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Genetic Variability and Clustering Patterns of Sugarcane (*Saccharum* spp.) Germplasms with Respect to Sucrose-Related Traits

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Abstract: Fifty-five sugarcane genotypes from around the world were collected and evaluated for potential use as parental material in the USDA ARS Canal Point (CP) sugarcane breeding program in Florida, USA. The genotypes were planted in a trial with four check cultivars on organic soils with four replications, and data were collected for two years [i.e., plant cane (PC) and first ratoon (FR) crops] to assess sucrose-yield-related traits and the cane-yield-related traits in PC. Using a multivariate analysis, variation was observed in all cane—[i.e., stalk weight, stalk population and cane yield] and sugar-yield-related traits [i.e., Brix, Pol, sucrose content and commercial recoverable sucrose (CRS)]. The mean CRS content was greater in the FR crop than the PC crop. Significant variations were attributed to genotype (G), crop cycles (C) and G × C effects. Variations between crop cycles were highly significant for all sucrose yield components, which could complicate the downstream selection of genotypes for sucrose yield. Based on CRS content, genotypes could be grouped into six distinct clusters. Based on plant cane data, cane yield traits (stalk weight, stalk population and cane yield) were used to estimate the breeding values of parents. Of the 55 genotypes, 8 had significantly greater *t*-BLUP values for cane yield, along with CP 00-1101. Combined sucrose yield traits, (Brix, Pol and sucrose content) from the two crops were used to estimate the breeding values of parents. Of the 55 genotypes, 10 genotypes had significantly greater *t*-BLUP values for CRS, along with CP 00-1101, CP 96-1252 and CP 01-2390, and can be considered as elite parents in future breeding efforts. These results provide a foundation for the efficient integration of genetic diversity in developing commercial cultivars, with improved sucrose yields, into the CP sugarcane breeding program.

Keywords: sugarcane; genetic diversity; commercial recoverable sucrose (CRS); best linear unbiased prediction (BLUP); *t*-BLUP

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1. Introduction

Sugarcane (*Saccharum* spp.) is an economically important crop in many tropical and subtropical regions, including the USA. It is the most valuable field crop in South Florida. In 2020, Florida ranked first nationally in the value of sugar produced from sugarcane (i.e., USD 737M), accounting for 50% of domestic sugarcane-derived sugar value. Florida's sugarcane contributes more than 23% of the total sugar produced from the combined yield of sugar beet and sugarcane in the United States [1]. The Canal Point (CP) breeding program, a tripartite collaboration among the USDA-ARS, the University of Florida (UF) and the Florida Sugar Cane League (FSCL), develops cultivars for commercial production in Florida.

High biomass production and sucrose content are important factors in increasing sugar production and reducing the cost per ton of sugar production and, therefore, are the two key selection criteria in the CP breeding program [2]. The CP breeding program has made critical contributions to commercial sugarcane productivity in two major soil types (organic and mineral) [3,4]. Edme et al. [3] demonstrated that between 1968 and 2000, improved cultivars released from the CP breeding program increased commercial recoverable sucrose (CRS, 26.0%), cane tonnage (5.5%) and sugar yield (47.0%) in Florida. In order to further improve the sustainable sugarcane production in Florida, breeding sugarcane cultivars with high performance for yield traits is critical to face the current challenges such as limited farmland area, competition with food crops and biotic and abiotic stresses [5]. In sugarcane, sugar yield (SY) is composed of two complex variables—cane yield (CY) and CRS [6]—thus, gains in SY are preferable through the increase in sucrose content along with CY, as described earlier by Legendre in 1992 [7]. Breeders from other breeding programs, specifically in Australia, also indicated that they increased SY by improving CY over time with negligible gains in CRS due to their high selection pressure on CY and insufficient selection pressure on sucrose yield [8–10]. The sucrose yield may have slow genetic gain [11]. Hence, selecting parents with high CRS content is necessary to make target cross combinations [11]. However, there are certain challenges and limitations in sugarcane genetics when breeding for sucrose yield. One of the key challenges is sugarcane's complex polyploid nature, which complicates genetic manipulation and breeding efforts [11–13]. The lack of a complete reference genome for sugarcane also hinders the identification and manipulation of genes related to sucrose production. In addition, the long breeding cycle of sugarcane, which means it can take several years to develop and evaluate new varieties, poses a challenge in the breeding process. Limited genetic diversity within commercial sugarcane varieties also limits the potential for introducing new traits through traditional breeding methods. Therefore, the characterization, maintenance and utilization of genetic diversity in sugarcane germplasm collections are critical [14]. This diversity is essential for selecting and combining desirable traits [15]. The CP breeding program curates a range of germplasms and actively collects new sugarcane genotypes to add to the collection to increase diversity. The CP breeding program exploits this diverse gene pool for high CRS, the primary trait of interest, as well as biotic and abiotic resistances [5] through strategic mating and subsequent offspring evaluation. Prior to making targeted crosses, a germplasm must be evaluated for commercial agronomic traits, specifically CRS and its related traits such as Brix, Pol and sucrose content, to avoid the downstream plateauing of sugar yield production [3]. Incorporating a foreign germplasm from different regions introduces genetic diversity and may have the potential to improve both sucrose content and cane tonnage. The objective of this study was to evaluate genetic and phenotypic variability in foreign sugarcane germplasms collected from various countries for the CRS content and its related traits and CY and its related traits to select sugarcane genotypes with high CRS content to be used in the CP breeding program.

2. Materials and Methods

2.1. Plant Material, Experimental Design and Data Collection

Fifty-five foreign sugarcane genotypes (Table 1) and four Canal Point (CP) check cultivars (Table 1) were planted in a trial randomized block design with four replications. Each plot consisted of two-row plots with 4.6 m rows and 1.5 m inter-row spacing, 1.5 m between plots and 6.1 m alleys between blocks. The field soil type (USDA-ARS Sugarcane Field Station, 26°52′0.16″ N and 80°37′37.78″ W, Canal Point, FL, USA) was classified as Torry muck (euic, hyperthermic typic haplosaprist) [16], which is a rich organic soil type in Florida's Everglades Agricultural Area (EAA). No fertilizer was added as the mineralization of organic soils is generally high and releases plant-available N to the growing crops [16]. No pesticides were applied during the trial. The trial area was surrounded by ditches which supplied water for seepage irrigation. All test materials were placed to fill the plot length in the furrow by hand, chopped into billet lengths and then covered mechanically.

The trial was planted on 5 October 2015 and harvested as a plant cane crop (PC) on 16 December 2016 and as a first ratoon crop (FR) on 19 January 2018.

Table 1. List of foreign sugarcane genotypes used in this study and their country of origin.

Cultivar	Country of Origin	Cultivar	Country of Origin
BR 97-1004	Dominican Republic	N 23	South Africa
BR 97-2001	Dominican Republic	N 25	South Africa
CC 48-0074	Colombia	N 37	South Africa
CC 84-75	Colombia	N 39	South Africa
CC 85-92	Colombia	N 41	South Africa
CC 93-4418	Colombia	Q 135	Australia
CG 00-102	Guatemala	Q 152	Australia
CG 05-1024	Guatemala	Q 153	Australia
CG 05-1292	Guatemala	Q 155	Australia
CG 96-01	Guatemala	Q 158	Australia
CG 97-100	Guatemala	Q 160	Australia
CR 00-0026	Dominican Republic	Q 167	Australia
CR 03-1009	Dominican Republic	Q 171	Australia
CR 93-1007	Dominican Republic	Q 172	Australia
CR 95-1007	Dominican Republic	Q 183	Australia
CR 97-1007	Dominican Republic	Q 190	Australia
GX 1	China	Q 191	Australia
GX 11	China	Q 196	Australia
GX 17	China	Q 197	Australia
GX 7	China	Q 200	Australia
ISD 20	Bangladesh	Q 201	Australia
ISD 27	Bangladesh	Q 208	Australia
ISD 28	Bangladesh	TUCCP 77-42	Argentina
ISD 29	Bangladesh	CB 41-76	Brazil
R 570	Reunion	CP 00-1101	USA
ROC 15	Taiwan	CP 01-2390	USA
S 97-19	Argentina	CP 78-1628	USA
SP 90-1638	Brazil	CP 96-1252	USA
SP 91-1049	Brazil		
SP 91-3011	Brazil		
SP 97-19	Brazil		

The four CP sugarcane check cultivars were CP 96-1252 [17], CP 00–1101 [18], CP 78-1628 [19] and CP 01-2390. CP 96-1252 is one of the highest-performing and most widely grown commercial sugarcane cultivars in Florida in both muck and sand soils [20]. CP 00-1101 has high sucrose yield with late maturation and is non-flowering under natural conditions in south Florida [18]. It is one of three commercial check cultivars regularly used for sucrose and sugar yield comparisons in the CP Cultivar Development Program in muck soils. CP 78-1628 was a commercial check cultivar in sand soils in Florida at the time of this study. Despite not being a commercial cultivar, CP 01-2390 is a favorable parent for developing offspring with high yield potential in the CP program but was not publicly

released due to smut susceptibility (caused by *Sporisorium scitamineum* (Syd., M. Piepenbr., M. Stoll & Oberw)) [21].

Daily minimum temperatures during pre-sampling were obtained from an onsite weather station for the two crops (PC and FR) (Figure 1). Four weeks before PC sampling (12 November through 12 December 2016), the daily average temperature was 16.7 °C, with the lowest temperatures ranging from 10 °C to 13.3 °C. The daily average minimum temperature preceding the ratoon crop harvest and sucrose analysis (19 December 2018 to 19 January 2019) was 16.3 °C, with the lowest temperatures ranging from 12.2 °C to 15.0 °C, and monthly weather data during the growing period are presented in Figure 1.

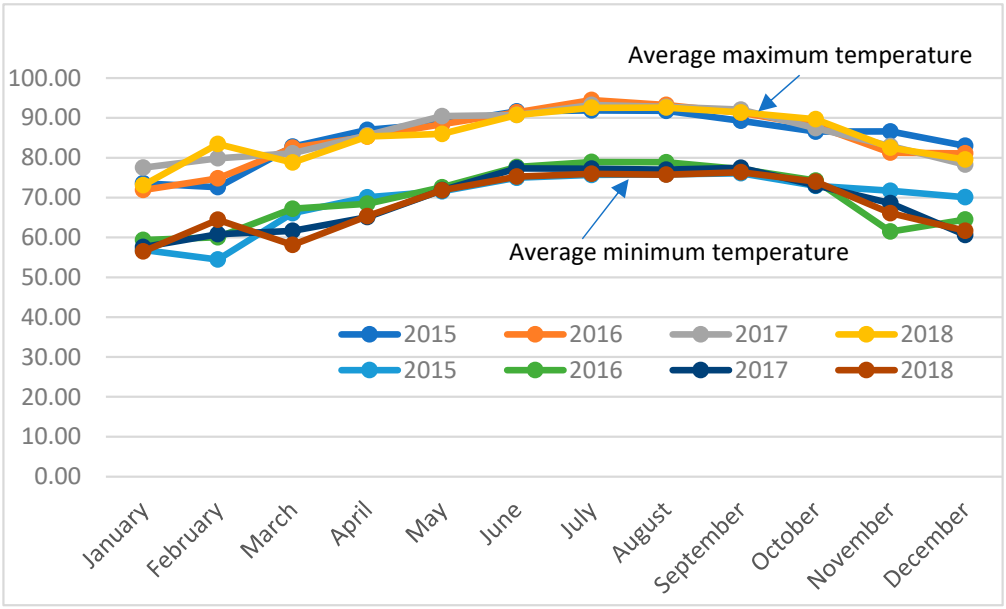

Figure 1. Average maximum and minimum monthly temperature during 2015 to 1018 at Canal Point, FL, USA.

Each plot was counted in August 2016 to estimate the number of millable stalks per hectare for the PC crop only. For both crops (PC and FR), 10-stalk bundles were collected from each plot, weighed and crushed in a roller mill to extract the juice. The juice was analyzed for Brix % and optical rotation (Pol; International Sugar Scale (ISS) units, °Z) using NIR spectrometry [FOSS 5000 beverage module NIR spectrometer (FOSS North America)]. Fiber content was arbitrarily assumed to be 100 g. kg^{-1} [9]. These inputs were used to calculate the theoretical recoverable sucrose (TRS, $kg{\cdot}Mg^{-1}$) as described by Legendre [7]. A correction factor of 0.86 was applied to the TRS value to approximate the commercial recoverable sucrose (CRS, $kg{\cdot}Mg^{-1}$) [10]. The cane yield for the PC crop ($t.ha^{-1}$) was estimated as follows:

$$\text{Cane yield } (t.ha^{-1}) = [\text{stalk weight } (Kg\ stalk^{-1}) \times \text{stalk number } (stalks\ ha^{-1})]/1000.$$

2.2. Data Analyses

2.2.1. Analysis of Variance

Tests to determine the homogeneity of variance and to detect inconsistencies and outliers for all variables were performed across the crop and with individual crops using Levene's test based on the residuals using JMP 17.0.0. Estimates of the variance components for genotypes using stalk weight; cane yield using plant cane data; Brix, Pol, and sucrose content; and CRS using both plant canes and the first ratoon crops were made using the REML model on JMP 17 (SAS Institute Inc., Cary, NC, USA) [22].

Variance components for individual crops were estimated using the following model:

$$Y_{ijk} = \mu + g_i + block_j(rep_k) + e_{ijk}$$

where Y_{ijk} = traits measured in the plot of genotype 'i' in block 'j' and replicate 'k'; μ = the trial mean; g_i = the random effect of genotype 'i'; $block_j(rep_k)$ = the random effect of the incomplete block 'j' within the 'k' replicate; and e_{ijk} = the residual associated with genotype 'i', block 'j' and replicate 'k'.

2.2.2. Prediction of Genotypic Value

The genetic merit of each genotype was predicted by Best Linear Unbiased Predictor (BLUP) values weighed by the studentized value (*t*-BLUP), which measures the ratio between the BLUP and its standard error [23]. The assignment as "elite genotypes" was based on the significance of a *t*-statistics test on the genotype BLUP values ($p < 0.05$). Based on this threshold level, the genotypes were classified as elite with scores '1' ($t > 1.67$), inferior with scores '−1' ($t < 1.67$), and intermediate with scores '0' ($1.67 < t > -1.67$) [24,25].

2.2.3. Cluster Analysis for CRS Content

The Shapiro–Wilk test was used to verify the normality of residues before performing the cluster analysis of the CRS content. Cluster analysis for CRS content was performed over all crop harvests using Ward's method [26] by scoring the squared Euclidian distance in JMP Pro 17.0.0 software [22]. The cluster analysis was used to generate a scatter plot matrix of PC_CRS and FR_CRS for the 55 sugarcane genotypes and four check cultivars.

2.2.4. Flowering Trait Data

Like any other breeding program, flowering is important to develop new cultivars with desired traits. To reveal the flowering behavior, flowering data (i.e., days to flower, absence/presence, and number of flowers per plant) were collected from all genotypes grown on the parental pots (38 L) naturally and in the photoperiod house during the 2015–2018 crossing season.

3. Results and Discussion

3.1. Variance Components for Cane- and Sucrose-Yield-Related Traits

Variance components were estimated for cane yield (CY) traits using PC data only and for sucrose-yield-related traits using combined data (PC and FR) using the restricted maximum likelihood (REML) method in JMP Pro. 17.

In the analysis of CY and its related traits, variations in CY and its components, i.e., stalk weight (StWt) and stalk populations (StPop), were attributed to the genotypic main effect, which were highly significant, indicates that the variation observed can be partitioned to differences among genotypes with high and low CY in the germplasm evaluated. Genotypes accounted for a significant amount (68.6%) of variation for StWt followed by a significant amount (77.9%) of variation for StPop and a significant amount (46.4%) of variation for CY (Table 2). Genotypes accounted for a significant amount of variation for Brix, Pol, sucrose content and CRS, explaining 50.5, 52.4, 52.1 and 56.2% of the total variation, respectively. (Table 2). Variance components were estimated across the crops data using sucrose-yield-related traits, Brix, Pol, sucrose and CRS. Variance components for CRS were further analyzed, and the variances found were attributed to crop age and genotype, and their interaction was highly significant (Table 2).

The CY ($t.ha^{-1}$) mean was 173.2 and the CV was 28.6% in the plant cane only. The CRS mean was 192.2 $kg{\cdot}Mg^{-1}$ (CV = 13.5%) and 216.0 $kg{\cdot}Mg^{-1}$ (CV = 9%) for the PC and FR crops, respectively (Table 3). The significantly higher CRS values in the FR crop may have been due to the crop age or low temperature (14.2 °C and 16.8 °C for the FR and PC crops, respectively) experienced before sampling, or maybe both, which is in agreement with the study of Kang et al. 1987. Kang et al. [27] also found higher genotype by crop effects on both sucrose concentration and sugar yield (tons per hectare). The significant variation in

CRS found in this study due to the crop cycle may complicate parental selection for sucrose yield from the trialed germplasms. Masri and Amein [28] and Milligan et al. [29] found that sugarcane genotypes varied more significantly for sucrose-yield-related traits in second ratoon crops than PC and FR crops. Shanti et al. [30] reported that lower air temperatures during the maturation phase to harvest could change enzymatic activities, favoring high sucrose accumulation in stalks. They also reported that an average daily temperature of 12–14 °C would be more desirable for proper ripening, and drastic declines in temperature below 8 °C may negatively affect enzyme activities and reduce sugar recovery. The results observed in this study (i.e., increased FR sucrose production at 14.2 °C) agree with the study of Shanthi et al. [30].

Table 2. Covariance parameter estimates for stalk weight (StWt), stalk population (StPop) and cane yield (CY) from plant cane and Brix, Pol, sucrose and CRS from two combined crops for genotypes and residual error (± SE) for the organic soil.

Traits	Genotype Estimate	Residual Estimate	Percent of Total	
			Genotype	Residual
StWt	0.03 ± 0.01 ***	0.01 ± 0.01 ***	68.6	31.4
StPop	8,767,278 ± 17,362,207 ***	24,710,510 ± 2,344,952 ***	77.9	22.1
CY	193.36 ± 45.54 ***	214.89 ± 20.44 ***	46.4	53.6
Brix	0.910 ± 0.18 ***	0.72 ± 0.05 ***	50.5	49.5
Pol	24.63 ± 4.93 ***	16.03 ± 1.14 ***	52.4	47.6
Sucrose	1.28 ± 0.26 ***	1.18 ± 0.08 ***	52.1	47.9
CRS	389.68 + 85.65 ***	303.24 + 29.70 ***	56.2	43.8
		CRS only		
Source	DF	F Ratio		
Genotype (G)	58	13.29 **		
Crop (C)	1	354.77 **		
G×C	58	1.80 *		

* significant at $p < 0.05$, ** significant at $p < 0.01$, *** highly significant at $p < 0.001$.

Table 3. Mean cane yield (CY, t.ha^{-1}), commercial recoverable sucrose (CRS) content of the genotype in plant cane (PC) and first ratoon (FR) crops and their cluster groups.

Cultivar	CY (t.ha^{-1})	CRS (kg·Mg^{-1})		Cluster	Cultivar	CY (t.ha^{-1})	CRS (kg·Mg^{-1})		Cluster
	PC	PC	FR			PC	PC	FR	
BR 97-1004	123.9	193.6	226.6	1	N 23	206.9	195.2	230.7	3
BR 97-2001	134.2	183.4	210.9	1	N 25	200.4	97.1	144.2	6
CC 48-0074	186.6	183.5	195.1	5	N 37	144.6	218.7	251.5	4
CC 84-75	196.5	168.8	192.0	5	N 39	167.9	198.9	213.7	2
CC 85-92	76.4	191.6	218.8	1	N 41	155.2	187.6	220.4	1
CC 93-4418	157.7	175.8	200.9	5	Q 135	159.8	189.9	224.2	1
CG 00-102	142.8	218.3	227.3	3	Q 152	186.6	197.0	218.7	1
CG 05-1024	201.1	175.0	217.2	1	Q 153	182.5	223.7	240.0	4
CG 05-1292	196.2	189.3	220.8	1	Q 155	169.4	228.2	245.0	4
CG 96-01	136.3	204.6	252.2	4	Q 158	169.4	221.3	225.9	3
CG 97-100	156.3	200.5	213.7	2	Q 160	249.4	168.4	214.1	1
CR 00-0026	86.9	171.0	205.1	2	Q 167	208.8	185.7	210.5	1
CR 03-1009	183.0	159.8	218.1	5	Q 171	146.5	200.9	232.4	3
CR 93-1007	203.9	172.6	218.7	1	Q 172	153.3	207.5	22.7	3

Table 3. *Cont.*

Cultivar	CY (t.ha^{-1})	CRS (kg·Mg^{-1})		Cluster	Cultivar	CY (t.ha^{-1})	CRS (kg·Mg^{-1})		Cluster
	PC	PC	FR			PC	PC	FR	
CR 95-1007	151.7	200.5	223.7	1	Q 183	196.6	200.7	233.2	3
CR 97-1007	144.1	219.3	233.6	3	Q 190	236.7	184.4	237.8	1
GX 1	189.4	182.1	221.5	1	Q 191	245.6	180.8	230.3	1
GX 11	148.2	206.0	229.9	3	Q 196	204.7	188.4	209.4	1
GX 17	209.8	196.0	221.7	1	Q 197	214.6	177.3	230.3	1
GX 7	193.8	176.2	223.3	1	Q 200	202.9	206.9	234.1	3
ISD 20	124.4	168.7	191.1	5	Q 201	169.3	180.9	224.4	1
ISD 27	189.9	186.0	219.6	1	Q 208	216.9	195.2	214.4	1
ISD 28	112.0	172.4	199.7	5	TUCCP 77-42	168.2	199.9	194.9	2
ISD 29	157.0	163.1	190.7	5	CB 41-76	113.7	171.1	190.2	5
R 570	132.6	203.6	225.8	1	CP 00-1101	221.3	247.5	252.4	4
ROC 15	134.1	200.1	234.8	3	CP 01-2390	131.7	205.6	231.1	3
S 97-19	95.8	203.5	209.7	2	CP 78-1628	190.9	201.9	210.8	1
SP 90-1638	233.9	186.6	225.5	1	CP 96-1252	187.3	202.4	227.2	1
SP 91-1049	196.9	204.3	210.5	2					
SP 91-3011	152.9	180.8	230.2	1	Mean	173.2	192.2	216.0	
SP 97-19	149.7	208.2	205.0	2	CV	28.6	11.2	8.4	

3.2. Cluster Analysis Based on CRS Content

The scatter plot matrix (Figure 2) illustrates the grouping of genotypes based on their CRS content in the PC and FR crops. The analysis grouped the 55 genotypes along with four check cultivars into five significant clusters, and 1 genotype, N25, was located far apart from any of the groups (Figure 2). The group frequency distribution of genotypes within the six scatter plot clusters were (1) twenty-one genotypes, (2) seven genotypes, (3) eleven genotypes, (4) five genotypes, (5) five genotypes and (6) one genotype (Table 3).

The genotypes in Group 4 (CG 96-01, N 37, Q 153, Q 155 and CP 00-1101) were the highest yielding genotypes in both PC and FR, followed by Group 3 including CP 01-2390, and had CRS values greater than the trial mean in both crops. The genotypes in Groups 3 and 4 were not influenced by the harvest time, as illustrated by their inclusion in the upper right matrix (i.e., high PC yield and high FR yield). Group 1 including CP 78-1628 and CP 96-1252 had genotypes with average CRS values, the Group 2 genotypes had above-average CRS in the PC crop but below-average CRS in the FR crop, and the poorest CRS values were derived from the Group 5 genotypes. One genotype (i.e., N 25) had the lowest CRS yields in both the PC and FR crops—placing it by itself in Group 6 (Table 3). The mean CRS values were higher in the FR crop than the PC crop, with CP 00-1101 having the greatest value (252.4 kg·Mg^{-1}), followed by CG 96-01 (252.2 kg·Mg^{-1}), N 37 (251.5 kg·Mg^{-1}), Q 155 (245 kg·Mg^{-1}) and Q 153 (240 kg·Mg^{-1}) in the FR crop (Table 3). An earlier study also showed that Q 155 ranked 1st, CP 00-1101 ranked 4th and TUCCP 77-042 ranked 110th in terms of CRS content out of 1084 genotypes evaluated in Florida sand soil in 2010–2011 (personal communication), which is in agreement with this study. Generally, ratoon crops had higher sucrose per ton yields but lower tonnage [31,32]. The sucrose content in CP 89-2143 changed over the crop cycles and was higher in the ratoon crops, demonstrating a yield increase with crop age and over the course of the harvest season [33].

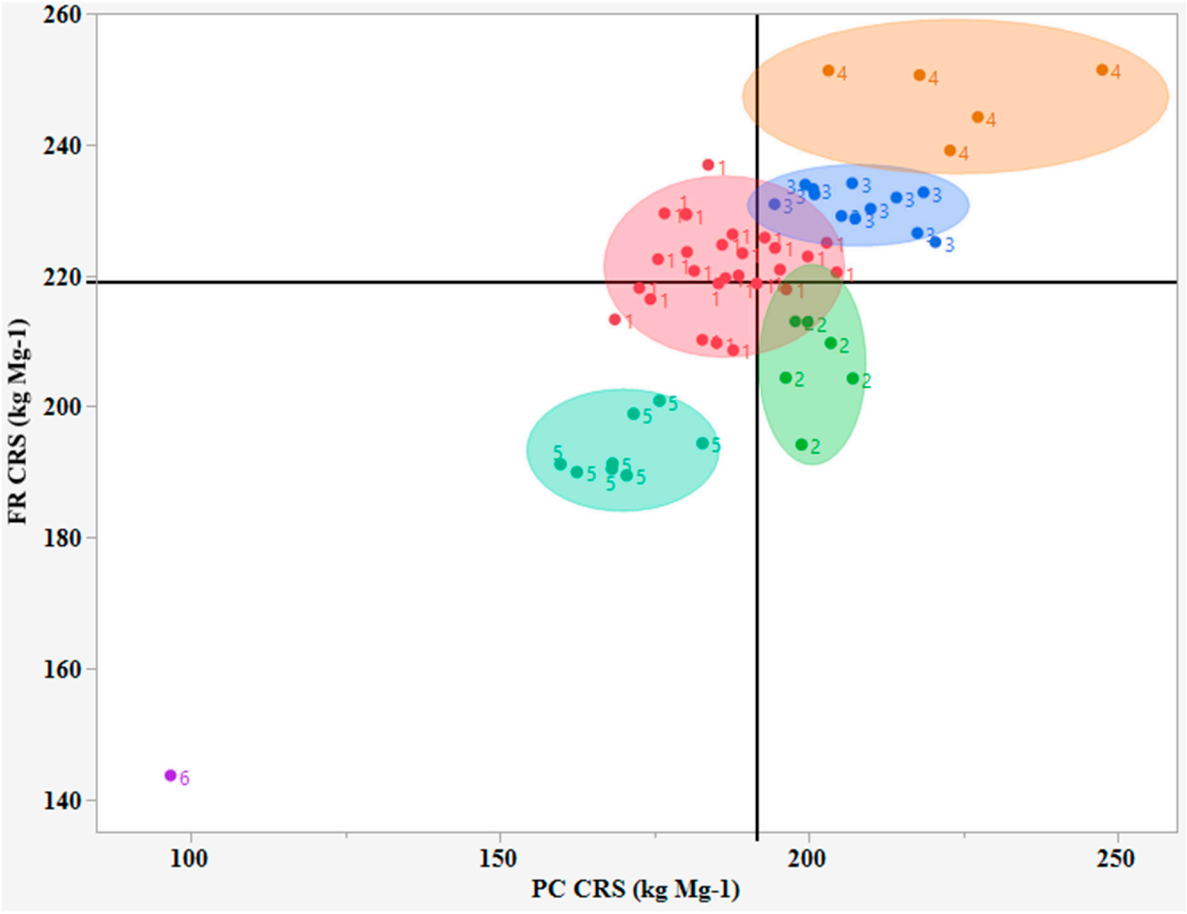

Figure 2. Scatter plot graph based on cluster analysis of commercial recoverable sucrose in plant cane crop (PC_CRS) and in first ratoon crop (FR_CRS) of the genotypes along with four checks. Assignment of trialed genotypes within clusters is described in Table 3.

3.3. Estimates of Genotypic Value via BLUP Analysis Using Sucrose, Cane Yield Related Traits

Genotypic variances and residual effects were highly significant ($p < 0.001$) for all CY and sucrose-yield-related traits in the overall analysis, indicating that genotypes differentially contribute to both yield variables.

Determining the genotypic breeding value is critical to increasing the combining ability and to plan target crosses between various sugarcane germplasms and maximize the probability of introgressing favorable alleles from both parents [23]. Usually, breeders select new parents in sugarcane breeding programs based on their phenotypic performance for key traits like yield, sucrose content and disease resistance. However, as CY has low heritability, the direct performance of any clone cannot provide an accurate prediction of its breeding value for this variable. As described by Zhou and Mokwele [34], BLUP estimates the breeding value relative to the population mean. In this study, *t*-statistics of the BLUP analysis were used to generate genotype scores for CY and its related traits, i.e., stalk weight (StWt) and stalk population (StPop), and also sucrose-yield-related traits, i.e., Brix, Pol and sucrose content as a measure of breeding reliability, as previously described by Yan et al. [25] and by Yan and Rajcan [24]. Genotypic scores that were significantly higher or lower relative to the grand means are presented in Tables 4 and 5.

According to the genotypic score shown in Table 4 for stalk weight, 13 genotypes had scores of '1' and were classified as elite genotypes, while 29 genotypes had scores of '−1' and were classified as non-elite genotypes. Sixteen of the foreign genotypes had scores of '0' and would be classified as intermediate genotypes in future breeding efforts [21]. The check cultivar CP 00-1101 had a score of '1', whereas CP 01-2390, CP 78-1628 and CP 96-1252 had scores of '−1'. For StPop, 17 genotypes had scores of '1' and were classified as elite parents,

while 27 genotypes had scores of '−1' and were classified as non-elite genotypes. Eleven of the foreign genotypes had scores of '0' and would be classified as intermediate genotypes for StPop. The check cultivars CP 00-1101, CP 78-1628 and CP 96-1252 had scores of '1', and CP 01-2390 had a score of '−1'. For CY, 9 genotypes had scores of '1' and were classified as elite genotypes, and 26 genotypes had scores of '−1' and were classified as non-elite genotypes. Twenty-two of the foreign genotypes had scores of '0' and would be classified as intermediate genotypes for CY in future breeding efforts. Only one check cultivar, CP 00-1101, had a score of '1'; two cultivars, CP 78-128 and CP 96-1252, had scores of '0'; and check cultivar CP 01-2390 had a score of '−1' (Table 4).

Table 4. Genotype scores obtained for stalk weight (StWt), stalk population (StPop) and cane yield (CY).

Genotypes	StWt [Ŧ]	StPop	CY	Genotypes	StWt	StPop	CY
BR 97-1004	1	−1	−1	N 23	0	0	0
BR 97-2001	1	−1	−1	N 25	0	0	0
CC 48-0074	−1	1	0	N 37	−1	−1	−1
CC 84-75	−1	1	0	N 39	−1	1	−1
CC 85-92	0	−1	−1	N 41	−1	1	−1
CC 93-4418	1	−1	−1	Q 135	−1	0	−1
CG 00-102	−1	−1	−1	Q 152	−1	1	0
CG 05-1024	1	−1	0	Q 153	−1	1	0
CG 05-1292	0	0	0	Q 155	−1	1	−1
CG 96-01	−1	−1	−1	Q 158	−1	0	−1
CG 97-100	0	−1	−1	Q 160	−1	1	1
CR 00-0026	−1	−1	−1	Q 167	−1	1	1
CR 03-1009	1	−1	0	Q 171	−1	0	−1
CR 93-1007	1	−1	0	Q 172	0	−1	−1
CR 95-1007	1	−1	−1	Q 183	0	−1	0
CR 97-1007	0	−1	−1	Q 190	1	−1	1
GX 1	0	−1	0	Q 191	1	1	1
GX 11	0	0	0	Q 196	−1	1	0
GX 17	1	−1	1	Q 197	1	−1	1
GX 7	1	−1	0	Q 200	−1	1	0
ISD 20	−1	1	0	Q 201	−1	0	−1
ISD 27	−1	1	0	Q 208	−1	1	1
ISD 28	−1	−1	−1	TUCCP 77-42	−1	0	−1
ISD 29	−1	−1	−1	CB 41-76	−1	−1	−1
R 570	0	−1	−1	CP 00-1101	0	1	1
ROC 15	0	−1	−1	CP 01-2390	−1	−1	−1
S 97-19	−1	−1	−1	CP 78-1628	−1	1	1
SP 90-1638	0	1	1	CP 96-1252	−1	1	0
SP 91-1049	1	−1	0				
SP 91-3011	−1	0	0				
SP 97-19	−1	−1	−1				

Note: [Ŧ] A score of '1' means significantly better than the grand mean; '−1' means significantly poorer than the grand mean; and '0' means not significantly different from the grand mean.

Table 5. Genotype scores obtained for Brix, Pol and sucrose content and commercial recoverable sucrose (CRS) content using overall data.

Genotypes	Brix ∓	Pol	Sucrose	CRS	Genotypes	Brix	Pol	Sucrose	CRS
BR 97-1004	0	0	0	0	N 23	0	0	0	0
BR 97-2001	−1	−1	−1	−1	N 25	−1	−1	−1	−1
CC 48-0074	−1	−1	−1	−1	N 37	1	1	1	1
CC 84-75	−1	−1	−1	−1	N 39	−1	−1	−1	0
CC 85-92	−1	−1	0	0	N 41	−1	−1	−1	−1
CC 93-4418	−1	−1	−1	−1	Q 135	−1	0	0	−1
CG 00-102	1	1	1	1	Q 152	−1	−1	−1	0
CG 05-1024	−1	−1	−1	−1	Q 153	1	1	1	1
CG 05-1292	−1	−1	−1	−1	Q 155	1	1	1	1
CG 96-01	1	1	1	−1	Q 158	1	1	1	1
CG 97-100	−1	−1	0	0	Q 160	−1	−1	−1	−1
CR 00-0026	−1	−1	−1	−1	Q 167	−1	−1	−1	−1
CR 03-1009	−1	−1	−1	−1	Q 171	0	0	0	1
CR 93-1007	−1	−1	−1	−1	Q 172	1	1	1	1
CR 95-1007	0	0	0	0	Q 183	1	1	1	0
CR 97-1007	1	1	1	1	Q 190	0	0	0	−1
GX 1	1	1	1	−1	Q 191	0	0	0	0
GX 11	0	1	1	0	Q 196	−1	−1	−1	−1
GX 17	−1	0	0	0	Q 197	−1	−1	−1	−1
GX 7	0	−1	−1	−1	Q 200	1	1	1	0
ISD 20	−1	−1	−1	−1	Q 201	1	1	1	−1
ISD 27	−1	−1	−1	−1	Q 208	−1	−1	−1	0
ISD 28	−1	−1	−1	−1	TUCCP 77-42	−1	−1	−1	−1
ISD 29	−1	−1	−1	−1	CB 41-76	−1	−1	−1	−1
R 570	0	0	0	1	CP 00-1101	1	1	1	1
ROC 15	1	1	1	0	CP 01-2390	1	1	1	1
S 97-19	1	1	1	0	CP 78-1628	−1	−1	−1	1
SP 90-1638	0	0	0	0	CP 96-1252	1	1	1	1
SP 91-1049	0	1	1	1					
SP 91-3011	0	0	0	−1					
SP 97-19	0	0	0	0					

Note: ∓ A score of '1' means significantly better than the grand mean; '−1' means significantly poorer than the grand mean; and '0' means not significantly different from the grand mean.

Eight genotypes, Q 160, Q 167, Q 190, Q 191, Q 197, Q 208, GX 17 and SP 90-1638, considered as elite genotypes, all had scores of '1', along with CP 00-1101 and CP 78-1628, for cane yield. All of them had cores of '1' for stalk population, except Q 190 and Q 197, which had scores of '−1'; however, they had scores of '0' for stalk weight. Fifteen of the intermediate parents out of twenty-two had either scores of '1' or 0' for stalk population or for stalk weight, which was in agreement with a study conducted on organic soil by Zhao et al. [21], where they found that StPop had a positive correlation with CY (R^2 = 0.548) and did not show any positive correlation with StWt. In addition, Sandhu and Saini, 1997, and Abu-Ellail, 2020, reported StPop as a very important trait correlated to CY.

Genotypic scores using overall Brix, Pol and sucrose content and CRS data are shown in Table 5. Among the 55 evaluated genotypes, 8 genotypes had scores of '1' for four traits and were classified as 'elite genotypes'; 3 had scores of '1' for Brix, Pol and sucrose and had scores of '0' for CRS. Six genotypes had scores of '0' for all four traits and were considered as intermediate parents. The check cultivars CP 00-1101, CP 01-2390 and CP 96-1252 also had higher genotypic scores relative to the grand means for all three traits, with all scoring '1' and being classified as elite parents. Meanwhile, CP 78-1628 had a score of '−1' for Brix, Pol and sucrose and scored '1' for CRS. Twenty-two genotypes had scores of '−1' for all four traits and were classified as non-elite parents in future breeding efforts [24]. Determining genotypic breeding values is critical to planning target crosses between various sugarcane germplasms and maximizing the probability of introgressing favorable alleles from both parents [35]. Among 55 genotypes, 10 genotypes (CG 00-102, CR 97-1007, N 37, Q 153, Q 155, Q 158, Q 171, Q 172, R 570 and SP 91-1049) were considered as elite parents for CRS for future breeding. Only two genotypes, SP 91-1049 and Q 153, out of eight genotypes had scores of '1' for CRS and '0' for CY, and rest of these had scores of '−1' for cane yield and were considered as non-elite parents, except for cane yield.

3.4. Flowering Behavior

The control of flowering is not only important for reproduction but also plays a key role in making target crosses. To reveal the flowering behavior, flowering data (i.e., days to flowering, absence/presence and number flowers per plant) were collected from all genotypes grown in pots (38 L) naturally and in the photoperiod house during 2015–2018. Among the tested genotypes, only 15 genotypes flowered, and the flowering percentage ranged from 0.2% (CC 85-92 and Q 200) to 5.0% for genotype CR 03-1009. Thirty-nine genotypes did not flower naturally in Florida's photoperiod environment (Table 6). Among the 39 non-flowering genotypes, 16 were grown in the photoperiod house, and only 7 of them subsequently flowered in response to photo induction (i.e., a constant photoperiod of 12 h 30 for 45 days, followed by a longer declining period of 45 to 60 s day^{-1}). Only one genotype (CG 96-01) from Group 4 (Table 4 and Figure 1) flowered naturally, two genotypes (N 37 and CP 00-1101) flowered under photoperiod conditions, and another two genotypes (Q 153 and Q 155) did not flower under any conditions. Among the 13 genotypes represented in cluster three (Figure 1), only two genotypes (Q 183 and SP 91-1049) flowered naturally (Table 6). In this study, most of the genotypes in Group 4 with high sucrose content did not flower under Florida's natural photoperiod condition, which agrees with the study of Khokar et al. [35]. However, in many plant species, high sucrose content is thought to promote floral transition as a signal molecule in general [36]. In sugarcane, the flowering of a genotype was reported to be controlled by both innate genetic makeup as well as other physiological stress conditions caused by the environment [37,38]. The study suggests that the genotypes that flowered naturally under the natural photoperiod can be used in the breeding program. Shy flowering varieties required artificially manipulated light to induce flowering in Canal Point. This was expected as sugarcane flowering behavior is complex and determined by many factors [38,39].

Table 6. Flowering status of sugarcane genotypes grown in pots and in photo induction house in 2015–2018.

Genotypes	Number of Flower/Plants *		Genotypes	Number of Flower/Plants	
	Natural Condition	Photo Induction		Natural Condition	Photo Induction
BR 97-1004	3.4	-	SP 97-19		-
BR 97-2001	0.9	-	N 23	0.0	-
CC 48-0074	0.0	-	N 25	0.0	0.0
CC 84-75	0.0	-	N 37	0.0	1.8

Table 6. *Cont.*

Genotypes	Number of Flower/Plants *		Genotypes	Number of Flower/Plants	
	Natural Condition	Photo Induction		Natural Condition	Photo Induction
CC 85-92	0.2	-	N 39	3.7	-
CC 93-4418	0.0	-	N 41	0.0	0.0
CG 00-102	0.0	0.0	Q 135	0.0	-
CG 05-1024	2.7	-	Q 152	0.0	-
CG 05-1292	0.5	-	Q 153	0.0	-
CG 96-01	3.5	-	Q 155	0.0	-
CG 97-100	0.0	-	Q 158	0.0	-
CR 00-0026	0.0	-	Q 160	0.0	-
CR 03-1009	5.0	-	Q 167	0.0	-
CR 93-1007	0.0	-	Q 171	0.0	-
CR 95-1007	1.9	-	Q 172	0.0	-
CR 97-1007	0.0	-	Q 183	0.0	0.0
GX 1	0.0	-	Q 190	0.0	-
GX 11	0.0	-	Q 191	0.0	0.0
GX 17	0.0	-	Q 196	0.0	0.2
GX 7	0.0	1.3	Q 197	0.0	-
ISD 20	0.0	-	Q 200	0.2	0.0
ISD 27	0.5	-	Q 201	0.0	-
ISD 28	0.3	-	Q 208	0.0	0.0
ISD 29	0.0	-	TUCCP 77-42	4.0	0.0
R 570	0.0	0.0	CB 41-76	0.0	-
ROC 15	0.0	-	CP 00-1101	0.0	0.2
S 97-19	-	-	CP 01-2390	3.3	-
SP 90-1638	0.0	0.0	CP 78-1628	5.8	-
SP 91-1049	0.4	0.4	CP 96-1252	4.5	-
SP 91-3011	0.7	1.0			

* Average number of flowers per plant grown in 2015 to 2018; '-' means that genotypes were not planted in photoperiod.

4. Conclusions

Fifty-five sugarcane genotypes differed in terms of cane yield in plant cane and sucrose-yield-related traits in both crops. Eight genotypes, GX 17, Q 160, Q 167, Q 191, Q 190, Q 197, Q 208 and SP 90-1638, were considered as elite genotypes, as they all had scores of with '1', along with CP 00-1101 and CP 78-1628 for cane yield. Based on a *t*-BLUP comparison using across crop data, 14 genotypes from the 55 tested had scores of '1' for Brix, Pol and sucrose, and 10 genotypes (CG 00-102, CR 97-1007, N 37, Q 153, Q 155, Q 158, Q 171, Q 172, R 570 and SP 91-1049) were considered as elite parents for CRS yield. The genotypes also differed in terms of sucrose content and CRS yield in the plant cane and first ratoon crops. In the first ratoon crops, genotypes had significantly higher mean CRS yields than in the plant cane crops. Clustering analysis showed some genotypes with high CRS yield based on the plant cane crops and first ratoon crops; these would likely be valuable sources of sucrose-related traits in the CP breeding program.

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