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Phenotypic evaluation of a diversity panel selected from the world collection of sugarcane (*Saccharum spp*) and related grasses

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Abstract

Long-term improvement of sugarcane and energy cane (complex hybrids of *Saccharum spp*) cultivars can be enhanced by breeding with the type of diverse germplasm available at the World Collection of Sugarcane and Related Grasses (WCSRG) maintained in Miami, Florida. To evaluate germplasm in the WCSRG for breeding purposes, a diversity panel was selected with approximately 300 accessions and planted at Canal Point, FL in three replications. These accessions were measured for stalk height and stalk number multiple times throughout the plant-crop growing season and for Brix and fresh biomass during the 2013 harvest. First-ratoon stalk height, stalk number, stalk diameter, internode length, Brix, and fresh and dry biomass were evaluated in 2014. The highest correlations were found between early season measurements and harvest traits. Hybrids had higher fresh weight and Brix while *Saccharum spontaneum* had higher stalk number and dry mass. According to the principal component analysis, the diversity panel was divided into two groups. One group had accessions with high stalk number and high dry biomass like *S. spontaneum* and the other had accessions with higher Brix and fresh biomass such as *S. officinarum*. In first ratoon, there were 110 accessions not significantly different in Brix from the sugarcane commercial standards, including 10 *S. spontaneum* accessions, and 17 and six accessions that were higher than commercial standards in dry and fresh mass, respectively. This study shows the variability in traits of interest and the breeding potential of accessions within the WCSRG for sugar-and energy-cane cultivar development.

Keywords: core collection, Erianthus, Miscanthus, biofuel, bioenergy

Introduction

The *Saccharum* genus is an important source of plants for sugar and fuel production. Breeding programs have not fully utilized the genetic potential of the Saccharum genus found within germplasm collections to enhance biomass yield and resistance to abiotic and biotic stresses. Two large germplasm collections called the World Collection of Sugarcane and Related Grasses (WCSRG) are located at the USDA-ARS Subtropical Horticulture Research Station in Miami, FL and the Research Centre at Kannur (Kerala, India) respectively. The Miami collection contains 20 Saccharum species and approximately 1,200 accessions from 45 countries. The most abundant species within the collection are *S. spontaneum* and *S. officinarum*, along with hybrid Saccharum genotypes. Based on molecular marker and phenotypic data, a core collection of 300 accessions and 10 checks with three replications was planted in Canal Point, FL to capture the majority of the diversity (Nayak et al, 2014; Todd et al, 2014) available in the WCSRG. Due to the labor required to maintain and evaluate the entire WCSRG, this smaller diversity panel with three replicates was more practical for accurately characterizing and analyzing existing variability in the entire collection. Because many of the clones in the collection are progenitors to modern sugarcane and energy cane cultivars (Bremer, 1961; Nair, 2008; Tew and Cobill, 2008), an intensive study of this replicated panel can improve sugarcane breeders' knowledge of the relationship between germplasm and plant phenotypic characteristics (Upadhyaya and Ortiz, 2001). Phenotypic characterization of the panel should be useful for parental selection for both energy cane and sugarcane breeding programs, and can be used as a diversity panel for identifying QTLs for yield, disease resistance, fiber content and other important traits for use in marker assisted selection(Perera et al, 2012).

The objectives of this study were to 1) phenotypically characterize a representative diversity panel derived from the WCSRG; 2) investigate how the measured phenotypic traits correlate with yield and how these correlations change at different developmental stages; 3) compare traits among species and determine how ranks among species for different traits

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change over a growing season; and 4) identify specific genotypes with positive breeding traits.

Materials and Methods

Plant material

Three-hundred accessions representing most of the diversity within the WCSRG (Miami collection) were selected (Nayak et al, 2014; Todd et al, 2014) and planted into 36.83 x 36.83 cm pots in March 2013. Three single-budded seed pieces (approximately 8 cm long) were planted per pot, and the pots were placed inside 29 x 41 x 52.5 cm containers with drain holes drilled 5 cm above the ground. The pots were filled with 5% fine pine bark (< 9.53 mm), 10% well point sand, and 85% compost and arranged in a randomized complete block design with three replications. Nine commercial cultivars, CP 00-1101 (Gilbert et al, 2008); CP 01-1372 (Edmé et al, 2009); CP 03-1912 (Gilbert et al, 2011); CP 72-2086 (Miller et al, 1984); CP 78-1628 (Tai et al, 1991); CP 88-1762 (Tai et al, 1997); CP 89-2143 (Glaz et al, 2000); CP 96-1252 (Edmé et al, 2005); CPCL 00-4111 (Glynn et al, 2011) and one new genotype, CP 01-2390 were included in the experiment as references. The genotype CP 01-2390 was included because of its high productivity in yield tests and its frequent use as a parent in the Canal Point breeding program (Glaz et al, 2010). Plants were watered with an automatic sprinkler system using shrubber emitters at a rate of 8 L per hour for five minute duration twice daily. Pots were weeded as needed. All plants were fertilized three months after planting with 25 g of 10-10-10 with micronutrients, and 100 g of 14-14-14 Osmocote® slow release fertilizer. The surrounding area was treated periodically with 2% Glyphosate to control weeds.

Measurements during growing season and harvest

During the 2013 growing season, plant height and tiller number were recorded seven times at 2-3 week intervals. Between September-December, 2013, aboveground stalks of all plants were hand harvested and the Brix of each harvested stalk was measured using a Bellingham + Stanley Ltd. Model 190 refractometer. Stalks were counted, and the plant fresh weight was measured at harvest. After harvest, the stubbles were allowed to re-grow into ratoon plants. During the July-August, 2014, plant height, leaf length and width, and stalk diameter were measured on the ratoon plants. Brix, (Index Instruments PTR model 46 Xrefractometer), total fresh weight, and stalk number were also recorded. Prior to harvest, a three-stalk sample was collected from each plant for analysis of fiber content. One stalk per sample was measured for height, diameter, internode length, leaf width, and leaf height on the leaf at the top visible dewlap. Three stalks were shredded, and the fresh weight was recorded to estimate total fresh biomass per pot. Shredded cane was subsequently dried at 60°C, and re-weighed to determine dry biomass yield per pot.

Data analysis

The 2013 incremental measurements of plant height and tiller number were subjected to repeated measures analysis using Proc Mixed in SAS (SAS Institute Inc SAS/STAT® 9.3 user's guide: the MIXED procedure) (SAS Institute Inc, 2011) with group, measurement time, and their interaction considered fixed effects. Measurement time was used as the repeated factor in the analysis and the genotype by replication interaction as the subject, based on procedures described by Tao et al (2002). The least square means (LS-means) for each measurement time were used for correlations. The LS-means for each genotype within the major groups were calculated for each trait at each measurement time. The height and stalk number at each measurement time was correlated using Pearson coefficients. The height and stalk means were also correlated with Brix and fresh weight yields. The correlations created by correlating the different traits were used to create a regression with time of measurement using Excel (R^2 coefficient).

Harvest fresh weight, stalk number, and Brix in 2013 and 2014 were analyzed using a mixed linear model with independent variables equal to group, crop cycle, and their interaction. The repeated measure of crop cycle was analyzed based on procedures described by Tao et al (2002). Replication and the replication within crop cycle interaction were treated as random. An analysis of the effects of genotypes was conducted in a similar manner, with group replaced by accession in the above mentioned equation.

Traits measured only in the ratoon crop, including dry mass, water content, internode length and diameter, and leaf width and length were analyzed using SAS Proc Mixed where the response variable was equal to group or accession depending on analysis and replication was considered a random effect. The LS-means of groups and accessions were compared with the diff option using PROC MIXED in SAS to determine LSD. Individual accessions were analyzed alongside the combined average of the 10 commercial checks in the LSD analysis (Saxton, 1998). The coefficient of variation (CV) was calculated using the same Proc Mixed model with the following formula CV = sqrt(RESID)*100/MEAN, where the RESID = the residual covariance and MEAN is the overall mean of the model.

The LS-means for stalk height, stalk diameter, internode length, leaf width, leaf length, fresh and dry biomass, water content, stalk number, and Brix data collected in 2014 were subject to principal component analysis (PCA) in the MULTIBIPLOT program using the single value decomposition method (Vicente-Villardón, 2010). For visualization purposes, the species *Saccharum barberi*, *S. edule*, *S. robustum*, and *S. sinense* were grouped together as «other Saccharum species» since this group is similar genetically (Nayak et al, 2014). Similarly, other non-Saccharum genera including Erianthus (*Erianthus bengalense*, *E. arun-*

dinaceum, *E. ravannae*, *E. procerum*, *E. kanashiroi*), Miscanthus (*M. floridulus* and *M. sinensis*), Sorghum species (*Sorghum arundinaceum*) and the species *Saccharum brevibarbe* and *E. rufipilus* which are actually non Saccharum species (Nayak et al, 2014) were grouped together. Correlations were performed on the data of each population in each crop cycle using Pearson coefficients using Proc CORR in SAS (SAS, 2011).

Results

Harvest traits of both plant cane and ratoon

Brix

For plant Brix in the combined plant-cane and first-ratoon crops, group ($p < 0.0001$) was highly significant at p=0.05 and its interaction with crops ($p = 0.0547$) was significant at $p = 0.1$. Within each crop Brix differed significantly between groups. Not surprisingly, the locally adapted check genotypes had the highest mean Brix, while the *S. spontaneum* and Other Genera groups had the lowest (Table 1). The group *S. spontaneum* and Other genera had the highest CVs in both years while checks, hybrids, and other Saccharum had the lowest CVs.

Biomass

Group and its interaction with crop had significant effect on fresh weight. When comparing each crop in a separate analysis, the checks had the highest fresh biomass in plant cane in 2013 followed by hybrids then *S. officinarum*, but in 2014 the checks and the hybrids were not significantly different (Table 1). In 2013, *S. spontaneum* and Other genera had the lowest fresh biomass but in 2014 *S. spontaneum* and Other genera were among the lower yielding genotypes, but the lowest was *S. officinarum*.

The estimated ratoon crop dry biomass was highest in *S. spontaneum*, Other genera, and Unknown groups, with the checks only significantly higher than *S. officinarum* that was significantly lower than other groups. Hybrid and Other Saccharum groups had similar yields next to the lowest (Table 2). The water content of the hybrids, checks, and Other Saccharum were the highest among the groups followed by the unknown group. The remaining entries did not differ significantly. The highest CV for dry weight was for the Unknown and Other genera groups.

Stalk number

Stalk number was significantly affected by group but not by crop or their interaction. On average *S. spontaneum* had a higher number of stalks than other groups within their respective crops. The Hybrid and *S. officinarum* groups had the highest CVs with the lowest being Other genera and the *S. spontaneum*, the groups with the highest stalk numbers (Table 1).

Stalk height

Stalk height had no significant difference between crops but was significantly different between groups and the group by crop interaction. The mean stalk height in 2013 was highest for Unknown, Checks, and *S. spontaneum* groups and it was lowest for *S. officinarum* and Other Saccharum (Table 1). In 2014 the highest stalk height was for the Unknown and Hybrids groups followed by *S. spontaneum*. The two groups with the shortest stalk heights were Other Genera and *S. officinarum* (Table 1). The group Other Genera had the highest CV and the checks had the lowest stalk height in both crops.

Harvest traits measured only on ratoon plants

Stalk diameter was significantly different among groups with Hybrids having the largest stalks, and *S. spontaneum* and Other genera the lowest (Table 2). The *S. spontaneum* group also had the highest CV for this trait and Hybrids had the lowest.

Leaf width and length were also significantly different among groups with the *S. officinarum* group having significantly higher leaf width than the others and the *S. spontaneum* group with the lowest leaf width. The CV of leaf width was the highest in *S. spontaneum* group and lowest in checks (Table 2). Leaf length was highest in Checks and Hybrids and lowest in *S. spontaneum* and Other genera. The Unknown and Other Saccharum groups had the highest

Table 1 - The estimated mean, standard deviation, least significant difference (0.05) and coefficient of variation for plant cane and ratoon harvest traits measured in 2013 in the diversity panel of WCSRG and the checks.

		Fresh biomass (kg)				Stalk Height (cm)				% Brix				Stalk Number			
2013	#	Mean	SE	diff	CV	Mean	SE	diff	CV	Mean	SE	diff	CV	Mean	SE	diff	CV
Overall	308	4.5	0.41		30.81	122.94	4.08		20.1	10.55	2.53		15.89	13.08	0.42,0.41		12.19
S. officinarum	78	4.75	0.42	BC	34.06	116.32	4.53	C	17.64	12.04	2.54	B	14.43	5.8	0.28,0.27	E	15.75
S. spontaneum	107	3.96	0.43	DE	26.1	128.42	4.42	AB	21.83	8.53	2.54	D	18.27	31.34	1.45,1.38	A	9.25
Hybrid	38	5.15	0.44	B	26.03	122.44	5.1	BC	14.71	12.42	2.54	B	14.31	6.24	0.39,0.37	E	16.15
Other Saccharum	33	4.53	0.45	C	35.45	115.75	5.42	C	16.97	10.81	2.55	C	13.83	8.52	0.55,0.52	D	13.83
Other Genera	18	3.63	0.5	E	35.2	117.4	6.66	BC	30.78	8.8	2.56	D	18.9	24.7	2.04,1.89	B	8.22
Checks	10	7.15	0.54	A	19.77	131.77	7.51	AB	10.09	15.65	2.56	A	12.45	7.87	0.84,0.76	D	11.54
Unknown	24	4.47	0.5	BCD	26.05	133.95	5.95	A	24.46	10.53	2.55	C	19.79	13.23	1.11,1.03	C	13.01
2014		Mean	SE	diff	CV	Mean	SE	diff	CV	Mean	SE	diff	CV	Mean	SE	diff	CV
Overall	289	3.14	0.25		57.35	127.60	4.67		23.32	12.32	0.34		19	16.77	0.92,0.87		62.24
S. officinarum	70	2.6	0.26	C	53.19	121.09	6.16	C	23.12	13.4	0.44	C	16.08	6.91	0.50,0.47	E	11.89
S. spontaneum	103	3.13	0.24	B	53.92	128.93	5.94	B	24.46	10.73	0.41	E	22.05	41.75	2.12,2.02	A	11.54
Hybrid	37	3.78	0.29	A	46.1	137.85	6.75	AB	19.59	14.39	0.47	B	15.55	7.32	0.62,0.57	E	11.99
Other Saccharum	28	3.62	0.31	AB	57.38	128.77	7.14	BC	20.77	12.28	0.49	D	15.17	10.97	0.98,0.90	D	16.88
Other Genera	18	3.03	0.36	BC	82.33	100.33	7.97	D	30.79	10.99	0.55	E	21.34	28.08	2.85,2.60	B	16.2
Checks	10	4.19	0.43	A	44.08	128.96	9.44	ABC	18.48	16.2	0.65	A	12.12	8.71	1.30,1.15	DE	12.41
Unknown	23	3.42	0.33	AB	61.49	143.04	7.51	A	22.13	12.9	0.52	CD	19.41	18.7	1.78,1.64	C	17.78

Table 2 - The estimated mean, standard deviation, least significant difference (0.05) and coefficient of variation for ratoon phenotypic traits measured in 2014 in the diversity panel of WCSRG and the checks.

	#	Dry biomass (kg)				Moisture content (kg)				Stalk Diameter (mm)				Stalk internode (cm)			
		Mean	SE	diff	CV	Mean	SE	diff	CV	Mean	SE	diff	CV	Mean	SE	diff	CV
Overall	289	1.04	0.13		72.47	2.12	0.15		58.77	16.33	0.58		25.95	10.09	0.60		33.98
S. officinarum	70	0.63	0.13	D	55.42	1.97	0.15	C	53.21	23.31	0.60	B	24.07	8.10	0.74	C	39.71
S. spontaneum	103	1.27	0.13	A	59.89	1.88	0.14	C	56.33	8.34	0.55	D	31.23	11.90	0.72	A	29.62
Hybrid	37	1.01	0.14	C	52.08	2.79	0.18	A	45.47	24.77	0.72	A	19.65	9.78	0.79	B	28.65
Other Saccharum	28	1.06	0.15	BC	53.58	2.65	0.19	AB	54.53	18.09	0.79	C	22.07	8.41	0.82	C	35.36
Other Genera	18	1.31	0.17	AB	81.35	1.84	0.24	C	73.93	10.06	0.95	D	26.88	10.59	0.94	AB	40.16
Checks	10	1.00	0.20	ABC	43.74	3.19	0.29	A	47.88	25.14	1.18	AB	20.00	9.27	1.03	BC	40.69
Unknown	23	1.35	0.16	A	108.77	2.18	0.21	BC	84.32	16.33	0.86	C	30.38	10.63	0.86	B	30.44

	#	Stalk Height (cm)				Leaf Length (cm)				Leaf Width (mm)			
		Mean	SE	diff	CV	Mean	SE	diff	CV	Mean	SE	diff	CV
Overall	289	127.60	4.67		23.32	119.24	1.65		18.17	27.06	1.42		42.76
S. officinarum	70	121.09	6.16	C	23.12	125.16	2.18	B	15.77	40.40	1.55	A	33.42
S. spontaneum	103	128.93	5.94	B	24.46	108.31	1.92	C	20.28	12.37	1.45	E	86.91
Hybrid	37	137.85	6.75	AB	19.59	133.09	2.82	A	12.08	36.96	1.80	B	28.22
Other Saccharum	28	128.77	7.14	BC	20.77	122.27	3.18	B	21.05	35.20	1.96	B	27.79
Other Genera	18	100.33	7.97	D	30.79	110.09	3.92	C	22.93	17.73	2.30	D	37.50
Checks	10	128.96	9.44	ABC	18.48	139.21	5.1	A	12.73	34.37	2.85	B	33.57
Unknown	23	143.04	7.51	A	22.13	120.58	3.52	B	21.94	27.45	2.12	C	57.08

CV and Checks and hybrids the lowest CV for leaf length. The leaves of the hybrids and *S. officinarum* had similar characteristics, with both species having wide leaves. However, the leaf length and width were significantly different between the two (Table 2).

Groups also significantly affected internode length. The *S. spontaneum* accessions had the longest internodes followed by Other Genera. *S. officinarum* and Other Saccharum had the shortest internodes. Other Genera and checks had the highest CV and Hybrid had the lowest.

Repeated measures analyses

For stalk number of the three largest species, groups, measurement time, and their interaction had significant effects. Regardless of measurement date, *S. spontaneum* had the highest stalk number (Supplementary Figure 1).

The plant height in plant cane was significantly affected by measurement date as well as the interaction between measurement date and species groups (data not shown). Trends were observed across the season when comparing the ranks among species groups. On the first day of measurement there were no differences between species (Supplementary Figure 1); however on days 2, 3, and 4, *S. spontaneum* was significantly taller than the others and on day 5, hybrid and *S. spontaneum* were not significantly different and *S. officinarum* had lowest height. On day 6 the order of each group was significant with spontaneum was tallest followed by hybrids. On day 7 hybrid and S. spontaneum were highest.

Repeated measurement correlations

Height and Tiller Number

In 2013, multiple traits were assessed at different times, making possible the exploration of correlations between traits across growth stages. A correlation analysis was conducted between tiller number and height on average as well as at specific time points, and these were, in turn, compared with biomass and Brix. The Pearson correlations between height and tiller number were higher in earlier measurements (Figure 1) among the groups tested. The correlations between height and tiller number of *S. officinarum* were high (positive) early in the season, but quickly dropped to non-significant by day five and were not significant for rest of the days. Because of this seasonal trend, there was a high negative slope (-0.11) for measurement date vs. correlation with high R^2 value (Figure 1). For *S. spontaneum* the correlations were lower and dropped with measurement time and had a large slope (-0.09) with high R^2. The only significant correlation was a negative correlation on day 7. When the correlation was regressed on the measurement date for the hybrid group, a line small with a weak negative slope (-0.02) was observed and weak R^2. Each of these correlations was significant.

To see if there were positive correlations between early and late measurements for some traits, the first stalk number or height measurement was correlated with each measurement of the other trait. The results of the correlation of first height with other stalk measurements were similar with the correlations between stalk number and height correlation each measurement with similar correlations and negative slopes.

When the initial stalk measurement was compared with each of the measurements of height, the same trend was apparent but all of the regression lines had high negative slopes and all of the lines had very high R^2. The correlations were all significant in hybrids and S. officinarum but only the first three correlations were significant in *S. spontaneum* (Results not shown).

Height and fresh weight

Each measurement of height was significantly and positively correlated with fresh biomass for each species. The same trend where early measurements of height were more highly correlated with biomass than late measurements in all three species was observed. The *S. officinarum* and *S. spontaneum* groups had higher slopes and R^2 values than hybrids (Figure 1).

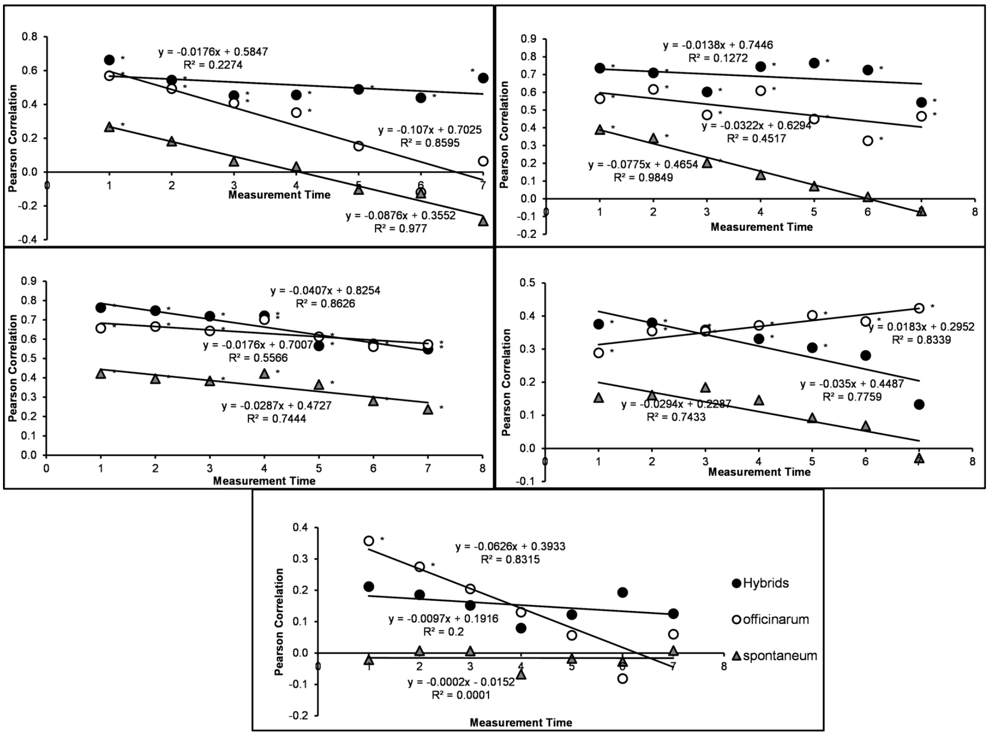

Figure 1 - Regression of measurement time and correlations between phenotypic traits for *Saccharum officinarum*, *S. spontaneum* and hybrid species. The * indicates a significant (0.05) correlation. Top left: plant heights and tiller count, Bottom left: height and fresh weight, Top Right, tiller number and fresh weight, Bottom Right tiller height and Brix, Bottom middle, tiller number fresh weight.

The regression analysis indicated height could be used as a significant predictor of fresh biomass specifically at early developmental stages.

Tiller number and Fresh Weight

The correlations between tiller number and harvest weight followed the same pattern of decreasing correlations. Early measurements were the most highly correlated (Figure 1). Correlations between stalk number and harvest weight were significant at each measurement time for hybrids and *S. officinarum*. The slope and R^2 were low for hybrids and moderate for *S. officinarum*, and highest for *S. spontaneum* (Figure 1).

Height and Brix

The same trends of decreasing correlations were seen in height and Brix except for *S. officinarum*, which showed a marked increase in correlation over time (Figure 1). The Hybrid and *S. spontaneum*, however, had decreasing correlations as the season progressed. The correlations between height and Brix at the first five times of measurement in the hybrid group were significant. No correlations between height and Brix were significant in the *S. spontanum* group. All of the height and Brix correlations in *S. officinarum* were significant.

Brix and Tiller number

The trend of higher correlations between Brix and tiller number at early measurements for hybrids and *S. officinarum* groups was also observed, but the regression slope lines and R^2 were lower for Hybrids and *S. spontaneum* groups and none of the correlations were significant. The phenotypic correlation trend across time was larger for *S. officinarum*, but correlations between Brix and tiller number at only the first two measurements were significant (Figure 1).

Correlations of traits measured at harvest

Several traits measured during growth were significantly correlated with those obtained at harvest. Overall in plant cane, fresh weight correlated negatively with stalk number and positively with Brix and stalk height (Table 3). Similar correlations were found within *S. spontaneum*, *S. officinarum*, and hybrid groups (Table 3), and in the last of the repeated measurements. But the negative correlation between stalk number and fresh weight was different in the whole collection from the *S. officinarum* and hybrid populations which had positive correlations. The correlation between Brix and stalk number in *S. spontaneum* group was negative and non-significant. In the whole population, harvest height and stalk number, and harvest height and Brix had significant positive correlations (Table 3). There were similar correlations in hybrids and *S. officinarum* groups, but hybrids had an additional significant correlation between stalk height and stalk number. In *S. spontaneum*, weight did not correlate with stalk height. Stalk number showed a negative correlation with stalk height (Table 3).

Table 3 - Correlations of traits for the total accessions and the three largest groups in the plant cane crop within the diversity panel of the World Collection of Sugarcane and Related Grasses.

Whole		***	***	***
Hybrid	**Biomass**	**	***	***
S. officinarum		***	**	***
S. spontaneum		NS	NS	NS
Whole	-0.22		***	NS
Hybrid	0.45	**Stalks**	NS	**
S.officinarum	0.50		*	NS
S. spontaneum	-0.06		NS	***
Whole	0.48	-0.57		*
Hybrid	0.62	0.07	**Brix**	**
S. officinarum	0.35	0.26		**
S. spontaneum	0.12	-0.08		***
Whole	0.24	0.01	0.13	
Hybrid	0.55	0.42	0.44	**SH**
S. officinarum	0.55	0.22	0.34	
S. spontaneum	0.17	-0.39	0.41	

Abbreviations: Whole, Whole collection; Hybrid, Hybrid accessions; S, Saccharum; Biomass, Fresh biomass; Stalks; Stalk number; Brix, %Brix; SH, Stalk Height. and *, **, and *** indicate significant correlations at 0.05, 0.01, and 0.001 respectively; NS, non-significant.

In ratoon crop, the majority of correlations among all the accessions were significant with only seven non-significant correlations (Table 4). Among highly significant correlations, some notables were positive correlations between stalk height and internode length, and fresh and dry weight with water content. There was also a small negative correlation between stalk number and stalk height. Stalk diameter had positive correlations between leaf width and length, Brix, and moisture content. Negative correlations were observed with internode length, stalk number, and dry weight. Internode length had small but significant negative correlation with leaf width and positive correlation with dry biomass. Other leaf width correlations include positive correlation with leaf length and Brix, and a large negative correlation with stalk number. Other leaf length correlations include a negative correlation with stalk number and positive with moisture content. The remaining fresh weight correlations were positive with Brix, dry weight, and moisture content. The three remaining stalk number correlations were negative correlations with Brix and moisture content and positive with dry matter. Brix and dry matter both correlated with moisture content. When looking at the three largest groups separately, a few differences were noticed. Hybrids did not have a significant correlation between stalk height and internode length or stalk height and water content like the other groups or the whole collection (Supplementary Table 1). For instance, the hybrid and officinarum groups did not have significant negative correlations between stalk height and stalk number like the whole population or *S. spontaneum* groups (Supplementary Tables 2 and 3). The *S. officinarum* group also had more correlations than hybrid or *S. spontaneum* groups; for example, a correlation between fresh weight and stalk diameter was observed in *S. officinarum* group, but not in the other groups (Table 4).

Comparisons of the harvest traits of individual accessions

In the model where genotype (each individual accession) was run separately for each crop, individual accession was significant for each trait. Individual accessions were compared with the average of the checks to find promising germplasm for specific traits. The mean Brix values of the checks in plant cane and ratoon were significantly (p = 0.05) greater than 265 accessions in plant cane and 172 accessions in ratoon crop. This suggests that many of the accessions will have their highest Brix values in ratoon rather than plant crops. However, no accession was identified with higher Brix than the check in either crop cycle. The diversity panel did not have accessions with Brix higher than the checks but did have 26 total accessions including 12 *S. officinarum*, 10 hybrids, one *S. sinense*, and three unknown in plant cane that were not significantly different from the checks. These accessions may be considered for use as parents for improved traits in commercial sugarcane breeding programs. In ratoon crop, there were 110 accessions that did not differ significantly from the mean of the checks for Brix including ten *S. spontaneum*, 28 hybrids, two *S. barberi*, one *Erianthus bengalense*, one *S. edule*, one *E. kanashiroi*, 45 *S. officinarum*, one *S. robustum*, six *S. sinense*, and 15 unknown.

For fresh biomass in plant crop, one accession was significantly greater than the mean of the checks (Diamond 10, Saccharum hybrid), 209 accessions were significantly less than the checks, and 82 not significantly different from the checks. In ratoon crop, there were 6 accessions significantly greater than the check (NG 77-196 *S. robustum*; Javari Kabbu, *S. officinarum*; SES 275, *S. spontaneum*, NG 28-020 *S. officinarum*, CO 0475 *S. hybrid*, SES 305 *E. ravennae*), There were 58 accessions significantly less than the checks, and 230 accessions not significantly different than the checks. Dry biomass values of 17 accessions were significantly greater than the checks including mostly *S. spontaneum* (70.6%), one *Sorghum arundinaceum*, one *E. ravennae*, and two unknowns. Dry biomass of only one accession was lower than the mean of the checks. The accessions with greatest deviation in dry biomass from the average of commercial checks (>1.5 kg) on a per-pot basis were *S. spontaneum* accessions US 61-037-01 (4.24±0.46), SES 275 (3.10 ± 0.46 kg), and SES 234 (2.98 ± 0.56 kg), and the *Sorghum arundinaceum* accession US47-001 3.84 ± 0.56 kg).

Regarding water content, 90 accessions had significantly less water content and four accessions significantly more than the checks. The majority of the low-water-content accessions were *S. spontaneum*, but there were 30 *S. officinarum* among the group.

For plant-cane stalk number, there were 130 accessions significantly greater than the average stan-

Table 4 - Correlations of traits for the total accessions in the ratoon crop within the diversity panel of the World Collection of Sugarcane and Related Grasses.

	SH	SD	Internode	LeafW	LeafL	Fresh	Stalks	Brix	Dry	Water
Height	**SH**	*	***	NS	NS	***	***	*	***	***
Diameter	0.14	**SD**	***	***	***	**	***	***	***	***
Internode	0.50	-0.31	**Internode**	***	*	**	***	**	***	NS
Width	0.02	0.82	-0.37	**LeafW**	***	NS	***	***	***	**
Length	0.00	0.53	-0.13	0.38	**LeafL**	**	***	***	NS	***
Biomass	0.44	0.18	0.18	0.00	0.19	**Fresh**	NS	***	***	***
Stalks	-0.21	-0.69	0.24	-0.63	-0.38	-0.03	**Stalks**	***	***	***
Brix	0.14	0.54	-0.19	0.36	0.20	0.23	-0.38	**Brix**	NS	***
Dry	0.33	-0.24	0.32	-0.34	-0.06	0.77	0.31	-0.06	**Dry**	***
Water	0.41	0.38	0.07	0.18	0.29	0.92	-0.20	0.34	0.55	**Water**

Abbreviations: Height, Stalk Height; Diameter, stalk diameter; Internode, Internode length; Width, Leaf Width; Length, Leaf length; Biomass, Fresh biomass; Stalks; Stalk number; Brix, %Brix; Dry, Dry weight, Water, Water content; and *, **, and *** indicate significant correlations at 0.05, 0.01, and 0.001 respectively; NS non-significant.

dard, 96 of which were *S. spontaneum*, and one was *S. officinarum* [Unknown (1995: R56-P50)]. Seventeen of the high-stalk number accessions also had significantly greater heights than the commercial standard. Out of 123 high-stalk-count accessions in first-ratoon, 95 were *S. spontaneum*, and none were hybrids or *S. officinarum*. Thirteen of these 123 accessions had Brix that was significantly equal to the commercial standard, and these included: nine *S. spontaneum* clones and three unknown. One high-stalk-population accession (SES 275 *Saccharum spontaneum*) which was also significantly greater than the commercial checks for fresh and dry biomass. There were 14 other accessions significantly higher than checks in both stalk number and dry weight which included eleven *S. spontaneum* and one *Sorghum arundinaceum* and two unknowns.

At plant cane harvest, 20 accessions had surpassed the height of the checks, and 55 remained shorter. The majority of the tall accessions were *S. spontaneum* (70%). In the ratoon crop, 37 accessions were taller than the average of checks and 49 were shorter. Again, *S. spontaneum* represented a majority of the taller accessions (46%) but there were some *S. officinarum* (22%) and a few other species. Among the tallest, there were 11 accessions with significantly high Brix, three with exceptionally high fresh biomass, five with high dry biomass, and 16 with high stalk populations. Four accessions had significantly higher height, dry biomass, and stalk number than the commercial standards. These four accessions were: (SES 011, unknown), (US 78-519 *S. spontaneum*), (SES 184 B, *S. spontaneum*), and (SLC 92-94, *S. spontaneum*)

Comparisons of other species

In the PCA of ratoon cane measurements, the majority of the variance was in the first two components (Supplementary Figure 2). The first component in PCA (PCA1) included stalk diameter, leaf width, leaf length, stalk number (negative), Brix, and water mass, and the second component (PCA2) included stalk height, internode length, fresh biomass, dry biomass and water mass. Both components include traits important to sugarcane and energy cane varieties. The PCA showed that all of the *S. spontaneum* clones clustered together on the left (negative) PC1, while the *S. officinarum*, hybrids and other Saccharum clones were on the right (Supplementary Figure 2). These results indicate that the diversity panel can be divided into two broad populations: one population, mostly composed of *S. spontaneum*, has high stalk number, high dry mass, low Brix, narrow stalk diameter, and narrow leaves, and the other population had fewer stalks, lower dry mass, higher Brix and broader leaves. Species other than *S. spontaneum* and *S. officinarum* fell into one of the two groups in a similar pattern. For example, the Other Genera group including species like *E. arundinaceum* and *E. rufipilus* clustered with the *S. spontnaeum*, whereas the Other Saccharum group including *S. robustum*, *S. edule*, and *S. barberi* tended to cluster with the *S. officinarum*. The analysis also showed that Brix and stalk number were not significantly negatively correlated and Brix was associated with low stalk number populations.

Discussion

Phenotypic traits

Phenotypic measurements from *S. spontaneum* and *S. officinarum* groups were consistent with previous reports, showing that *S. officinarum* had a small number of large-diameter stalks with wide leaves, high Brix, and high water content, whereas *S. spontaneum* clones had a high stalk population with small diameter, narrow leaves, and low Brix (Matsuoka et al, 2014; Allison, 1978; Roach, 1978; Gravois and Milligan, 1992; Tai et al, 1992; Ramdoyal and Badaloo, 2007). The hybrids group in the diversity panel was intermediate between the two groups; however they were more similar to *S. officinarum* with higher Brix in ratoon. The high Brix values in the hybrids and checks were not surprising since each had been advanced through intense selection pressure in sugarcane breeding program. Hybrid clones were not significantly higher in stalk number than the *S. officinarum*, indicating simultaneous selection for Brix and stalk number was somewhat successful, yet limited by the correlation between stalk number and fiber (Jackson, 1994). Breaking the link between stalk number and fiber has the potential to greatly increase sugar yields through breeding. The 110 accessions with Brix statistically equal to the standard genotypes might be useful as breeding stock to broaden the genetic base of sugarcane, particularly those accessions that were not significantly different in other traits such as biomass, stalk number, and height.

The fresh biomass of the plants comprised primarily dry mass, total soluble solids, and water contained within the plant. *S. officinarum* had a strong fresh weight yield decline and rank change from 2013 to 2014 and this was the probable cause of the groups by crop interaction. Consequently, in ratoon-crop in 2014, the *S. spontaneum* clones performed better than *S. officinarum* while the opposite was true in plant cane in 2013 for fresh weight. The water contents of the hybrid and check populations were significantly higher than the S. officinarum population and there was a rank change from fresh weight to dry weight. The reason could be that hybrids were bred for high sugar and low fiber, and fiber and water are negatively correlated (Gravois and Milligan, 1992). The Hybrid group also had the lowest correlation between water and dry weight.

Repeated correlations

The interaction between group and time of measurement for stalk number and height was expected given the differential growth rate of the plants. Correlations between height or tiller number with harvest traits like Brix and fresh biomass at early developmental stages could be an indication of hybrid vigor. The correlation of stalk number and height at early stages with later harvest yield measurements likely reflected the relationship between early plant establishment with overall health and improved development throughout the lifecycle. There was an opposite trend with height and Brix seen in the *S. officinarum* group. It was unknown why *S. officinarum* height and stalk number had lower correlations at early stages with Brix while the hybrid group had higher correlations earlier. It would be useful to repeat this study under field conditions to determine if the correlated response holds true in larger scale trials. If this trend is repeatable in commercial material then early selection or crop predictions for Brix could be made using early stalk height and stalk number.

A similar study was conducted by (Silva et al, 2008) who also concluded that plant height and tiller number could be used to estimate yield. Sandhu et al (2012) measured growth and leaf area index throughout the growing season of sugarcane and determined that their correlation with final yield was highest for late-season measurements. For visual ratings of plant vigor, mid-season ratings were the most highly correlated with final yield (Silva et al, 2008; Sandhu et al, 2012). In our study, a linear decrease in correlation between height or stalk number and Brix throughout the season was observed. Possible reasons for differences include that the measurements in our study were actual measurements rather than the subjective visual ratings used in other studies.

Correlations

In both the whole and *S. spontaneum* populations, height and stalk number were negatively correlated, indicating that selection for one trait should have a simultaneous negative influence on the other. But there was no significant correlation for these traits in the hybrid and *S. officinarum* populations. According to Milligan et al (1990a, b), genotypic correlations between stalk number and height were negatively correlated in plant cane (r = -0.33) and first ratoon (r = -0.17), but positively correlated in second ratoon (r = 0.43; Milligan et al, 1990a, b). Kang et al (1983) also found non-significant negative correlations between stalk height and stalk number. Correlations between stalk number and the other mass traits were not as strong.

The correlation between Brix and stalk number in this study were positive in the hybrid population and negative in the whole population, suggesting that the selection of Brix may have a positive or negative response for stalk number depending on population. Since stalk diameter was negatively correlated with stalk number in all populations, it is difficult to make selections for large diameter stalks and high population sugarcane genotypes (James, 1971; Kang et al, 1983; Milligan et al, 1990b; Pisaroglo de Carvalho et al, 2014). Older high Brix genotypes of sugarcane had large stalk diameters (Vijayalakshmi, 1967; Daniels and Roach, 1987). In this study, there were low non-significant positive correlations between stalk diameter and Brix in all subpopulations but the whole population had a significant positive correlation. Other studies disagree, such as Kang et al (1983) found a significantly negative phenotypic correlation and a non-significant genotypic correlation between the traits. Milligan et al (1990b) found significant negative correlations between Brix and stalk diameter in both plant cane (r = -0.64) and first ratoons (r = -0.67). This suggests that stalk diameter was not always indicative of Brix concentration.

Stalk height and fresh biomass were significantly correlated except in *S. spontaneum* group. This indicated that selecting tall plants could result in a corresponding increase in yield of fresh biomass. In this study, stalk height and stalk diameter were positively correlated in *S. officinarum* and *S. spontaneum* populations and negatively or non-significantly correlated in hybrids and weakly correlated in the whole collection. Kang et al (1983) found a non-significant positive correlation between these traits (r = 0.01). Kang et al (1983) and Milligan et al (1990b) found significant negative correlations in plant cane (r = -0.13) and non-significant correlations in the first ratoon (Kang et al, 1983; Milligan et al, 1990b). Pisaroglo de Carvalho et al (2014) found larger positive genotypic correlations between stalk diameters and stalk height. These results indicated that the correlations between these traits were population specific and should be investigated further (Pisaroglo de Carvalho et al, 2014).

Conclusions

The hybrid and check populations had the highest fresh biomass and Brix. *S. spontaneum* population had the highest average stalk number. Dry matter was also the highest among the *S. sponatneum*, which had early plant vigor (stalk height and stalk number) when compared with hybrids and *S. officinarum*. Early height and stalk number were correlated positively with the two important harvest traits, fresh biomass and Brix. The correlations varied by population and crop cycle. The related traits of fresh, dry, and water mass were highly correlated in all populations, but dry biomass and water mass correlated less among the *S. spontaneum* populations. Stalk number and stalk height correlated positively in hybrids in plant cane but not in first ratoon. Stalk height and stalk number were negatively correlated in *S. spontaneum*. In *S. spontaneum*, fresh and dry biomass did not correlate with stalk number. All the accessions in the diversity panel can be divided into two populations based on phenotypic traits, one population with higher stalk number and dry biomass and the other with higher stalk diameter, leaf width and Brix. Individual accessions that were positive extremes from these two populations were identified in this study that could be used in breeding for improved energy cane and sugarcane cultivars.

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