleptic evaluation] in a completely randomized experimental design. Fifty-six accessions with sweet and juicy stalks and PH greater than 1.8 m were selected for further replicated evaluations. In addition, 15 accessions that showed high sugar content [Brix values from 13.5 to 19.42 %] at Manhattan, Kansas, U.S. (Wang et al. 2009) and are being employed in biofuel breeding programs were included in the evaluation, as well as two U.S. traditional sweet sorghum cultivars [Rio (PI 651496) and HoneyNo2 (PI 562716)] (Table 1).

Table 1 Plant introduction number, name, place of origin and germplasm classification of 73 sweet sorghum accession evaluated for biofuel components during the wet season of 2011 and 2012, at the USDA Research Station, Isabela, Puerto Rico

PI	Name	Origin[a]	Type	PI	Name	Origin[a]	Type
144133	MN394/Haakdoorn	ZAF	Cultivar	511355*	Smith	USA	Advanced
144331	MN400/Isidomba	ZAF	Cultivar	525013	MW262	MWI	Cultivar
144335	MN404/Ufutane	ZAF	Cultivar	525041	MW515	MWI	Cultivar
146890	MN591/Sugar Drip	COG	Cultivar	533998*	Brawley	USA	Advanced
147172	MN467/Rahi Jowar	IND	Cultivar	535783	N98	USA	Advanced
152593	MN732/Ankolib black	SDN	Cultivar	562267	FAO 55049	SDN	Cultivar
152747	MN876/Nyan bau	SDN	Cultivar	562716[+]	HoneyNo2	USA	Advanced
152881	MN984/Lwel Kochung	SDN	Cultivar	566819	Della	USA	Advanced
152914	MN1152/Waxi club	USA	Cultivar	583832*	Top 76-6	USA	Advanced
153874	MN1280/Katemu	KEN	Cultivar	586541*	Tracy	AUS	Cultivar
155518	MN1707/Misali	ZMB	Cultivar	641815*	Early Folger	USA	Cultivar
155555	MN1751/Maila	ZMB	Cultivar	641821*	Honey Drip	USA	Cultivar
155631	MN1837/Mbikiloni	ZMB	Cultivar	641834*	Planter	USA	Cultivar
155642	MN1850/Marangomwa	ZMB	Cultivar	641835*	Rex	USA	Advanced
155672	MN1882/Misali	MWI	Cultivar	641838	Saccaline	USA	Cultivar
155676	MN1886	MWI	Cultivar	641855	MN41	USA	Advanced
155755	MN1968/Kapire	MWI	Cultivar	641862*	MN715/Collier	USA	Cultivar
156140	MN1757/Maila	ZMB	Cultivar	643003	MN2720/Variety A	USA	Cultivar
156180	MN2016/Neseguku	MWI	Cultivar	643466	MN4548	USA	Cultivar
156210	MN2102/Mckotta	MWI	Cultivar	648068	MN3373	USA	Cultivar
156358	MN2244	ZMB	Cultivar	648080	MN3456	USA	Advanced
156424	MN2305/Movari	TZA	Cultivar	648087	MN3463	USA	Advanced
156699	MN2421	KEN	Cultivar	648091	MN3467	USA	Advanced
183001	MN2946/Ghaonla	IND	Cultivar	648098	MN3479	USA	Advanced
196073	MN3077	ETH	Cultivar	648114	MN3528	USA	Advanced
197548	MN3104	DZA	Cultivar	648118	MN3532	USA	Advanced
19770	MN258/Collier	ZAF	Cultivar	648161	MN3751	USA	Advanced
198885	MN3131/Sweet Saccaline	AUS	Cultivar	648184	MN4111	MMR	Cultivar
257602	No.8 Gambela	ETH	Cultivar	648206	MN4552/MO Gray Top	KEN	Cultivar
267124	Orange	MEX	Cultivar	651493*	Ramada	USA	Advanced
30204	MN2705/Japanese Dwarf Broomcorn	USA	Advanced	651495*	Dale	USA	Advanced
454500	ETS 3080	ETH	Cultivar	651496[+]	RIO	USA	Advanced
455286	ETS 3488	ETH	Cultivar	651497*	Theis	USA	Advanced
500958	ZM-1038	ZMB	Cultivar	653411*	M81-E	USA	Advanced
500989	ZM-1325	ZMB	Cultivar	653616*	Wray	USA	Advanced
500990	ZM-1328	ZMB	Cultivar	653617*	Keller	USA	Advanced
501079	ZM-2476	ZMB	Cultivar				

[a] Three letter country code

* Accessions selected from Wang et al. (2009)

[+] Control accessions

Experimental Design

The 73 sweet sorghum accessions were evaluated at the USDA-ARS Tropical Agriculture Research Station experimental farm at Isabela, Puerto Rico during the wet season of 2011 and 2012. The 2011 experiment was planted in August, while the 2012 experiment was planted in October to reduce the photoperiod response of the accessions. The experimental design in both years was a randomized block design (RCBD) with three blocks. Seed from each accession were planted in a single row of 1.8 m of length with 0.9 m spacing between rows. Plants were maintained using standard management practices, and weeds were controlled with mechanical tillage and hand hoeing.

Phenotypic Evaluation

Agronomical Traits

Flowering time (FL), PH, plant and mid-rib color were recorded for each accession/plot. FL was defined as the numbers of days when 50 % of the plants within a row reached anthesis. PH refers to the distance from the base of the plant (i.e. soil) to the top of the panicle recorded at maturity and represents the average height for three plants within an accession/plot. Plant and mid-rib color classification were according to the Sorghum Crop Germplasm Committee (SCGC), and both were recorded at maturity.

Biofuel Traits

Biofuel traits were evaluated at physiological maturity, although few late flowering accessions were evaluated at the hard dough stage. The traits evaluated were plant lodging (PL), fresh plant weight (FrW; leaves and stalks), dry weight (DrW), extracted juice volume (JVol), brix (soluble solids content), percent moisture (PMo), and total sugar (TSu). PL per plot was measured using a 1–10 scale where: 1 = no lodging and 10 = 100 % lodged (refer to plants no longer upright). FrW refers to the average weight of three representing plants from each accession/plot cut within 3 cm of the ground. These three plants were dried in an oven at 70 °C until reaching a constant weight to determine the DrW per plot. PMo was determined by the equation: [(DrW)/(FrW)] ×100. Brix was measured using a handheld refractometer (Atago U.S.A. Inc., Bellevue, WA). In 2011, the brix refer to the average measure of three plants per plot pressed at the bottom, middle and top of the stem, while in 2012 the brix was determined from the total juice obtained from three plants pressed with a three-roller sugarcane mill (Raja-1, US Ice Machine Manufacturing Co. FL, USA). JVol refers to the total juice obtained from three plants pressed in 2012 and the TSu was determined by multiplying JVol and brix values.

DNS Analysis

Brix is a measure of the mass ratio of soluble solids to water, and for sorghum is highly correlated to TSu content (sucrose, glucose, fructose) obtained from the stalk (Kawahigashi et al. 2013). Nevertheless, reduced sugars (i.e. sucrose) are the most favorable for fermentation to ethanol (Smith et al. 1987). The dinitrosalicylic (DNS) method (Miller 1959) was used to quantify reducing sugars in sorghum juice samples from 2012 as implemented by Vandenbrink et al. (2010). The DNS solution contained (per liter): 10 g dinitrosalicylic acid, 0.5 g sodium sulfite, and 10 g sodium hydroxide. First, sorghum juice samples were diluted tenfold with double distillated water and 50 µl of the diluted sample was combined with 50 µl DNS solution in a 96 well PCR plates. Standard curves of 12 different sucrose concentrations (0.025–1 mg/µl) were included in each plate. The plates were heated at 90 °C for 10 min to promote color change, and later 17 µl of 40 % potassium sodium tartrate were added to each well to stop the color change reaction. Last, 100 µl of each sample was transferred to ELISA plate, and the absorbance was measures at 565 nm using a plate reader (BioRad Ultramark Microplate Imagine System). The quantity of reducing sugar in the juice sample was quantified by comparing its absorbance with the obtained from the standard sucrose curve.

Statistical Analysis

Year data for each experiment were initially combined to perform analyses of variance (ANOVA) using the *Proc mixed covtest* method *type 3* procedure of SAS. Variance components were estimated employing restricted maximum likelihood (REML), and each variance tested for significance using the likelihood ratio statistic (Littell et al. 1996). The linear effect model for such ANOVA was the following: $Y = \mu + L + B(L) + P + L \times P + e$; where Y is the trait (FL, PH, PL, FrW, DrW, brix, and PMo), µ is the common effect, L is the year effect, $B(L)$ is the block within location effect, P is the effect of accession, $L \times P$ is the year × accession interaction and e is the plot to plot variation within accessions. The year effect (L) was considered fixed, while others effects and interaction were treated as random effects. Likewise, each year experiment was analyses independently for traits having one year data (JVol, TSu, DNS) and/or significant interaction among year and accession ($L \times P$). The linear random effect model for such ANOVA was the following: $Y = \mu + B + B(P) + P + e$; where B and $B(P)$ are the block effects and the block × accession interaction, respectively.

In order to access whether year × accession ($L \times P$) interactions were due to trait magnitude changes between years or changes in the ranking performance of accessions, Spearman correlation coefficients (r_s) were calculated using accessions data for FL, PH, FrW, DrW, PMo, and brix across year according to Yan and Rajcan (2003). When the correlation coefficient between data across years was ≤ 0.5, L × P interactions were considered more likely to be due by ranking changes, and when $r_s \geq 0.5$, L × P interactions were considered more likely to be due to trait magnitude changes between years.

Performances of the accessions were determined by least-squares means and comparisons using the Tukey–Kramer P value adjustment for multiple comparisons in

SAS. The variability of each trait was estimated based on means, range, coefficients of variation, and phenotypic and genotypic coefficient of variation. Phenotypic and genotypic coefficients of variation (PVC and GCV, respectively) were estimated according to Singh and Chaudhary (1977), and refer to the square root of the phenotypic and genotypic variance divided by the trait mean. Phenotypic correlation between biofuel traits (FL, PH, FrW, DrW, brix, PMo, JVol, and DNS) were calculated as Pearson coefficients.

Diseases Resistance Response

Anthracnose

The inoculation and disease assessment methods were similar to those described by Prom et al. (2009). Briefly, fungal cultures were prepared with five different isolates of *C. sublineolum*, which represent the pathotypes present at the Isabela experimental farm, and used to colonize sorghum seeds. Subsequently, the sorghum plants were inoculated 30 days after planting by placing approximate 10 *C. sublineolum*-colonized grains into the leaf whorls. Disease assessments were conducted before harvesting, and the ratings were based on a scale 1–5, where 1 = no symptoms or chlorotic flecks on leaves; 2 = hypersensitive reaction on inoculated leaves but not acervuli in the center; 3 = lesions on inoculated leaves but no acervuli formation and no symptoms observed on other leaves; 4 = necrotic lesions with acervuli observed on inoculated and bottom leaves with the infection spreading to middle leaves; 5 = most leaves necrotic due to infection including infection on the flag leaf. This rating system was then categorized into resistant (rated as 1 and 2) and susceptible (rated as 3–5) reaction classes. The inbred lines BTx623 and SC748-5 were randomly distributed in experimental field as susceptible and resistant checks, respectively.

Rust

Disease assessment was based on the rust severity of leaves from the middle of the stalk to the top of the plant. Rust severity was based on a scale of 1–5; where 1 = no rust, leaves free of disease; 2 = 1–10 % leaf area infected; 3 = 11–40 % leaf area infected; 4 = 41–65 % leaf area infected; 5 = 66–100 % leaf area infected (Wang et al. 2006). This scale was further categorized into three reaction classes: 1 or 2 are considered resistance, accessions rated 3 are considered moderately resistant, while accessions rated as 4 or 5 are susceptible. The accession PI 609251 from Mali, and the inbred lines BTx623 and Sureño were randomly distributed in experimental field as susceptible and resistance checks, respectively.

Results

Phenotypic Evaluation

Variance component analysis indicated significant ($P \leq 0.05$) main effects of accessions and year × accessions interactions for FL, PH, FrW, DrW, PMo, and brix (Table 2). Likewise, results of variance components analyses conducted independently for each year indicated significant ($P \leq 0.05$) accession effects for all evaluated traits. The largest variance components of FrW, DrW, PMo, and brix belong to the accessions effects. Year × accession ($P \times L$) interaction and residual effect were the largest variance components for FL and PH, respectively. Coefficient of variation ranged from 5.81 (flowering) to 29.82 (fresh weight) in the combined year analysis (Table 2). In 2011, coefficient of variation ranged from 3.16 (FL) to 32.64 (FrW), and from 8.39 (FL) to 39.14 (DNS) in 2012 (Table 3). Spearman correlation (r_s) between years indicated that the interaction between year and accessions was mainly due to changes in trait magnitude for PH (0.60), FrW (0.61), DrW (0.64), and Brix (0.67); and to ranking changes for FL (0.33) and PMo (0.37).

Given that accessions performance differed between years and significant accessions x year interactions effects were detected, data are hereafter presented by year. The means of FL, PH, FrW, and DrW were higher in 2011 than 2012 (Table 3). In 2011, FL ranged from 60 to 101 days with a mean of 78 days, while in 2012 ranged from 51 to 91 days with a mean of 64 days. The mean of PH were 277.03 and 222.35 cm in 2011 and 2012, respectively. The lowest PH were similar in both years (90.0 and 93.33 cm, respectively), however, maximum PH values were 376.3 and 334.7 cm during 2011 and 2012, respectively. The FrW averaged 471.0 g/plant and ranged from 54.12 to 1411.0 g/plant during 2011, and averaged 345.49 g/plant and ranged from 71.0 to 946.0 g/plant in 2012. The 2011 DrW was higher than in 2012 (79.49 vs. 57.92 g/plant, respectively), and varied from 8.33 to 268.67 g/plant in 2011, and from 16.0 to 218.33 g/plant in 2012. For brix, the average and range were consistent across years. In 2011, brix varies from 3.61 to 17.54 with an average of 9.14, and in 2012 varied from 3.17 to 17.80 with an average of 9.64. The DNS values were in accordance with brix, being 2.31 and 14.63 the lowest and highest values with an average of 6.60. The JVol ranged from 4.33 to 216.78 ml/plant with an average of 79.76 ml/plant, and TSu ranged from 18.6 to 4,513.50 g/plant with an average of 785.07 g/plant. PL differed among the 73 sweet sorghum accessions. In 2011 and 2012, 19 and 15 accessions, respectively, had lodging plant within the plot. Seven accessions (PI 147172, PI 152747, PI 155631, PI 501079,

Table 2 Variance component analysis for flowering time, plant height, fresh and dry weight, moisture and brix for the analysis of 73 sweet sorghum accessions evaluated at the USDA Research Station, Isabela, Puerto Rico, during the wet season of 2011 and 2012

	Flowering[a]	Height[b]	Fresh weight	Dry weight	Moisture	Brix
Year	0.28***	0.90***	27.24**	0.00**	0.00	0.00
Year (block)	1.44***	40.52*	96.06	0.46	0.03	0.04
PI	14.33***	1,278.44***	16,562.00***	655.05***	5.48***	6.57***
PI*year	28.72***	640.25***	4,252.64**	294.24***	3.48***	1.35***
Residual	17.04	1,316.68	14,840	332.83	6.96	2.40
CV	5.81	14.59	29.82	26.59	15.60	16.50
R^2	0.89	0.79	0.76	0.85	0.72	0.85
r_s	0.33**	0.60***	0.61***	0.64***	0.37**	0.67***

[a] Flowering refers to days to 50 % flowering of the plot

[b] Plant height refer to the distance from the base of the main stalk to the top of the panicle, CV refers to coefficient of variation

*, ** and *** refers to significant effects at $P \leq 0.05$, 0.01, and 0.001, respectively

Table 3 Agronomical performances of 73 sweet sorghum accessions evaluated for biofuel components during the wet season of 2011 and 2012 at the USDA Research Station, Isabela, Puerto Rico

Trait	2011							2012						
	Means	S.D.[a]	Min.[b]	Max.[c]	CV[d]	PCV[e]	GCV[f]	Means	S.D.[a]	Min.[b]	Max.[c]	CV[d]	PCV[f]	GCV[f]
Flowering time[g]	77.61	8.05	60.67	97.56	3.16	10.42	10.26	64.34	5.89	53.17	78.56	8.39	8.96	7.55
Plant height (cm)[h]	274.06	52.45	96.67	376.34	17.26	19.71	17.06	222.79	41.11	101.11	334.73	7.95	18.40	17.82
Fresh weight (g)	476.71	177.57	78.11	1,052.89	32.64	37.09	31.93	346.19	142.74	80.00	759.55	21.45	39.11	37.01
Dry weight (g)	80.52	39.10	13.89	200.00	28.20	48.82	46.08	58.10	25.79	18.88	151.78	21.98	41.67	39.79
Brix	9.13	2.87	3.61	17.54	16.03	31.52	30.13	9.60	3.07	3.17	17.80	16.97	31.56	30.04
% moisture	16.54	3.73	9.43	26.24	17.75	20.82	18.10	16.92	3.43	7.05	32.02	13.02	18.59	17.09
Volume (ml)[i]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	79.76	42.46	4.33	216.78	31.70	54.11	50.90
Total sugar (g)[j]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	785.07	580.62	18.6	4,513.5	35.90	71.51	68.48
DNS[k]	n.a	n.a	n.a	n.a	n.a	n.a	n.a	6.60	3.25	2.31	14.63	39.14	49.59	44.17

[a] S.D. refers to standard deviations

[b] Minimun observed value

[c] Maximun observed value

[d] Coefficient of variation

[e] Phenotype coefficient of variation

[f] Genetic coefficient of variation

[g] Flowering time refers to days to 50 % flowering of the plot

[h] Plant height refers to the distance from the base of the main stalk to the top of the panicle

[i] Volume refers to the average juiciness per plant

[j] Total sugar per plant was determined as the product of Brix value × Juicy Volume/plant

[k] 3,5 Dinitrosalicylic acid to estimate amount of reducing sugars in g/ml; n.a data was not obtained

PI 641834, PI 648080, and PI 648114) had lodged plants within the plot in both years, and a total of 27 accessions had lodged plants within the plot at least one of the years.

Coefficients of variations varied across years (Table 3). The lowest coefficient of variation in 2011 was for FL (3.16), followed by brix, PH, PMo, DrW and FrW (16.03, 17.26, 17.75, 28.20 and 32.64, respectively). In 2012, the lowest coefficient of variation was in PH (7.95) later by FL, PMo, brix, FrW, DrW, JVol, TSu, and DNS (8.39, 13.02, 16.97, 21.45, 21.98, 31.7, 35.9, and 39.14, respectively). The PCV and GCV were consistent across years (Table 3), and the largest difference between both values was observed for FrW (5.16) in 2011. Traits evaluated only in 2012 showed high PCV [TSu (71.51), TVol (54.11) and DNS (49.59)], and the largest difference between PCV and GCV was observed for DNS (5.42).

Phenotypic correlations among FL, PH, FrW and DrW were observed during both years (Table 4). Brix was

Table 4 Pearson correlation coefficients between biofuel traits among 73 sweet sorghum accessions evaluated during the wet season of 2011 (upper diagonal) and 2012 (lower diagonal), at the USDA Research Station, Isabela, Puerto Rico

	Flowering[a]	Plant height[b]	Fresh weight	Dry weight	Brix	% moisture[c]	Volume[d]	DNS[e]
Flowering[a]		0.65***	0.68***	0.73***	0.02	0.43***	*n.a*	*n.a*
Plant height[b]	0.45***		0.71***	0.76***	0.13	0.39***	*n.a*	*n.a*
Fresh weight	0.43***	0.77***		0.86***	0.07	0.21	*n.a*	*n.a*
Dry weight	0.40***	0.72***	0.82***		0.21	0.64***	*n.a*	*n.a*
Brix	0.28*	0.17	0.25*	0.27*		0.38***	*n.a*	*n.a*
% moisture[c]	−0.06	−0.14	−0.30**	0.25*	0.12		*n.a*	*n.a*
Volume[d]	0.34**	0.66***	0.90***	0.55***	0.18	−0.47***		*n.a*
DNS[e]	0.07	0.04	0.14	0.00	0.39***	−0.25*	0.19	

[a] Flowering time refers to days to 50 % flowering of the plot

[b] Plant height refers to the distance from the base of the main stalk to the top of the panicle

[c] Percent moisture determined as (dry weight/fresh weight) × 100

[d] Volume refers to the average juiciness per plant

[e] 3,5 Dinitrosalicylic acid to estimate amount of reducing sugars in g/ml

*, **, *** refers to significant at the < 0.05, < 0.01, and < 0.001 probability levels, respectively

positively correlated with PMo (0.38) in 2011, and with FL (0.28), FrW (0.25), DrW (0.27) and DNS (0.39) in 2012. The PMo was positively correlated to FL (0.43), PH (0.39), and DrW (0.64) in 2011, however, was negatively and positively correlated to FrW (−0.30) and DrW (0.25), respectively, in 2012. The JVol was positively correlated to FL (0.34), PH (0.66), FrW (0.90), and DrW (0.55), and negatively to PMo (−0.47). The correlation between DNS and PMo was negative (−0.25).

Twenty eight accessions had higher brix and DrW values than the two references accessions (Rio and Honey-No2) in both years. In 2011, brix and DrW could be divided into 21 and 12, respectively, based on means comparisons using the Tukey-Kramer test. The highest brix group included 8 accessions (PI 653617, PI 653616, PI 641815, PI 643003, PI 648098, PI 500990, PI 648091, and PI 535783) with values ranging from 12.5 to 17.54, while the highest DrW group included 11 accessions (PI 648068, PI 454500, PI 455286, PI 586541, PI 155642, PI 562267, PI 155631, PI 156699, PI 155755, PI 196073, and PI 155518) with values ranging from 122.70 to 200. In 2012, brix and DrW could be divided into 8 and 7 groups, respectively. The highest brix group included 25 accessions with values from 10.9 to 17.80, while the highest DrW group included 5 accessions (PI 586541, PI 455286, PI 653616, PI 648088 and PI 648080) with values from 96.39 to 151.80. Remarkably, two accessions (PI 653616 and PI 455286) from U.S. and Ethiopia, respectively, combined high brix (>10.0) and DrW (>112) during both years evaluations (Table 5). In addition, other nine accessions (PI 653617, PI 144335, PI 155518, PI 648080, PI 643003, PI 648098, PI 648091, PI 155555, and PI 562267; Tables 5 and 6) have great biofuel potential due to their consistent results across years (brix > 10.0 and DrW > 67 in 2011; brix > 10.0 and DrW > 60) (Table 5).

Disease Resistance

The anthracnose and rust response differed among the 73 sweet sorghum accessions. Anthracnose resistance response was observed in 47 and 28 accessions in 2011 and 2012, respectively. Nineteen accessions rated as resistant in 2011 were susceptible in the 2012 rating evaluation, while, two accessions rated as susceptible in 2011 were resistant in the 2012 rating evaluation. Hypersensitive reactions (i.e. Score = 2) was observed in all resistant accessions being red and red/purple the most frequent plant colours. Rust resistance responses were observed in 22 and 13 accessions in 2011 and 2012, respectively. Nine accessions rated as resistant in 2011 were susceptible in the 2012 evaluation. Five accessions (PI 156424, PI 566819, PI 535783, PI 653616 and PI 651493) showed resistance to both anthracnose and rust during both years.

Discussion

The development of bioenergy crops is an important objective for many nations. In the United States, the majority of ethanol production for bioenergy is from grain, mainly corn and to lesser extent, sorghum. Because these are food and feed grains, alternative sources are being evaluated (Godoy and Tesso 2013). Sweet sorghum is a source of easily fermentable sugars, produces large biomass, has a short growing cycle, is tolerant to drought, and capable of growing in marginal soils making it a desirable

Table 5 Performance of reference accessions (HoneyNo2 and Rio) and 11 superior sweet sorghum accessions identified from 73 accessions evaluated for biofuel traits during the wet season of 2011 and 2012 at the USDA Research Station, Isabela, Puerto Rico. Different letters indicate the accessions are significantly different at $P \leq 0.05$ by Tukey–Kramer test

PI	Origin[a]	Sugars (g)[a]	Volume (ml)[b]	DNS[c]	Brix		Dry matter (g)		Fresh tissue (g)		Plant height (cm)[d]	
		2012	2012	2012	2011	2012	2011	2012	2011	2012	2011	2012
HoneyNo2	USA	0374.81 e–i	061.11d–i	05.71 a–f	05.26 p–u	06.13 g–j	037.6 g–l	028.44 g–i	307.83 b–e	211.67 e–h	210.11 c–h	202.33 e–j
RIO	USA	0215.81 g–i	023.89 g–i	02.45 f	06.53 k–u	09.03 c–j	052.33 d–l	030.56 g–i	334.72 b–e	157.78 g–h	187.78 f–h	144.22 j–m
PI_653616*	USA	3,426.35 a	192.28 a-c	04.01 b–f	15.69 a–b	17.80 a	111.88 b–j	115.24 a–c	487.33 b–e	708.44 a–c	290.00 a–g	247.23 b–g
PI_144335	ZAF	1,991.73 b	197.22 a–b	09.86 a–f	11.27 b–m	10.13 b–h	112.67 b–i	091.33 b–e	646.78 a–e	680.77 a–b	312.22 a–g	280.00 a–c
PI_648080	USA	1,824.47 b–c	143.33 a–d	10.95 a–f	11.54 b–k	12.70 a–d	088.89 c–l	096.39 b–d	553.46 a–e	561.56 a–d	291.67 a–g	284.44 a–b
PI_643003	USA	1,793.77 b–c	130.00 a–e	10.76 a–f	13.77 a–d	14.00 a–b	108.66 b–j	072.06 c–g	565.00 a–e	462.45 a–f	275.56 a–g	235.00 b–g
PI_455286	ETH	1,587.00 b–d	153.11 a–d	05.31a–f	10.63 b–m	10.55 b–h	156.87 a–c	127.04 a–b	674.86 a–e	731.41 a	376.11 a–b	334.73 a
PI_648098	USA	1,382.90 b–e	109.44 b–h	04.97 b–f	13.15 a–d	12.67 a–d	067.11 c–l	069.89 c–g	367.06 b–e	429.89 a–g	276.61 a–g	252.78 b–g
PI_648091	USA	1,362.03 b–e	134.78 a–d	11.42 a–e	12.67 a–d	10.23 b–h	081.67 c–l	067.11 c–g	752.33 a–c	419.56 a–g	268.00 b–g	248.89 b–g
PI_653617*	USA	1,213.50 b–g	069.06 d–i	04.84 b–f	17.54 a	17.40 a	112.22 b–j	070.00 c–g	497.89 b–e	362.72 d–h	312.50 a–g	247.39 b–g
PI_155518	ZMB	1,022.43 b–i	096.67 b–h	10.14 a–f	10.05 c–m	10.60 b–h	122.70 a–h	071.22 c–g	672.36 a–e	416.11 a–g	354.44 a–d	271.67 a–d
PI_155555	ZMB	0934.43 b–i	087.22 c–i	10.85 a–f	11.79 b–j	10.63 b–h	117.78 b–h	062.22 d–g	582.89 a–e	386.00 d–h	293.33 a–g	202.78 e–j
PI_562267	SDN	0925.10 c–i	088.33 c–i	05.45 a–f	11.70 b–j	10.13 b–h	135.22 a–d	059.89 d–g	677.67 a–d	350.56 d–h	335.28 a–g	246.44 b–g

[a] Total gram of sugar per plant was determined as the product of Brix value × Juicy Volume/plant

[b] Volume refers to the average juicy per plant

[c] 3,5 Dinitrosalicylic acid to estimate amount of reducing sugars in g/ml

[d] Plant height refers to the distance from the base of the main stalk to the top of the panicle

* refers to advanced germplasm materials

Table 6 Mean agronomic performance of controls (HoneyNo2 and Rio) and 11 superior sweet sorghum accessions identified from 73 accessions evaluated for biofuel traits during the wet season of 2011 and 2012 at the USDA Research Station, Isabela, Puerto Rico. Different letters indicate the accessions are significantly different at $P \leq 0.05$ by Tukey–Kramer test

PI	Origin[f]	Flowering[a]		Anthracnose[b]		Rust[c]		Lodging[d]		Plant color[e]	Mid rib[e]
		2011	2012	2011	2012	2011	2012	2011	2012	2011	2011
HoneyNo2	USA	81.33 b	65.78 abcd	4.5	5.0	2.0	5.0	1	1	R	Y
RIO	USA	75.67 bcd	58.56 cd	2.0	4.7	5.0	5.0	1	1	R/P	CL
PI_653616*	USA	77.55 bcd	69.00 abcd	2.0	2.0	1.0	1.3	1	1	P	CL
PI_144335	ZAF	81.67 b	68.67 abcd	2.3	4.3	3.3	4.3	3	1	R/P	CL
PI_648080	USA	75.89 bcd	76.94 abc	2.0	2.0	3.7	5.0	7	3	R/P	CL
PI_643003	USA	79.89 bc	78.56 a	2.0	2.0	3.3	5.0	1	1	P	CL
PI_455286	ETH	93.28 a	76.00 abc	2.0	2.0	2.3	5.0	1	1	R	CL
PI_648098	USA	74.44 cd	68.22 abcd	2.0	2.0	3.7	5.0	5	1	R/P	CL
PI_648091	USA	72.11 d	61.44 abcd	2.0	3.0	4.3	5.0	8	1	R/P	CL
PI_653617*	USA	79.56 bc	77.50 ab	2.0	2.0	3.0	5.0	4	1	P	GY
PI_155518	ZMB	82.44 b	66.89 abcd	2.0	3.0	1.0	1.3	1	1	R/P	GR
PI_155555	ZMB	79.00 bc	59.67 bcd	2.0	2.0	3.0	4.3	1	1	R/P	CL
PI_562267	SDN	75.67 bcd	55.11 d	3.0	4.7	3.0	4.7	1	1	R/P	CL

[a] Flowering time refers to days to 50 % flowering of the plot

[b] Anthracnose resistance responses according to Prom et al. (2009) based on a scale of 1–5; where 1 = no symptoms; 2 = hypersensitive reaction; 3, 4 and 5 = necrotic lesion with acervuli in 30, 60, and 100 % of the leaves from bottom to top of the plant

[c] Rust resistance response according to Wang et al. (2006), based on a scale of 1–5; where 1 = no rust; 2 = 1–10 % leaf are infected; 3 = 11–40 % leaf are infected; 4 = 41–65 % leaf are infected; 5 = 66–100 % leaf area infected

[d] Lodging based on a scale of 1–10; where 1 = no lodging in the plot; 10 = 100 % of lodging in the plot

[e] Plant and midrib color according to the Sorghum Crop Germplasm Committee (SCGC); *R* red, *P* purple, *GR* green, *Y* yellow, *GY* green yellow, and *CL* colorless

[f] Three letter country code

alternative bioenergy crop. Sweet sorghum has been used to produce syrup and molasses on a small scale in the United States for over 150 years and in the 1970s was evaluated for granule sugar production (Broadhead 1972). The sugar content of the juice extracted from the stalks of sweet sorghum can range from 5 to 23 % (Saballos 2008). The production of fermentable sugars vary between varieties and across environments (Olweny et al. 2013) and breeding programs are underway to develop new varieties (Umakanth et al. 2012; Olweny et al. 2013; Rooney et al. 2007).

The genetic diversity present in the NPGS sweet sorghum germplasm collection is crucial for the development and improvement of biofuel breeding programs. For instance, for the 925 accessions evaluated for key bioenergy traits, 535 of these accessions had a juicy and a sweet tasting stalk. From this group, 56 accessions had brix values ranging from 3.17 to 14.20. Most of the accessions had similar or higher values as compared to the sweet sorghum line Rio, which has been used as a parent in sweet sorghum breeding programs for the development of high sugar varieties [e.g. Wray (Broadhead et al. 1981), Keller (Broadhead 1982), etc.]. In fact, previous inheritance studies indicate additive gene action for brix suggesting this trait can be improved through breeding (Umakanth et al. 2012). In this regard, the high brix accessions identified herein originally from Ethiopia, Sudan, Zimbabwe, South Africa and USA, also include the sorghum race Durra, Guinea, and Kafir-bicolor, thus, it is highly probable that they should enclose different genes/alleles for brix. Hence, the incorporation of these lines into breeding programs can benefit the development of new high sugar varieties. Moreover, since brix values were similar across years, this would suggest a large scale evaluation of NPGS sweet sorghum collection would be desirable to identify new high brix genes/alleles.

The development of biofuel sweet sorghum germplasm requires the evaluation of factors associated with ethanol production (Vandenbrink et al. 2010). Although the average brix of the 17 U.S. reference accessions was higher than the NPGS accessions (9.78 vs. 9.55, respectively), their average DNS values were lower than the NPGS accessions (5.54 vs. 6.93, respectively). Remarkably, the DNS value of PI 653616 and PI 653617, the two U.S. reference accessions with the higher brix (17.8 and 17.4, respectively; Table 5), were 4.01 and 4.84, respectively. In

contrast, the DNS of five NPGS accessions with high brix (>10.0) ranged from 9.86 to 11.42. The difference between brix and the DNS value may be attributed to multiple factors associated with the chemical structure of the juice. For instance, the profile of sucrose, glucose, and fructose in sweet sorghum juice can be very different among varieties (Prasad et al. 2007). Typically, sucrose levels increase at the expense of glucose and fructose levels as the plant matures (Almodares et al. 2007), and this ratio varies among sweet sorghum varieties (Whitfield et al. 2012). Indeed, "sugar" varieties have high levels of sucrose at maturity, while "syrup" varieties would contain a relative low level of sucrose (Whitfield et al. 2012). Therefore, the sugar composition of many sweet sorghum accessions present in NPGS should be favorable for biofuel. Further screening should include the brix value followed by an analysis of the sugar composition to identify the most valuable accessions for biofuel.

The place of origin for accessions resistant to anthracnose and rust includes Ethiopia, Kenya, Malawi, Tanzania, Zaire, Zambia, India, and the U.S. suggesting the presence of multiple resistance sources. Remarkably, the Tanzanian accession PI 156424 was resistant to both diseases, but its brix and DNS values were among the lowest. Olweny et al. (2013) and Umakanth et al. (2012) reported positive general combining ability (GCA) for the brix trait indicating additive gene action that would suggest the trait can be improved with breeding and selection. Thus, accessions with lower brix values, but having other desirable traits should be considered in breeding programs to increase genetic diversity. Indeed, the genetic diversity present in the NPGS subset evaluated herein can be employed to enhance the narrow genetic base present in the biofuel breeding programs worldwide. For instance, the total dry matter content of PI 455286, PI 454500 and PI 648068 (originally from Ethiopia and U.S.) were higher than the 17 U.S. references accessions (>156.87 g/plant). The positive correlation between dry matter with juicy volume and brix indicates new biofuel varieties combining these traits are achievable for high ethanol recovery. Thus, the use of these accessions in breeding programs could be valuable for the development of new biofuel sweet sorghum varieties.

Photoperiodic sensitive sorghum is a unique approach to enhance biomass yield in temperate regions (Rooney et al. 2007), however, breeding programs is affected by the lack of flowering.

In this study, the ranking order of the majority of the accessions for PH, FrW, DrW, and Brix were consistent across the years ($r_s > 0.60$), being brix values the most reliable ($r_s > 0.67$). Moreover, the brix values of some accessions were consistent with previous evaluations in temperate regions (Wang et al. 2006; USDA-ARS 2013), thus, results obtained herein are also reliable for temperate regions. Indeed, the CV, PCV and GCV of these traits indicate that genetic gain can be achieved in tropical regions. Since the majority of the accessions in the NPGS sweet sorghum collection are photoperiod sensitive, the development of new biofuel sweet sorghum varieties might be initiated in tropical regions by the intercrossing of superior complementary accessions, followed by the selection of superior recombinants and their evaluation in replicated trials in temperate regions.

The narrow genetic diversity among US sweet sorghum varieties is well documented (Murray et al. 2009; Ritter et al. 2007; Ali et al. 2008; Wang et al. 2009). Indeed, improved sweet sorghum lines from the U.S. are derived from six landraces originally from Sudan (MN960, MN1048, MN1054, MN1056, and MN1060) and Uganda (MN1500) (Murray et al. 2009; Ritter et al. 2007), thus, the majority of these lines enclose similar genes/alleles (i.e. identical by descent) for high brix. In this study, the NPGS sweet sorghum collection was evaluated to identify promising accessions with high biofuel potential. Accessions with higher DNS values, dry matter content than traditional U.S. sweet sorghum cultivars were identified, as well as new source of resistance to anthracnose and rust. The integration of these accessions into biofuel sorghum breeding programs will aid to increase genetic diversity and to the development of new biofuel varieties. Further, these results showed that the NPGS sweet sorghum collections enclose valuable genes/alleles for biofuel that are not being employed in breeding programs. Thus, further research should be directed to tap this collection. Sorghum researchers worldwide can obtain seed samples of the NPGS sweet sorghum collection through the Germplasm Resources Information Network (GRIN; www.ars-grin.gov).

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