agriculture

MDPI

Article

Selection of New Sugarcane Genotypes for Sandy Soils in Florida with Enhanced Sucrose Content

Orlando Coto Arbelo [1,*], Aliya Momotaz [1], Hardev S. Sandhu [2], Sushma Sood [1], Wayne Davidson [3], Miguel Baltazar [3] and Duli Zhao [1]

[1] USDA-ARS Sugarcane Field Station, Canal Point, FL 33438, USA; aliya.momotaz@usda.gov (A.M.); sushma.sood@usda.gov (S.S.); dulizhao22@gmail.com (D.Z.)
[2] Everglades Research and Education Center, University of Florida, Belle Glade, FL 33430, USA; hsandhu@ufl.edu
[3] Florida Sugar Cane League, Inc., Clewiston, FL 33440, USA; caneagronomist@gmail.com (W.D.); miguel.baltazar@usda.gov (M.B.)
* Correspondence: orlando.cotoarbelo@usda.gov

Abstract: The selection of sugarcane genotypes with high sucrose content and good ratooning ability (RA) is an important objective of the Canal Point breeding program to improve the current profitability levels of the Florida sugarcane industry. In this study, thirteen test sugarcane genotypes and three checks were evaluated in three sand locations, Pahokee Produce Inc. (PP), Townsite farm (TS), and Lykes Brothers Inc. (PF), during three crop cycles (plant cane, first ratoon, and second ratoon). Multi-environment best linear unbiased predictors (BLUPs) were highly significant for commercially recoverable sucrose (CRS) and not significant for cane yield (CY) among genotypes. The ANOVA based on the RA values produced significant genotypic effects but a reduced RA diversity among the genotypes. The simultaneous selection for BLUP_CRS, BLUP_CY yield, and RA identified CP 14-4165 and CP 13-2340 as the most outstanding genotypes. The BLUP_GGE biplots method showed that the PP location was the most discriminative for BLUP_CY, whereas the TS was the ideal location. For BLUP_CRS, the three locations had similar abilities to discriminate genotypes and were positively and strongly correlated. The which-won-where graph indicated that CP 13-2340 showed the highest BLUP_CRS levels in TS and PP locations, while CP 14-4165 and CP 14-4588 were the top genotypes in the PF location. The results suggest that selecting genotypes with high CRS values is possible without compromising the genotype discrimination for CY. CP 14-4165 and CP 13-2340 are resistant to most diseases and genetically diverse.

Keywords: sugarcane genotypes; commercial recoverable sucrose (CRS); cane yield; ratooning ability; BLUP-GGE

Citation: Coto Arbelo, O.; Momotaz, A.; Sandhu, H.S.; Sood, S.; Davidson, W.; Baltazar, M.; Zhao, D. Selection of New Sugarcane Genotypes for Sandy Soils in Florida with Enhanced Sucrose Content. *Agriculture* **2023**, *13*, 1079. https://doi.org/10.3390/agriculture13051079

Academic Editor: Paulo Mazzafera

Received: 17 April 2023
Revised: 11 May 2023
Accepted: 12 May 2023
Published: 18 May 2023

1. Introduction

Sugarcane (*Saccharum* spp.) is a preferred crop for sugar production worldwide. Florida is the top sugarcane-producing state in the United States, accounting for 1.89 million tons of sugar from sugarcane grown on over 160,929 ha during the 2021–2022 cropping season [1]. New sugarcane cultivars are released in Florida as part of a cooperative sugarcane cultivar development program, hereafter referred to as the Canal Point (CP) program. The CP program is a successful collaborative research effort among the USDA-ARS, the University of Florida, and the Florida Sugar Cane League. The CP program is focused on the selection of new genotypes with broad adaptation to the two major soil types (muck and sand). In 2021, 46,687 ha of sugarcane was grown on sandy soil, which encompassed 29% of the total sugarcane acreage in Florida [1]. Sugarcane production in Florida has expanded to sandy (mineral) soil due to the limited availability of muck (organic) soils and because of the loss of depth of muck soils due to microbial oxidation, a process known as "soil subsidence" [2]. Additionally, sugarcane production on sandy soils is expanding

into fallow citrus land where citrus cultivation is abandoned due to disease pressure (e.g., citrus's Huanglongbing disease).

Commercial recoverable sucrose (CRS) is substantially higher on Florida sandy soils due to nutritional stress in plants that reduces vegetative growth and triggers sucrose accumulation; however, cane yield (CY) is lower in sandy soils than in muck soils [3]. Traditionally, sugarcane breeding was directed to muck soil, but a breeding program was started, especially for sandy soils, in 2011 [4].

Ratooning ability (RA) is usually considered a CY-associated trait and a key criterion during the selection of new genotypes [5]. In sugarcane, the ratoon crop yields decline with years (crops). The term RA is used to describe the yield performance of genotypes in relation to older crop yields to plant crop yields [6,7]. Genotypes with good RA could increase profitability because of the reduction in costs associated with more frequent replanting. Several factors impact RA, including soil, temperature, humidity, water supply [8], and experimental locations [9].

In the CP program, the two yield variables, CY and CRS, are used to select new genotypes. There is limited information about the trend of the sugar recovery rate on sandy soil in Florida. Experimental trials planted on sandy soils with historical cultivars released from 1930 to 2004 showed a linear trend in CRS gain [10]. Florida experienced a phenomenal annual growth of 0.2380% in its sugar recovery rate from 1928 to 1941 [11]. However, its annual growth slowed to 0.0531% from 1942 and has decreased further in recent years. Additionally, Zhao and Yang-Rui [12] reported that an increase in sugar yield (SY) in most recently released cultivars on sandy soils in Florida was mainly associated with an increase in CY rather than an increase in CRS content. Research conducted in Australia also indicated that breeding programs have improved CY, but gains in CRS content have been negligible [13,14]. Jackson [13] proposed two hypotheses for slow genetic gain in sucrose yield: (1) insufficient selection pressure has been applied for sucrose yield in breeding programs for the selection of parents because of a dominating effect of variation in CY and (2) a small number of key genotypes used as parents [15].

The CP program for sandy soils still lacks enough data to study the genotype by environment interaction (GEI) in the final selection stage (Stage 4). GEI analysis evaluates the performance of genotypes in several environments and at the same time assesses the representativeness and discriminating ability of test locations; all of this is visualized in biplot graphs [3]. The number of sand locations to test new genotypes was increased from two to four based on the GEI studies conducted in muck and sand locations in Florida and demonstrated that including more locations would be a successful approach to improve the selection of cultivars for sandy soils [3,16,17].

Genotypes advanced to Stage 4 of the CP sugarcane cultivar development program are evaluated in three sand locations for three crop cycles. However, Florida's sandy soils are not uniform from location to location or within a location [17]. The high variability of sandy soils in Florida is likely to affect the selection of non-replicated genotypes of the early selection stages [18]. Significant genotype × crop × location interaction on CY was reported in CP genotypes evaluated in sand Stage 4 trials, emphasizing the complexity associated with breeding for CY on sandy soils in southern Florida [19]. However, studies on the use of GGE interaction analysis to support in selecting outstanding genotypes for sucrose yield on sand locations are limited [20]. It is imperative to conduct GGE interaction studies with CP genotypes exclusively planted on sand locations to enhance the efficiency of the CP program for this type of soil.

Several procedures have been proposed to evaluate the GE interaction accurately. Among the most commonly used are the additive main effects and multiplicative interaction (AMMI) analysis [21], the genotype plus genotype × environment (GGE) biplot analysis [22], the factor analysis [23], and the BLUP-GGE combined analysis [24,25]. GGE analysis has attracted increasing attention because it provides more complete evaluation for multilocation trials, distinguishes and represents the test environment, and detects the most appropriate genotypes in a specific location and those with high and stable yields [26].

The BLUP-GGE joint analysis method combines spatial analysis with factor analysis to obtain the BLUP value of each genotype at each location. This method was proposed to avoid the restrictions of the fixed effects model, homogeneity of test locations, and bias due to missing data. Breeders found that the GGE biplot analysis of BLUP data is more reliable than the original data [25]. Therefore, the objective of this study was to select sugarcane genotypes with high CRS content, CY, good RA, and performance stability/adaptability across Florida sand locations.

2. Materials and Methods

2.1. Plant Material, Locations, Experimental Design, and Data Collection

In the CP sugarcane breeding program for sandy soils, the selection process involves a first stage (seedling stage) followed by three clonal stages (Stages 2, 3, and 4). The seedling stage consists of 12,000–18,000 seedlings transplanted yearly; this number is reduced to approximately 900 genotypes in Stage 2. There is no Stage 1 in the sand program, and selection of genotypes in seedling stage, Stage 2, and Stage 3 is conducted in ratoon crops. A selection intensity of approximately 10% is applied during selection at Stages 2 and 3. Every year, up to 135 genotypes are advanced to Stage 3 trials, which are tested at two sand locations. In the final stage, Stage 4, 10–13 test genotypes plus 3 checks with 6 replicates are planted at three sand locations and evaluated during three crop cycles (plant cane, first ratoon, and second ratoon).

Thirteen test sugarcane genotypes and three commercial cultivars (checks) were evaluated in Stage 4 trials planted on sandy soils in southern Florida. The 13 test genotypes included one CP 11 that was released for muck soil [27], two CP 13 genotypes advanced to the final selection stage (Stage 4) on muck soils, and nine CP 14 genotypes advanced to Stage 4 on sandy soil. Upon planting in Stage 2 trials in the CP breeding program, each genotype is named CP YY-XXXX with YY being the last two digits of the year of advancement to Stage 2 followed by a sequence of 4 digits beginning at 1001 for muck soil and 4001 for sand soil. Thus, CP 11, CP 13, and CP 14 indicate genotypes advanced to Stage 2 in 2011, 2013, and 2014, respectively. Genotype names and their parentage are described in Table 1.

Table 1. List of sugarcane genotypes evaluated in the trials and parentage information.

Genotypes	Parents	
	Female	Male
CP 96-1252	CP 90-1533	CP 84-1198
CL 88-4730	CL 82-3160	CL 78-1600
CPCL 97-2730	CL 75-0853	CL 88-4730
CP 11-2248 †	CP 06-2664	Polycross
CP 13-1644	CP 06-2406	CP 06-2664
CP 13-2340	CPCL 05-1102	Polycross
CP 14-4165	CP 04-1566	CPCL 02-8021
CP 14-4277	CP 04-1566	Polycross
CP 14-4326	CP 04-1844	CP 06-2664
CP 14-4330	CL 88-4730	Polycross
CP 14-4380	CP 06-2897	CL 88-2747
CP 14-4430	CP 04-1566	Polycross
CP 14-4482	CP 03-1912	Polycross
CP 14-4588	CP 84-1591	CP 07-2547
CP 14-4602	CP 04-1844	CL 88-4730
CPCL 14-4066	CL 88-4730	Polycross

† Released to Florida growers for organic (muck) soils in June 2018 [27].

The three check cultivars were CP 96-1252 [28], CPCL 97-2730 [29], and CL 88-4730 (a proprietary cultivar selected by the United States Sugar Corporation). These cultivars were the top commercially grown cultivars on sandy soil in Florida when these trials were planted. CL 88-4730 was the major commercial cultivar on sandy soil in Florida, and CPCL 97-2730 became the main cultivar on sandy soil because of its acceptable CY and CRS [29]. CP 96-1252 was released because of its high CY in muck and sandy soils [30], and it is the most widely grown sugarcane cultivar in muck and sandy soils in Florida [1]. Stage 4 trials were planted on three test locations (farms) on different sandy soils: Lykes Brothers, Inc. (PF), Townsite farm (TS), and Pahokee Produce Inc. farm (PP) (Table 2).

Table 2. Information of sand locations used to assess CP series of sugarcane genotypes in the CP breeding and selection program.

Locations	Acronym	Latitude (N)	Longitude (W)	Sandy Soil Type
Lykes Brothers Inc. farm	PF	26°53′08′	81°08′41′	Pompano fine sand
Townsite farm	TS	26°44′37′	80°58′95′	Margate sand
Pahokee Produce Inc. farm	PP	26°47′29′	80°25′09′	50% Pineda sand and 50% Riviera fine sand

Trials were planted on 8 November 2018, 16 November 2018, and 5 December 2018, at PF, TS, and PP locations, respectively, using randomized complete block design (RCBD) with six replications. Seed cane of each genotype was increased at each location before planting Stage 4 trials to guarantee highest seed quality. Field trials were prepared for planting, furrowed, fertilized, and covered by the growers following standard practices for sugarcane in Florida [31]; this strategy is used to mimic the grower's commercial practices as much as possible, testing how genotypes perform under commercial growing conditions. During planting, two stalks are laid out at the bottom of each furrow in parallel with the top of one stalk alternated with the bottom of the second parallel stalk, and seeds are cut into 2′ pieces. Trials at each location were surrounded by ditches which supply the water for seepage irrigation. Because screening for resistance to main sugarcane diseases is an integral part of the CP program, no pesticides are applied during any stage of the CP program. Each plot was arranged as three rows 10.5 m long, with 1.5 m inter-row spacing and 1.5 m alleys between plots. The millable stalks were counted in the two interior rows of each plot in each crop in July–August from 2019 to 2021, and the number of stalks per hectare (stalks ha^{-1}) was estimated and used to calculate CY. The trials were sampled following the production scheme in Florida, i.e., harvest dates of the plant-cane (PC), first-ratoon (FR), and second-ratoon (SR) crops were 3 February 2020, 16 November 2020, and 17 October 2022, respectively, at PF; 6 February 2020, 28 January 2021, and 25 January 2022, respectively, at TS; and 21 January 2020, 9 February 2021, and 3 January 2022, respectively, at pp. These dates relied on the dates when the industry harvested adjacent commercial sugarcane fields. At harvest, 10-stalk bundles were randomly collected from the 2nd and 3rd rows of each plot for yield estimates. Average stalk weight (kg $stalk^{-1}$) for each replicate was estimated by weighing each 10-stalk bundle. Cane yield (CY, Mg ha^{-1}) was calculated based on the number of stalks per ha and the average stalk weight as follows:

$$CY = \frac{STW \times STN}{1000}$$

Brix, Pol, and fiber content were measured via NIR spectroscopy of shredded cane using a Cane Presentation System (CPS, Bruker Optics). Brix and Pol values were used to calculate sucrose concentration; these three quality yield components and fiber values were used to calculate the theoretical recoverable sucrose (TRS, kg Mg^{-1}) as described by Legendre [32]. A correction factor of 0.86 was applied to the TRS value to approximate CRS content (kg Mg^{-1}).

2.2. Data Analysis

Data from each trial were inspected to detect inconsistencies and outliers, and the homogeneity of variance for each parameter was evaluated by Levene's test based on the residuals using the JMP Pro 17.0.0 software. Statistical analyses were conducted based on replication data of plant cane and the two ratoon crops from each location. The genetic merit of each genotype was evaluated by best linear unbiased predictors (BLUPs). The BLUPs across the environments and for each genotype by environment were calculated with the META-R software [33] using the following model:

$$Y_{ijk} = \mu + L_i + R(L)_{j(i)} + G_k + G_k \times L_i + E_{ijk}$$

where Y_{ijk} is the trait of interest; μ is the overall mean effect; L_i is the fixed effect of the ith location; $R(L)_{j(i)}$ is the random effect of the *j*th replicate within location; G_k is the random effect of the *k*th genotype; and $G_k \times L_i$ is the random effect of the location × genotype interaction. Differences between genotype BLUPs were analyzed by the LSD test ($p < 0.05$). Cluster analysis based on Ward's method was used to generate the scatter plot matrices of BLUP_CY versus BLUP_CRS among the 16 sugarcane genotypes. Cluster analysis was conducted using the JMP Pro package 17.0.0.

The selection of new sugarcane genotypes with the good RA becomes a key breeding objective to improve the efficiency of the CP program for sandy soils because replanting sugarcane is an expensive task affecting profitability to growers. Ratooning ability (RA) was calculated as described by Dunckelman [34] using the following formula:

$$\text{RAi} = \left[\left(\frac{\text{FR}}{\text{PC}}\right) + \left(\frac{\text{SR}}{\text{PC}}\right)\right]/2$$

where PC = plant cane crop, FR = first ratoon crop, and SR = second ratoon crop.

Values of RA calculated were subjected to ANOVA (GLIMMIX model procedure in SAS), and genotypes (G) were considered as fixed effects, whereas location (L), replicates within locations (Rep(L)), and genotype × location (GL) interaction were considered random effects. Genotypes were ranked from 1 (highest RA) to 16 (lowest RA). Ward's method was used to generate a scatter plot matrix of RA versus BLUP_CY among 16 sugarcane genotypes to reduce the risk of a high pressure on RA or BLUP_CY affecting the selection efficiency. The scatter plot graph was drawn using the JMP Pro package 17.0.0.

The BLUPs data for CY and CRS for each location in the form of adjusted means were subjected to genotype plus genotype for environment interaction (BLUP-GGE) analysis [24]. The analyses were based on data scaled by the standard deviation (SD-scaled) and environmental-centered (G + GE). Row or column metrics were selected according to the type of graph [35–37]. GGE biplot analyses for BLUP_CY and BLUP_CRS were conducted using GEA-R (Genotype × Environment analysis with R) software version 4.1 [38].

2.3. Screening and Evaluation for Disease Reactions

Reactions to main diseases affecting sugarcane in southern Florida were evaluated by inoculation testing and/or monitoring for natural infection of brown (caused by *Puccinia melanocephala* H. and P. Sydow) and orange rusts (caused by *P. kuehnii* E.J. Butler), leaf scald (caused by *Xanthomonas albilineans* (Ashby) Dowson), ratoon stunt (caused by *Leifsonia xyli* subsp. *xyli* Evtushenko et al.), smut (caused by *Sporisorium scitamineum* (Syd.) M. Piepenbr., M. Stoll and Oberwand), and *Sugarcane mosaic virus strain E (mosaic)*, in greenhouses or under field conditions or both using standard methods followed in the CP program [39]. Screening for natural disease infestation under field conditions was conducted in 2020. The rating scales used for orange and brown rusts in field plots for natural infection have been described by Zhao et al. [40]. Inoculation tests for susceptibilities were based on the percentage of infected plants with mosaic and leaf scald, the percentage of plants with sori for smut, and the number of colonized vascular bundles for ratoon stunt.

Susceptibility to smut, leaf scald, and mosaic was compared with CP 78-1628 [41], CP 80-1743 [42], and CP 72-2086 [43], respectively. In artificial inoculation tests for ratoon stunt susceptibility from 2017 to 2019, the test genotypes were compared with CP 72-1210 [44]. Comparisons were made with these reference cultivars because their susceptibilities to specific diseases are at the upper limits of acceptability for commercial sugarcane production in Florida. In addition, test genotypes and check cultivars were screened for the presence of the *Bru*1 brown rust resistance locus [45] using methods described by Costet et al. [46] and Glynn et al. [47].

2.4. Genetic Diversity Analysis

The genetic diversity of the most outstanding test genotypes was investigated using six SSR markers (SMC1493CL, mSSCIR53, mSSCIR14, and SMC221MS, SMC222CG, and SMC179A) developed through the International Consortium for Sugarcane Biotechnology [48]. Forward primers were fluorescent-labeled at the 5′ end with FAM 96-carboxy fluorescein or HEX (4,7,2′,4′,5,7-hexachloro-carboxyfluorescein). Two primer pairs with two different dyes were multiplexed in each PCR reaction. DNA fragments were separated using an ABI 3730XL Genetic Analyzer (Life Technologies, Holdings Pte Ltd. | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256). GeneScan-500 ROX was used as an internal DNA size standard. Electropherogram processing and SSR allele-size scoring data were performed using GeneMarker V2.4.0 software (SoftGenetics LLC, 100 Oakwood Ave, Suite 350, State College, PA 16803, USA). The genetic fingerprinting of the most outstanding test genotypes was compared with that of five commercial cultivars grown on sandy soils. These commercial cultivars included CP 96-1252, CPCL 05-1201 [20], CP 03-1912 [49], CPCL 97-2730, and CP 06-2042 [50] along with CP 13-4474 [51], a recently released CP cultivar for sandy soils in Florida. The commercial cultivars represent 39.9, 18.2, 8.1, 6.9, and 3.9% of the total sugarcane acreage in Florida in sandy soils, respectively [1]. Additionally, two commercial cultivars were also included in the analysis: CP 00-1101 [52], a high yielding cultivar recommended for muck and sandy soils, and "CP 06-2400" [53], released because of its high cane and sucrose yields and acceptable CRS on muck soils. Each polymorphic SSR fragment was scored 1 for presence and 0 for absence in each genotype. Genetic similarity between the ten genotypes was calculated based on Dice similarity coefficients (s) using PAST software ver. 4.03 [54]. The genetic distance (d) between the genotypes was calculated as $d = 1 - s$, where s is the genetic similarity value. Cluster analysis was performed based on the genetic distance matrix using Ward's hierarchical method in JMP® Pro 17.0.0, and clusters were visualized using a constellation plot.

3. Results and Discussion

The estimates of multi-environment BLUP and the genotype variance were highly significant for CRS content and not for CY (Table 3). The estimate of genotype variance was greater than the variance due to GEI for BLUP_CRS, indicating that there are opportunities to detect suitable genotypes for CRS content in the germplasm evaluated. The estimate of genotype variance for BLUP_CY was lower than the variance attributable to GEI, indicating that all evaluated genotypes showed similar BLUP_CY estimates. One possible explanation for the lack of significance and lower variation in BLUP_CY estimates could reside in higher selection pressure for the stalk population during the early selection stages of the breeding program on sandy soils in Florida. The BLUP_CY and BLUP_CRS in the evaluated sugarcane germplasm were significantly affected by GEI (Table 3). Significant GEI effect will cause differences among genotypes in different environments; therefore, GEI should be accurately evaluated to select genotypes that adapt to each environment or genotypes with a wide range of adaptations. The broad-sense heritability (H^2) value across trials was moderate (0.33) for BLUP_CY, which indicates that only 33% of the differences among the 16 test genotypes across three crops cycles and three locations were due to genotypic rather than environmental differences. The coefficient of variation (CV) for BLUP_CY (13.5%) was similar to the CV for CY reported in sugarcane trials [17,19,55,56]. In addition, the relatively

large GEI variance for BLUP_CY in relation to the genotypic variance indicates that the principal genotype effects between the environments are better explained, and the patterns explained in the biplot analysis are reliable. These results suggest that successful selection for BLUP_CY in this population might be accomplished by testing the genotypes in several environments [57]. The broad-sense heritability (H^2) value across trials for BLUP_CRS among the thirteen genotypes and three checks evaluated across three crops cycles at three locations was high (0.94); this value indicates that 94% of the detected differences were due to genotype rather than environmental difference. The more significant contribution of the genotype effect in relation to the environmental effect indicates that the selection of genotypes is more effective for BLUP_CRS. The BLUP_CRS exhibited a relatively small CV (3.3%), indicating that this parameter was relatively stable in the germplasm across different environments and making the selection for BLUP_CRS relatively easier. CP 14-4165 and CP 13-2340 had significantly higher BLUP_CRS than the main check (CP 96-1252), whereas genotypes CP 14-4165 and CP 13-2340 had numerically but not significantly higher BLUP_CRS than the checks CPCL 97-2730 and CL 88-4730.

Table 3. Genetic parameters from across environments' BLUPs for CY (Mg ha^{-1}) and CRS (kg Mg^{-1}) content.

Statistic Parameters	BLUP_CY	BLUP_CRS
Heritability (H^2)	0.33	0.94
Genotype variance (G)	11.96	26.88
GEI variance (GEI)	32.11	2.66
Residual variance	238.17	16.81
Genotype significance (*p* value)	0.35	<0.0001
GEI significance (*p* value)	0.02	0.008
CV	15.18	3.3

CV = Coefficient of variation.

Results of multi-environment BLUP for CY and CRS content of sugarcane genotypes are shown in Table 4. No significant differences were detected among the genotypes for BLUP_CY. Based on the BLUP_CY estimates, the check CP 96-1252 was the genotype with the highest CY across locations and crop cycles (ranked first), while the test genotypes CP 14-4486 and CP 13-2340 were ranked second and third, respectively. The difference in BLUP_CRS was also observed among genotypes. Based on the BLUP_CRS estimates, CP 14-4165 and CP 13-2340 were ranked as first and second in the germplasm evaluated, respectively. Based on the percentages, across the three crop cycles, CP 14-4165 and CP 13-2340 had 3.44 and 3.08% higher BLUP_CRS content than the average of three check cultivars. A scatterplot matrix based on BLUP_CY and BLUP_CRS estimates showed that genotypes CP 14-4165 and CP 11-2340 had the highest BLUP_CRS and BLUP_CY estimates above the mean, while the check CP 96-1252 had the highest BLUP_CY value (Figure 1).

Table 4. Comparative BLUP performance for CY and CRS content.

Genotypes	Mean	BLUP_CY	Rank †	Mean	BLUP_CRS	Rank
CL 88-4730 (G1)	104.67	102.68	5	127.07	126.89 abc	6
CP 11-2248 (G2)	99.42	100.93	13	119.20	119.52 de	13
CP 13-1644 (G3)	105.17	102.84	4	115.73	116.27 e	15
CP 13-2340 (G4)	105.71	103.02	3	131.37	130.92 a	2
CP 14-4165 (G5)	102.61	101.99	7	131.89	131.41 a	1
CP 14-4277 (G6)	102.55	101.97	8	124.06	124.07 bcd	10
CP 14-4326 (G7)	100.21	101.19	11	117.31	117.75 e	14
CP 14-4330 (G8)	99.70	101.02	12	128.09	127.85 ab	4
CP 14-4380 (G9)	90.41	97.93	16	125.21	125.15 bc	9
CP 14-4430 (G10)	92.16	98.51	15	123.86	123.89 bcd	11

Table 4. *Cont.*

Genotypes	Mean	BLUP_CY	Rank †	Mean	BLUP_CRS	Rank
CP 14-4486 (G11)	107.57	103.64	2	113.62	114.29 e	16
CP 14-4588 (G12)	101.61	101.66	9	127.59	127.38 ab	5
CP 14-4602 (G13)	104.38	102.58	6	126.35	126.22 bc	7
CP 96-1252 (G14)	115.23	106.20	1	125.76	125.66 bc	8
CPCL 14-4066 (G15)	100.43	101.26	10	122.39	122.51 cd	12
CPCL 97-2730 (G16)	95.07	99.48	14	128.39	128.12 ab	3
Overall mean	101.68			124.34		
$LSD_{0.05}$		8.28			4.63	

G1–G16 are genotype codes used for clarity in the biplot graphs. † Rank of genotypes (1–16) based on BLUP estimates. Estimates with different letters differ one from the other (LSD test, $p < 0.05$).

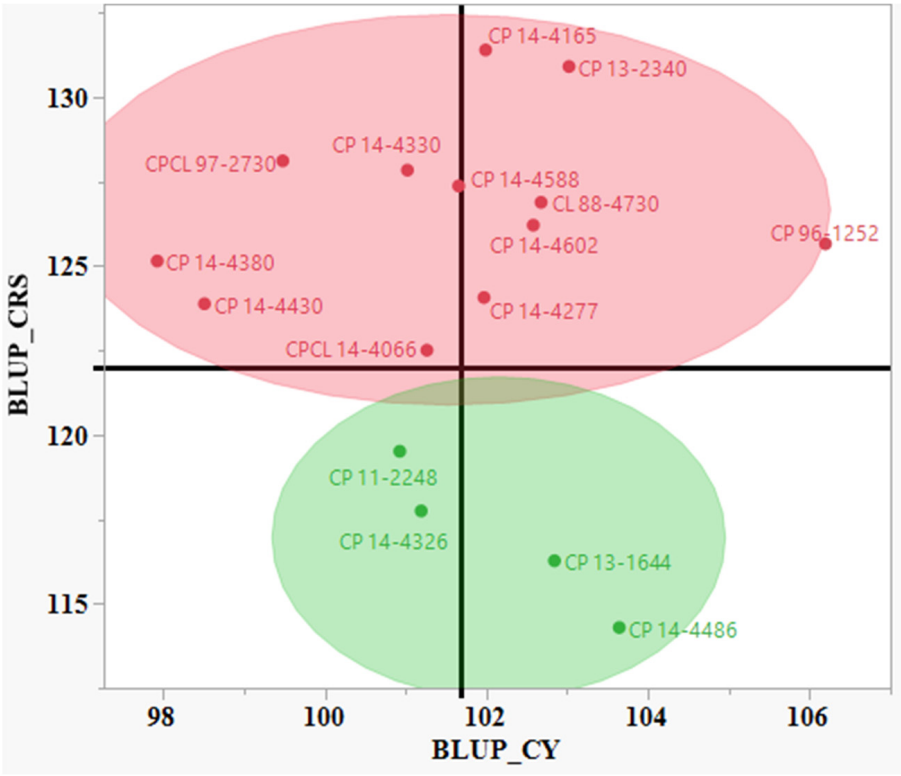


Figure 1. Scatter plot graph based on cluster analysis with Ward's method of BLUP_CY and BLUP_CY estimates of 16 sugarcane genotypes evaluated in three sand locations and across three crop cycles. Vertical and horizontal lines in the graph indicate the average BLUP_CY (Mg ha^{-1}) and BLUP_CRS (kg Mg^{-1}) estimates, respectively.

The overall performances of sugarcane genotypes across three locations are shown in Table 5. The genotypes evaluated produced higher BLUP_CY estimates (123.97 Mg ha^{-1}) and BLUP_CRS estimates (126.36 kg Mg^{-1}) in the TS location, whereas in PP and PF locations the genotypes produced lower BLUP_CY (70.01 Mg ha^{-1}) and BLUP_CRS estimates (121.32 g ha^{-1}), respectively. Genotypes varied significantly in BLUP_CY only in the PF location. According to the LSD (0.05) test, the most outstanding genotype was CP 14-4486, on a percentage basis; this test genotype was 3.8% higher than the best check (CP 96-1252) in this location.

Table 5. Mean performance of BLUP_CY and BLUP_CRS estimates of the evaluated sugarcane genotypes across three locations.

Genotypes	BLUP_CY (Mg ha^{-1})			BLUP_CRS (kg Mg^{-1})		
	PF	PP	TS	PF	PP	TS
CP 14-4486	126.25 a	69.60	123.19	111.84 h	114.58 h	117.21 g
CP 96-1252	121.44 ab	72.23	121.58	124.46 abcd	127.75 cd	127.32 cd
CP 13-1644	118.04 abc	67.51	127.67	113.81 gh	117.29 bc	118.31 fg
CP 14-4602	117.68 abc	70.05	123.35	123.44 bcd	126.38 cdef	128.68 cd
CP 13-2340	117.64 abc	70.86	123.66	121.68 cde	134.30 a	136.33 a
CP 14-4277	114.69 abc	69.37	123.95	120.83 def	124.39 cdef	127.02 cd
CL 88-4730	113.44 abc	70.96	124.64	125.39 abc	127.75 cd	127.32 cd
CP 14-4165	112.55 abc	69.46	125.03	127.94 a	133.28 ab	132.46 ab
CP 14-4588	110.08 bcd	72.45	120.96	126.99 ab	126.99 cde	127.89 cd
CPCL 14-4066	108.74 bcd	70.77	122.82	117.58 efg	121.86 fg	128.26 cd
CP 14-4326	106.42 bcd	69.74	125.31	114.71 gh	117.39 gh	121.65 ef
CP 14-4330	105.77 bcd	71.79	122.16	124.19 abcd	128.45 bc	130.64 bc
CP 14-4380	103.17 cd	66.11	121.58	122.33 cd	126.48 cdef	126.58 cd
CP 11-2248	102.88 cd	69.60	126.55	116.54 fg	123.24 def	119.10 fg
CP 14-4430	102.75 cd	69.95	121.07	123.34 cd	122.78 ef	125.55 de
CPCL 97-2730	94.42 d	72.92	121.58	126.15 abc	129.08 bc	128.83 bcd
H^2	0.68	0.25	0.37	0.91	0.91	0.92
$LSD_{0.05}$	16.11	8.99	9.39	4.47	4.74	4.29
Mean	111.0	70.01	123.97	121.32	125.04	126.36
δ^2g (p value)	0.002	0.451	0.236	<0.001	<0.001	<0.001
CV	15.34	22.16	10.92	3.36	3.45	3.08

PF, PP, and TS = location abbreviations shown in Table 1. H^2 = broad-sense heritability; LSD = least significant differences; δ^2g = genotype variance; CV = coefficient of variation (%). Different letters are least significantly different at 5%.

Results showed smaller differences (not statistically significant) in BLUP_CY estimates among the genotypes in PP and TS locations. The genotypes varied significantly in BLUP_CRS estimates in the three locations. Test genotypes CP 14-4165 and CP 14-4588 produced the higher BLUP_CRS estimates in the PF location, which were 1.40% and 0.66% higher than the best check genotype (CPCL 97-2730). CP 13-2340 and CP 14-4165 remained consistently superior for BLUP_CRS at PP and TS locations.

The ANOVA to test the difference among genotypes for RA values produced significant genotypic effects (p = 0.04). The pairwise comparison of genotypes based on the RA values is shown in Table 6. The check CP 96-1252 showed higher RA (ranked first), and two test genotypes (CP 14-4165 and CP 14-4326) ranked second compared to checks CL 88-4730 and CPCL 97-2730, which ranked fifth and sixth, respectively. Test genotypes CP 13-1644 and CP 14-4588 ranked 11th and 12th, and both exhibited significantly lower ($p < 0.05$) RA compared with checks CP 96-1252 and CL 88-4730, indicating the poorest RA. The other test genotypes showed similar RA ($p > 0.05$) to the checks CL 88-4730 and CPCL 97-2730.

Cluster analysis based on BLUP_CY and RA data and Ward's method divided all the genotypes into two main clusters (Figure 2), plus the check CP 96-1252 located farther apart, the two groups showing the highest BLUP_CY estimates and the best RA. Cluster 1 grouped ten genotypes and two test genotypes (CP 14-4165 and CP 13-2340), and the check CL 88-4730 had BLUP_CY estimates and RA above average, while five additional test genotypes (CP 14-4430, CP 14-4326, CPCL 14-4066, CP 11-2248, and CP 14-4380) and the check CPCL 97-2730 had RA values above average but BLUP_CY estimates below average. Cluster 2 grouped five test genotypes with the lowest RA values, and three test genotypes (CP 14-4486, CP 14-4277, and CP 13-1644) had BLUP_CY estimates above average, whereas CP 14-4330 and CP 14-4588 had BLUP_CY below average.

Table 6. Estimates of RA of 16 sugarcane genotypes tested at three sand locations and across three crop cycles.

Genotypes	RA †	Rank
CP 96-1252	1.15 a	1
CP 14-4165	1.02 ab	2
CP 14-4326	1.02 ab	2
CP 14-4430	1.01 bc	3
CPCL 14-4066	0.99 bcd	4
CL 88-4730	0.98 bcd	5
CP 11-2248	0.98 bcd	5
CP 14-4380	0.91 bcde	6
CP 13-2340	0.91 bcde	6
CPCL 97-2730	0.91 bcde	6
CP 14-4602	0.88 bcde	7
CP 14-4486	0.80 bcde	8
CP 14-4330	0.74 cde	9
CP 14-4277	0.73 de	10
CP 13-1644	0.71 e	11
CP 14-4588	0.69 e	12

† RA values estimated using the method reported by Dunckelman [34]. Rank of genotypes from 1 (highest RA) to 12 (lowest RA). Genotypes with different letters are least significantly different at 5%.

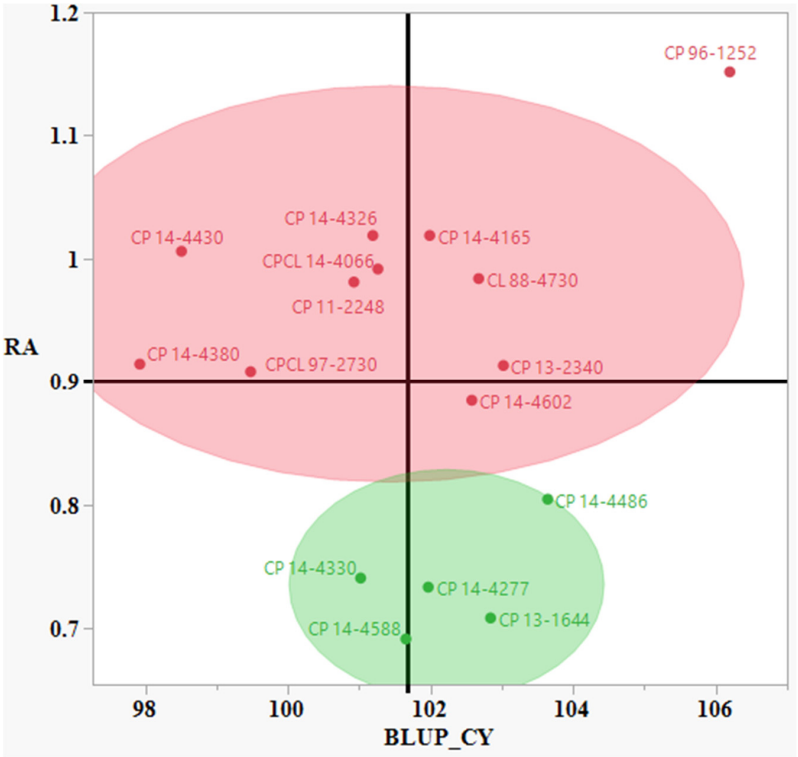


Figure 2. Scatter plot graph based on cluster analysis with Ward's method of BLUP_CY estimates and RA values of 16 sugarcane genotypes evaluated in three sand locations and across three crop cycles. Vertical and horizontal lines in the graph indicate the average BLUP_CY (Mg ha^{-1}) estimates and RA values, respectively.

These results emphasize the risk of using an unbalanced selection strategy of putting high pressure in BLUP_CY or RA, affecting the selection efficiency because of the increase in type II error [19]. For example, test genotypes CP 14-4326 and CP 14-4430 ranked second and third, based on RA, but both had BLUP_CY below average. Low plant cane yield

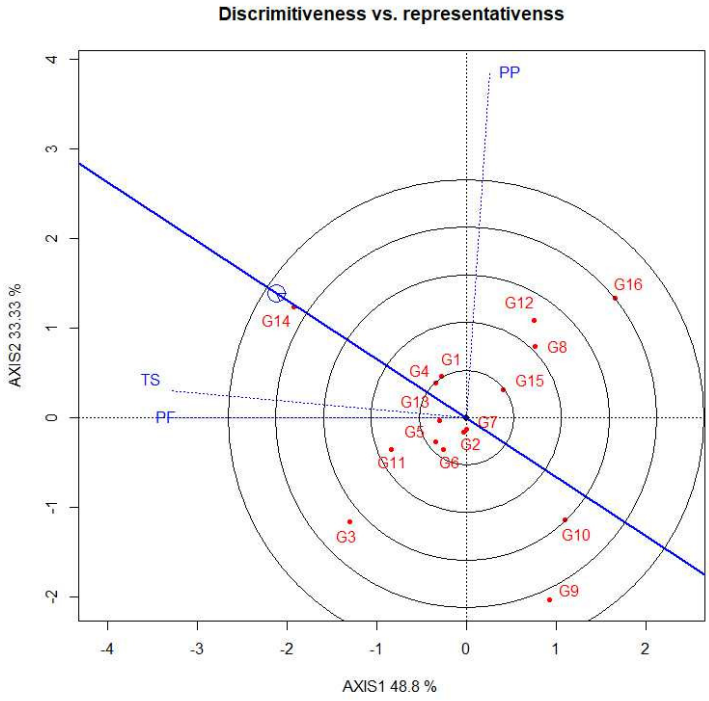
inflates RA; ratooning indexes described by Dunckelman [34] and Milligan [7] use point estimates, ignoring the effect of ratooning cycles [58]. Similarly, CP 14-4486 was ranked second based on overall BLUP_CY estimates, but it ranked eight for RA and was grouped together with the lowest RA test genotypes. Based on the combined selection for BLUP_CRS, BLUP_CY, and RA, two test genotypes can be considered: CP 14-4165 showed the highest BLUP_CRS in the population, ranked second in RA, and it had BLUP_CY above average, and CP 13-2340 ranked second in BLUP_CRS and had BLUP_CY and RA above average. In sugarcane, the number of ratoons crops harvested with appropriate CY represents an important contribution to the income of sugarcane growers. In Florida, during the 2020–2021 crop season, plant cane represented 28.5% of the total acreage [1]. If genotypes with improved RA are released, 28.5% of the total sugarcane acreage replanted may be reduced. In the CP breeding program, elite cultivars are recommended for commercial release by the Sugarcane Variety Committee based on CY, sucrose content, sugar yield, and disease resistance as main selection criteria. Ratooning ability is indirectly considered during the recommendation process by comparing genotypes for CY across the ratooning cycles, but no statistical method is used to evaluate the CY decline that occurs across the cycles. In addition, the CP breeding program has been releasing high-tonnage cultivars but usually with low sucrose content [11]. Our hypothesis for the low gain in sucrose content in Florida includes the use of an economic index [59] to rank the genotypes during the final selection stages (Stages 3 and 4). This index is based on CY and SY, because SY is the product of CY and CRS, the selection unconsciously underweighting sucrose content. In Australia, the selection of genotypes based solely on economic index might include genotypes with higher CY but with similar or lower commercial cane sugar (CCS) [13]. Our results indicate that the CP breeding program for sandy soils is being impacted by the two hypotheses stated by Jackson [13] in relation to CRS content. The CP program, like most sugarcane breeding programs, applies a recurrent selection scheme. The use of an economic index [59] to assist the selection ranking the genotypes in the CP program prioritizes the CY and the underweight sucrose content. In the CP program, genotypes that advanced from Stage 3 to Stage 4, with high CY, are used as parents, and then genes linked with CRS content are fixed within the genotypes released. In addition, variability in sandy soils also complicates the selection based on CRS content in the CP program during Stage 2 trials, and efforts have already started with the use of spatial analysis to improve the efficiency of the selection in this stage for CY and CRS content. However, no study has conclusively demonstrated the existence, or lack thereof, of genetic advancement in sucrose concentration or content [60]. Some have suggested that breeding has reached a sucrose plateau [61,62]. Others point to the progressive increase in sucrose concentration until 1980 as evidence that breeding has not reached a sucrose plateau [63]. For example, sugarcane breeding in Louisiana appears to have yet to reach a plateau in sucrose juice traits or sucrose yield [60].

Using an unbalanced selection strategy by putting high selection intensity on CY or RA also affects the selection efficiency due to an increase in type II error. For instance, none of the test genotypes could succeed the primary check cultivar, CP 96-1252, in terms of CY. However, the test genotype CP 14-4165 exhibited similar BLUP_CY and RA to CP 96-1252 but significantly higher BLUP-CRS than CP 96-1252. In sugarcane, the number of ratoon crops harvested with appropriate CY and high CRS represents an important contribution to the income of sugarcane growers and industry in Florida.

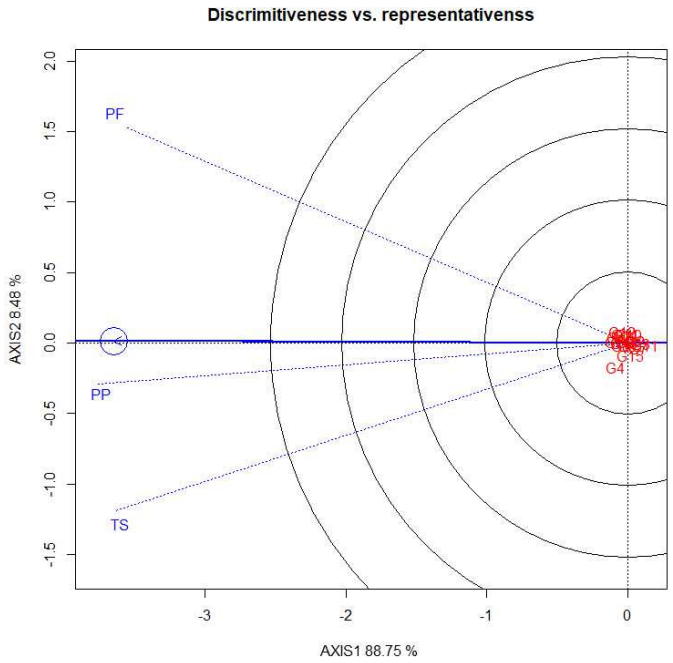
3.1. BLUP-GGE Analyses for CY and CRS Content

The BLUP-GGE biplots were used to visualize the GEI. The GGE biplots for BLUP_CY and BLUP_CRS representing the locations' vector views are shown in Figure 3. Results showed that the sum of the first and second principal components (Axis) explained 82.13% and 97.23% in the BLUP_CY and BLUP_CRS, respectively. These values indicate that the variability due to GEI is adequately represented. Location vectors explain the discriminativeness and representativeness of the test locations, and test locations with longer vectors are more discriminative than shorter vectors. The cosine of the angle between the

test locations vector and the Average Environmental Axis (AEA) (the single arrowed line) indicates the representativeness of the test location: the smaller the cosine value (acute angle), the more representative the test location [64].



(**a**)



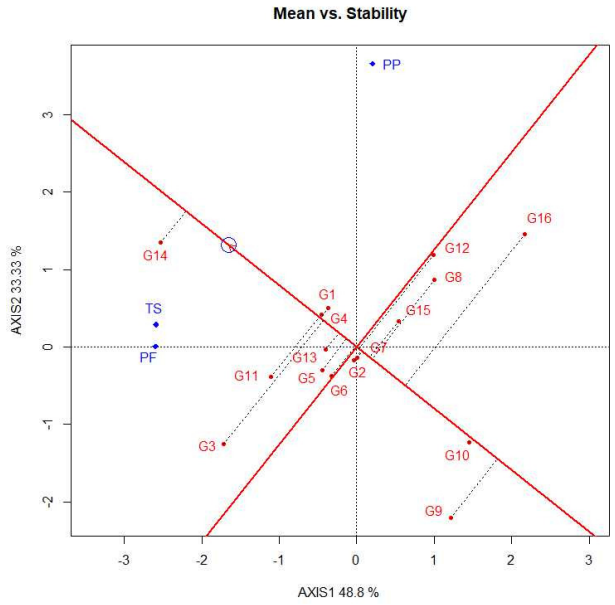
(**b**)

Figure 3. GGE biplot representing the locations' vector views to test the overall BLUP_CY (**a**) and BLUP_CRS (**b**) (plant-cane through second-ratoon crop cycles) of 16 CP sugarcane genotypes. The genotypes are shown as G1-G16, and full names are displayed in Table 4. The locations' abbreviations are shown in Table 1.

The vector length indicates that the test location PP is the most discriminative for BLUP_CY in the sandy soils of Florida, and TS and PF are the least discriminative, whereas TS was the ideal location for BLUP_CY (Figure 3a). Coto et al. [19] showed the same results with the CP 11 series genotypes. Zhao et al. [65] reported that dry biomass yield was significantly lower at the PP location than that at the TS location and associated the difference to the low amount of nitrogen regularly applied at the PP location. Despite the lack of information about the PF location regarding fertilization doses and rates, our study suggests that TS and PF have similar fertility levels based on the acute angles between them for CY and CRS content.

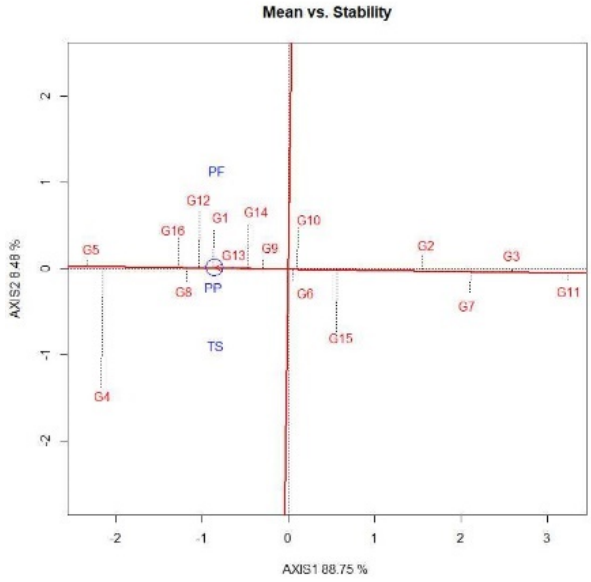
The genetic correlation between TS and PF locations for BLUP_CY was positive but not significant (r_g = 0.92, p = 0.24), whereas a negative and not significant genetic correlation was detected among TS and PF locations with respect to the PP location (obtuse angle). The three test locations had nearly the same long vectors for BLUP_CRS, so they had a similar ability to discriminate between genotypes (Figure 3b). The three locations were positive and strongly correlated for BLUP_CRS, indicated by the acute angles between them, and the genetic correlation (r_g) ranged from 0.83 between PF and TS, though it was 0.98 between PP and TS. The GGE interaction analysis also indicated that the PP location could be considered an ideal location to test genotypes for CRS in Florida's sandy soils. Results for BLUP_CRS correspond to the series of genotypes evaluated in this study; additional CP series of genotypes should be studied to confirm the representativeness and discriminative ability of these locations.

The mean versus stability assessment of the BLUP-GGE biplots is shown in Figure 4. In the graphs, each vertical dotted line of the Average Environmental Coordinate (AEC) represents the average and the stability of BLUP_CY and BLUP_CRS estimates of each genotype across all test locations. The solid line perpendicular to the AEC is the mean population of the evaluated germplasm. The BLUP_CY and BLUP_CRS of the genotypes on the left of the solid line are higher than the population means, and the farther away any genotype is from this line, the higher the BLUP_CY or BLUP_CRS estimates. Thus, genotypes G14 (CP 96-1252), G11 (CP 14-4486), G1 (CL 88-4730), G3 (CP 13-1644), G4 (CP 13-2340), G13 (CP 14-4602), and G5 (CP 14-4165) had the highest BLUP_CY mean (Figure 4a). CP 13-2340 was the most stable genotype for BLUP_CY.



(**a**)

Figure 4. *Cont.*

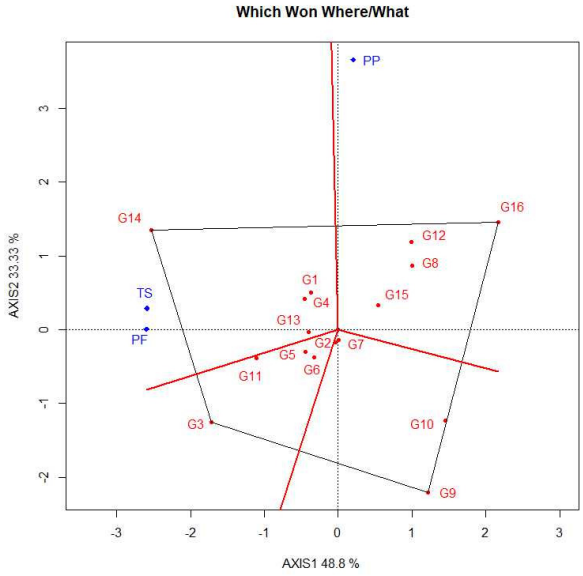


(**b**)

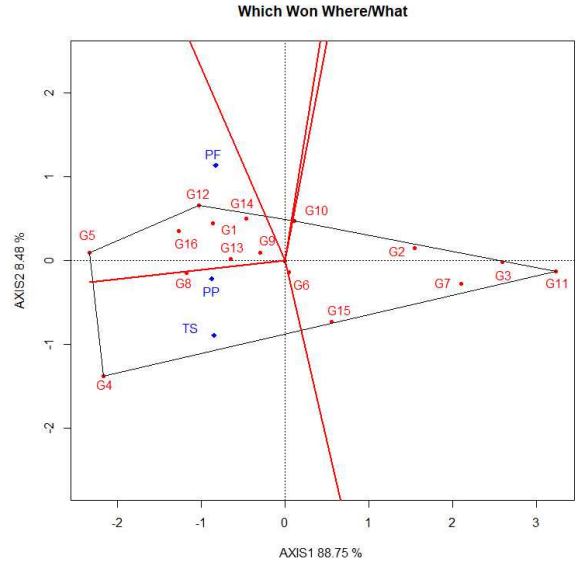
Figure 4. GGE biplot showing the rank of 16 CP sugarcane genotypes for BLUP_CY (**a**), BLUP_CRS (**b**), and stability performance over three locations and three crop cycles. The genotypes are shown as G1-G16, and full names are displayed in Table 4. The locations' abbreviations are shown in Table 1.

The GGE biplot analysis for BLUP_CRS showed that test genotypes G5 (CP 14-4165) and G4 (CP 13-2340) showed the BLUP_CRS estimate to be higher than the best performance check (G14, CP 96-1252) (Figure 4b). CP 14-4165 (G5) had both the highest BLUP_CRS performance and the highest stability. As discussed by Yan [57], high yielding but less stable genotypes may be mistakenly discarded if too much weight is given to instability during the selection process. A good strategy to improve the selection on sandy soils in Florida might be simultaneous selection for yield, RA, and stability [19]. If the ranks based on average BLUP_CY, BLUP_CRS, RA, and stability are weighted equally, CP 14-4165 (G5) would be selected. CP 13-2340 (G4) showed BLUP_CY to be above the mean, with an average RA and high stability, whereas it showed BLUP_CRS to be above the mean but with high instability across locations.

The which-won-where polygon is an important component of the GGE analysis. It helps visualize the interaction patterns between genotypes and environments and shows the presence of crossover GE interaction, mega-environment differentiation, and specific adaptation [57]. Genotypes at the corners of the polygons divide the biplot area and test locations into sectors; simultaneously, it reveals the suitable genotypes for test locations within each sector or mega-environments (one environment or the combination of several environments) [57]. The which-won-where graph shown in Figure 5a shows that the three locations fall into two mega-environments, and the vortex genotypes of each mega-environment have the highest BLUP_CY in the locations that fall in the sector. The first mega-environment includes the PF and TS locations, with check CP 96-1252 (G14) being the winner. Check CPCL 97-2730 (G16) was the winner in the second mega-environment containing the PP location. Test genotypes CP 11-2248 (G2), CP 14-4165 (G5), CP 14-4277 (G6), CP 14-4326 (G7), CP 14-4602 (G13), CP 13-2340 (G4), and check CL 88-4730 (G1) were located close to the center of the biplot, indicating that they showed a stable performance of BLUP_CY across the test locations [60]. The polygon graph of the BLUP_CRS presented in Figure 5b revealed that the three locations also fall into two mega-environments. Locations TS and PP were positioned in one mega-environment with test genotype CP 13-2340 (G4) showing the highest BLUP-CRS in these two test environments (Table 4). Two test genotypes, CP 14-4165 (G5) and CP 14-4588 (G12), are the winning genotypes in the second mega-environment associated with the PF location (Figure 5b).



(**a**)



(**b**)

Figure 5. The which-won-where view of 16 CP sugarcane genotypes for overall BLUP_CY (**a**) and BLUP_CRS (**b**) over three locations and three crop cycles. The genotypes are shown as G1-G16, and their full names are displayed in Table 4. The locations' abbreviations are shown in Table 1.

The detection of two winning genotypes in the second mega-environment is beneficial to divide the target environments into meaningful mega-environments [66]. The detection of different sugarcane genotype winners in the mega-environments for BLUP_CRS indicated the presence of crossover as revealed by differential yield ranking and stability across environments (Figures 4b and 5b).

In sugarcane, high-yielding genotypes generally have a low sucrose content, and genotypes with a high sucrose content generally have low cane yield. Obtaining an ideal sugarcane genotype with a high cane yield and sugar content is relatively difficult [67]. The GEI analysis enables the identification of genotypes with narrow adaptation, which can significantly improve crop productivity in specific regions [68]. For example, in the sugarcane breeding program of Reunion Island, the economic profitability of new genotypes is correlated little between many selection sites, and the selection strategy is firstly oriented

toward selecting genotypes adapted to specifically targeted environments to enhance the mean productivity rather than selecting for broad adaptation [69]. In response to sugarcane market demands, the Florida sugarcane industry has increased the acreage of sand land. The three experimental areas considered in this investigation represent the dominant area where sugarcane grows in south Florida sand land. The selection of CP 14-4165 and CP 13-2340 for those specific test locations suggests that sugarcane breeders in the CP program need to consider the evaluation of GEI to release new sugarcane genotypes. Our results suggest that the strategy of selecting simultaneously for BLUP_CY, RA, and GEI analysis in sandy soils should be extended to the selection of genotypes specifically adapted for BLUP_CRS.

3.2. Screening and Evaluation for Disease Reactions

The CP sugarcane breeding program releases genotypes with good economic index and disease resistance to several economically important diseases for commercial production. Therefore, the disease resistance of CP 13-2340 and CP 14-4165 is an essential selecting factor along with higher CRS and CY. The average natural infection and inoculation ratings for diseases of the thirteen genotypes and the three checks are summarized in Table 7. CP 13-2340 had no plants infected with brown rust across 52 field plots, and 1 out of 52 plots was infected with orange rust. CP 14-4165 had 32 out of 52 plots infected with brown rust, while 5 out of 52 plots in the field were observed to have plants with orange rust. No plants with leaf scald, mosaic, ratoon stunt, and smut were observed in the 52 field plots rated in both genotypes. Artificial inoculation tests for mosaic showed an average of 5.6% of plants of CP 13-2430 infected and no plants of CP 14-4165 infected, compared with 20.5% for the susceptible reference CP 72-2086. For ratoon stunt disease, the artificial inoculation tests conducted for two years showed an average of 0.2 and 0.7 colonized vascular bundles for CP 13-2430 and CP 14-4165, respectively, compared with 10.6 bundles (2-year average) for CP 72-1210, the susceptible check. For smut, 2 years of artificial inoculation tests showed no infection in CP 13-2340 and CP 14-4165, while 3.6% of infection was detected in the susceptible check CP 78-1628. Two years of artificial inoculation tests for leaf scald showed an average of 1.5% and 0.0% of plants infected for CP 13-2340 and CP 14-4165, respectively. In addition, CP 13-2340, and CP 14-4165 tested negative for *Bru*1.

Table 7. Disease response of sugarcane genotypes compared with reference cultivars in Florida.

Cultivar	Brown Rust	*Bru*1 ‡	Orange Rust	Leaf Scald	Mosaic	Ratoon Stunt	Smut
CL 88-4730	S	-	MS	MR	R	R	MR
CP 11-2248	MS	ND	R	R	R	MR	R
CP 13-1644	R	ND	R	MR	R	R	R
CP 13-2340	R	-	R	R	R	R	R
CP 14-4165	S	-	R	R	R	R	R
CP 14-4277	R	+	S	R	R	R	MS
CP 14-4326	S	ND	R	MS	R	MR	R
CP 14-4330	S	ND	MR	S	R	MR	R
CP 14-4380	MR	-	MR	MS	R	MS	R
CP 14-4430	R	ND	R	MR	R	MR	R
CP 14-4486	S	ND	R	S	R	MS	R
CP 14-4588	S	+	R	R	R	R	R
CP 14-4602	R	ND	R	R	R	MS	S
CP 96-1252	S	-	R	MR	R	R	R
CPCL 97-2730	R	+	R	MR	R	R	R
CPCL 14-4066	R	ND	R	R	R	R	R

R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible. ‡ + denotes the presence, - denotes the absence of *Bru*1 gene, and ND denotes no data.

Based on artificial inoculation tests and observations of field plots under natural infection, CP 13-2340 is considered resistant to the major diseases of concern in Florida. CP 14-4165 was susceptible to brown rust, but its higher CRS makes it a valuable addition to existing commercial sugarcane cultivars in Florida or to the parental pool in the breeding program or to both (Table 7). Other genotypes, CP 13-1644, CP 14-4430, and CPCL 14-4066, were resistant to several economically important diseases (Table 7) but were not selected for CRS. There was no significant location effect on the diseases, but the genotype effect on diseases was significant.

3.3. Genetic Diversity Analysis

The six SSR markers used to evaluate genetic diversity generated 68 polymorphic allele fragments among the ten genotypes tested, and they were utilized for hierarchical clustering analysis (Figure 6). The 68 fragments ranged from 106 to 260 base pairs. The genetic distance among 10 genotypes based on 68 polymorphic alleles ranged from 0.26 to 0.67. The least genetic distance of 0.26 was observed between the commercial cultivar CPCL 05-1201 and the check CP 96-1252, whereas the commercial cultivar CP 00-1101 and check and commercial cultivar CP 96-1252 displayed the highest genetic distance of 0.68. The average genetic distance in the population was moderate (0.52). The hierarchical clustering analysis revealed two groups of genotypes (Figure 6). Each group comprised five genotypes, and the genotypes of group 1 were three of the commercial cultivars (CP 96-1252, CPCL 05-1201, and CPCL 97-2730), the recently released cultivar for sandy soil (CP 13-4474), and the test genotype CP 14-4165.

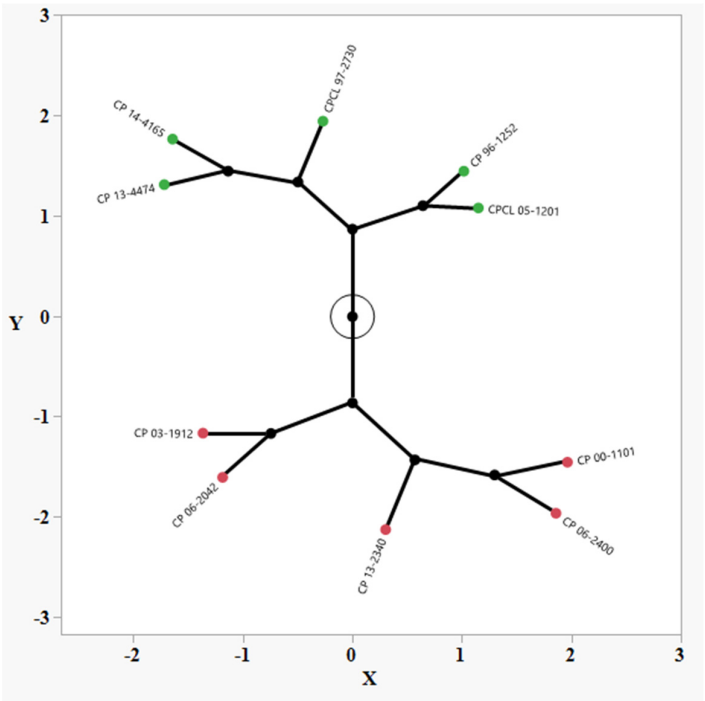


Figure 6. Constellation graph displaying the relationships between 10 sugarcane genotypes. Genotypes possessing the same color belong to the same cluster. Red stands for genotypes of group 1 and green for genotypes of group 2.

The genotypes of group 2 were the commercial cultivars CP 06-2400, CP 00-1101, CP 06-2042, CP 03-1912, and the test genotype CP 13-2340. The average genetic distance within group 1 was 0.55, whereas within group 2 it was 0.41. The genetic distance between groups 1 and 2 was 0.56. CP 14-4165 and CP 13-2340 showed an average genetic distance of 0.45 and 0.59, respectively, compared with the other seven genotypes. Genetic distance between CP 14-4165 and CP 13-2340 was moderate (d = 0.58). The moderate genetic dissimilarity

of CP 14-4165 and CP 13-2340 with respect to commercial germplasm is an important contribution to diversify the germplasm available for sugarcane breeders and growers in Florida. In sugarcane breeding programs, the selection of parents with high sugar content should be effective in producing progeny with high sugar content, more so than with cane yield [13]. One of the reasons proposed for the observed yield plateaus has been the small number of genotypes used as parents [70]. These two genotypes tend to possess an acceptable genetic variation; hence, they can further be exploited commercially and as parents in the breeding program to improve sugarcane genotypes. CP 14-4165 and CP 13-2340 have fertile pollens and were included as parents in the CP breeding program.

4. Conclusions

The simultaneous selection based on BLUP_CRS, BLUP_CY, and RA led to the conclusion that test genotypes CP 14-4165 and CP 13-2340 are the most outstanding test genotypes. Both genotypes exhibited significantly higher BLUP_CRS than the others, including the checks in the PP and TS locations. In the PF location, CP 14-4165 was the most outstanding genotype. The test genotype CP 14-4165 had similar BLUP_CY and RA to the current leading cultivar, CP 96-1252, in the Florida sugarcane industry. The GEI analysis revealed that the PP location is the most discriminative for CY and the ideal location to test genotypes for CRS content, whereas TS is the ideal location for CY. The three test locations had a similar discriminative ability to differentiate genotypes for CRS. Based on the findings obtained in the GEI analyses, it is necessary to conduct additional analyses with other CP series of genotypes to decide for the most appropriate locations to select new genotypes in sandy soils for CRS content but without compromising the genotypes' discrimination for CY. The combined analysis of CY and CRS content based on BLUP-GGE joint analysis, the pathological evaluation, and the molecular diversity analysis permitted the selection of new sugarcane genotypes with specific adaptation to sand locations in Florida. The selection of CP 14-4165 and CP 13-2430 represents continuous breeding efforts made by the CP breeding program to develop superior sugarcane genotypes with enhanced sucrose yield, resistance to major sugarcane diseases (particularly orange rust), and acceptable genetic diversity when they are used as parents in breeding cycles.

Author Contributions: O.C.A.: conceptualization, formal analysis, investigation, methodology, supervision, validation, visualization, writing—original draft, writing—review and editing, and software. A.M.: conceptualization, investigation, methodology, writing—review and editing, validation, and visualization. H.S.S.: conceptualization, investigation, methodology, and writing—review and editing. S.S.: conceptualization, data curation, investigation, methodology, supervision, writing—review and editing, validation, and visualization. W.D.: data curation, investigation, methodology, supervision, writing—review and editing, resources, and validation. M.B.: conceptualization, data curation, investigation, methodology, and supervision. D.Z.: investigation, methodology, supervision, writing—review and editing, funding acquisition, project administration, resources, and validation. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the U.S. Department of Agriculture–Agricultural Research Service CRIS projects 6030-21000-006-000D and the Florida Sugar Cane League.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Nick Pachter for his assistance in collecting data for this manuscript. The authors thank Md S. Islam for testing the *Bru*1 gene. We also thank the Stage 4 crew from CP station for planting, harvesting, and sample processing. We also acknowledge two internal reviewers (James Todd and Keo Corak) for their valuable suggestions. We thank the United States Sugar Corporation, Florida Crystal Corporation, and the Florida Sugarcane League for their support in providing sugarcane acreage for the trials.

Conflicts of Interest: The authors declare no conflict of interest. Mention of trade names or commercial products does not imply recommendation or endorsement by the U.S. Department of Agriculture. The USDA is an equal opportunity provider and employer.

References

1. van Weelden, M.; Swanson, S.; Davidson, W.; Baltazar, M.; Rice, R. Sugarcane variety census: Florida. *Sugar J.* **2022**, *85*, 8–20.
2. Roka, F.M.; Baucum, L.E.; Rice, R.W.; Alvarez, J. Comparing costs and returns for sugarcane production on sand and muck soils of southern Florida, 2008–2009. *J. Am. Soc. Sugar Cane Technol.* **2010**, *30*, 50–66.
3. Glaz, B.; Kang, M.S. Locations contribution determined via GGE Biplot analysis of multienvironment sugarcane genotype-performance trials. *Crop Sci.* **2008**, *48*, 941–950. [CrossRef]
4. Edmé, S.J.; Miller, J.D.; Glaz, B.; Tai, P.Y.P.; Comstock, J.C. Genetic contribution to yield gain in the Florida sugarcane industry across 33 years. *Crop Sci.* **2005**, *45*, 92–97. [CrossRef]
5. Plunkett, D.L.; Evenson, J.P.; Sanford, W.C. Ratooning crops. *Adv. Agron.* **1970**, *22*, 285–330.
6. Jackson, P. Genotype x environment interactions in sugarcane. II. Use of performance in plant cane as an indirect selection criterion for performance in ratoon crops. *Aust. J. Agric. Res.* **1992**, *43*, 1461–1471. [CrossRef]
7. Milligan, S.B.; Gravois, K.A.; Martin, F.A. Inheritance of sugarcane ratooning ability and the relation of younger crop traits to older crop traits. *Crop Sci.* **1996**, *36*, 45–50. [CrossRef]
8. Xu, F.; Wang, Z.; Lu, G.; Zeng, R.; Que, Y. Sugarcane Ratooning Ability: Research Status, Shortcomings, and Prospects. *Biology* **2021**, *10*, 1052. [CrossRef]
9. Ricaud, R.; Arceneaux, A. Some factors affecting ratoon cane yield and longevity in Louisiana. In Proceedings of the 8th Conference of the Australian Society of Sugar Cane Technologist, Townsville, Australia, 28 April–1 May 1986; Volume 19, pp. 18–24.
10. Edmé, S.; Suman, A.; Kimbeng, C. Sugarcane. In *Yield Gains in Major U.S. Field Crops*; CSSA Special Publication; ACSESS: Madison, WI, USA, 2014; pp. 397–431.
11. Kennedy, P.L.; Schmitz, A.; Zhang, F. Sugar recovery rates in Louisiana, Florida, and Australia (1901–2018): Sugarcane varietal development and cultural practices. *Crop Sci.* **2021**, *61*, 331–341. [CrossRef]
12. Zhao, D.; Li, Y.-R. Climate Change and Sugarcane Production: Potential Impact and Mitigation Strategies. *Int. J. Agron.* **2015**, *2015*, 10. [CrossRef]
13. Jackson, P.A. Breeding for improved sugar content in sugarcane. *Field Crop. Res.* **2005**, *92*, 277–290. [CrossRef]
14. Jackson, P.A.; McRae, T.A. Selection of sugarcane clones in small plots: Effects of plot size and selection criteria. *Crop Sci.* **2001**, *41*, 315–322. [CrossRef]
15. Berding, N.; Owens, W.G.; le Brocq, D.G. Genetic diversity: Breeding to avoid future vulnerability. *Proc. Aust. Soc. Sugar Cane Technol.* **1998**, *20*, 140–147.
16. Brown, J.S.; Glaz, B. Analysis of resource allocation in final stage sugarcane clonal selection. *Crop Sci.* **2001**, *41*, 57–62. [CrossRef]
17. Del Blanco, I.A.; Glaz, B.; Edmé, S.J. Improving efficiency of sugarcane genotypes. *Crop Sci.* **2010**, *50*, 1744–1753. [CrossRef]
18. Coto Arbelo, O.; Sandhu, H.S.; Sandoya, G.; Momotaz, A.; Poudyal, C. Spatial analysis improves the efficiency of selection in the first clonal stage of the Canal Point sugarcane breeding program for sandy soils. *Proc. ASSCT Sugar J.* **2022**, *85*, 27–28.
19. Coto Arbelo, O.; Sandhu, H.; Momotaz, A.; Davidson, W.; Sandoya, G.; Baltazar, M.; Barreto, E.; LaBorde, C. Selection via Cane Yield and Ratooning Ability of Sugarcane Genotypes in Sandy Soils in Florida. *Sugar Tech.* **2021**, *23*, 986–998. [CrossRef]
20. Edmé, S.J.; Davidson, R.W.; Zhao, D.; Comstock, J.C.; Sandhu, H.S.; Glaz, B.; Milligan, S.; Hu, C.-J.; Sood, S.; McCorkle, K.; et al. Registration of 'CPCL 05-1201' Sugarcane. *J. Plant Regist.* **2016**, *10*, 14–21. [CrossRef]
21. Arnold, G.C. Statistical analysis of regional yield trials: AMMI analysis of factorial designs. *Comput. Electron. Agric.* **1992**, *12*, 81–82. [CrossRef]
22. Yan, W.; Sheng, H.A.; Szlavnics, Q. Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Sci.* **2000**, *40*, 597–605. [CrossRef]
23. Cullis, B.R.; Jefferson, P.; Thompson, R.; Smith, A.B. Factor analytic and reduced animal models for the investigation of additive genotype-by-environment interaction in outcrossing plant species with application to a Pinus radiata breeding programme. *Appl Genet.* **2014**, *127*, 2193–2210. [CrossRef] [PubMed]
24. Dia, M.; Wehner, T.C.; Perkins-Veazie, P.; Hassell, R.; Price, D.S.; Boyhan, G.E.; Olson, S.M.; King, S.R.; Davis, A.R.; Tolla, G.E.; et al. Stability of fruit quality traits in diverse watermelon cultivars tested in multiple environments. *Hortic. Res.* **2016**, *3*, 16066. [CrossRef] [PubMed]
25. Cheng, L.; Zhang, X.; Zhang, X.; Zhang, W.; Lin, Y. Forestry multi-environment trial analysis based on BLUP and GGE biplot. *J. Northeast. Univ Nat. Sci. Ed.* **2018**, *46*, 87–93. (In Chinese)
26. Ling, J.; Xiao, Y.; Hu, J.; Wang, F.; Ouyang, F.; Wang, J.; Weng, Y.; Zhang, H. Genotype by environment interaction analysis of growth of *Picea koraiensis* families at different sites using BLUP-GGE. *New For.* **2021**, *52*, 113–127. [CrossRef]
27. Zhao, D.; Davidson, R.W.; Gordon, V.S.; Md, S.I.; McCord, P.H.; Sandhu, H.S.; Sood, S.; Comstock, J.C.; Baltazar, M.; Singh, M.P. Registration of 'CP 11–2248' sugarcane for Florida organic soils. *J. Plant Regist.* **2020**, *14*, 318–327. [CrossRef]
28. Edmé, S.J.; Tai, P.Y.P.; Glaz, B.; Gilbert, R.A.; Miller, J.D.; Davidson, J.D.; Dunckelman, J.W.; Comstock, J.C. Registration of 'CP 96-1252' Sugarcane. *Crop Sci.* **2005**, *45*, 421–423. [CrossRef]

29. Milligan, S.B.; Davidson, R.W.; Edmé, S.J.; Comstock, J.C.; Hu, C.-J.; Holder, D.G.; Glaz, B.; Glynn, N.C.; Gilbert, R.A. Registration of 'CPCL 97-2730'. *Sugarcane J. Plant Regist.* **2009**, *3*, 158–164. [CrossRef]
30. Rice, R.W.; Baucum, L.E.; Davidson, W. Sugarcane variety census: Florida. 2013. *Sugar J.* **2014**, *77*, 10–19.
31. Muchovej, R.M.C.; Luo, Y.; Shine, J.M., Jr.; Jones, J.C. Nutritional problems associated with low yield of sugarcane on mineral soils. *Soil Crop Sci. Soc. Fla.* **2000**, *59*, 146–150.
32. Legendre, B.L. The Core/Press Method for Predicting the Sugar Yield from Cane for Use in Cane Payment. *Sugar J.* **1992**, *52*, 1–7.
33. Alvarado, G.; Rodríguez, F.M.; Pacheco, A.; Burgueño, J.; Crossa, J.; Vargas, M.; PérezRodríguez, P.; Lopez-Cruce, M.A. META-R: A software to analyze data from multi-environment plant breeding trials. *Crop. J.* **2020**, *8*, 745–756. [CrossRef]
34. Dunckelman, J.W. Breeding Behavior of Ratooning Ability in Sugarcane. Ph.D. Thesis, Louisiana State University, Baton Rouge, LA, USA, 1982; p. 3714.
35. Yan, W. Singular value partitioning for biplot analysis of multi-environment trial data. *Agron. J.* **2002**, *94*, 990–996.
36. Yan, W.; Hunt, L.A. Biplot analysis of diallel data. *Crop Sci.* **2002**, *42*, 21–30. [CrossRef] [PubMed]
37. Frutos, E.; Galindo, M.P.; Leyva, V. An interactive biplot implementation in R for modeling genotype-by-environment interaction. *Stoch. Environ. Res. Risk Assess.* **2014**, *28*, 1629–1641. [CrossRef]
38. Pacheco, A.; Vargas, M.; Alvarado, G.; Rodríguez, F.; Crossa, J.; Burgueño, J. GEA-R (Genotype × Environment Analysis with R for Windows) Version 4.0. CIMMYT Research Software, Mexico. 2015. Available online: https://hdl.handle.net/11529/10203 (accessed on 20 December 2022).
39. Sood, S.; Davidson, W.; Comstock, J.; Gordon, V.; Islam, S.; Baltazar, M.; Sandhu, H.; Zhao, D. Development of disease-resistant CP sugarcane cultivars in Florida. *Proc. Int. Soc. Sugar Cane Technol.* **2019**, *30*, 1882–1887.
40. Zhao, D.; Davidson, R.W.; Baltazar, M.; Comstock, J.C. Field evaluation of sugarcane orange rust for first clonal stage of the CP cultivar development program. *Am. J. Agric. Biol. Sci.* **2015**, *10*, 1–11. [CrossRef]
41. Tai, P.Y.P.; Miller, J.D.; Glaz, B.; Deren, C.W.; Shine, J.M., Jr. Registration of 'CP 78-1628' Sugarcane. *Crop Sci.* **1991**, *31*, 236. [CrossRef]
42. Deren, C.W.; Glaz, B.; Tai, P.Y.P.; Miller, J.D.; Shine, J.M., Jr. Registration of 'CP 80-1743' Sugarcane. *Crop Sci.* **1991**, *31*, 235–236. [CrossRef]
43. Miller, J.D.; Tai, P.Y.P.; Glaz, B.; Dean, J.L.; Kang, M.S. Registration of 'CP 72-2086' Sugarcane. *Crop Sci.* **1984**, *24*, 210. [CrossRef]
44. Miller, J.D.; Rice, E.R.; Dean, J.L.; Tai, P.Y.P. Registration of 'CP 72-1210' Sugarcane. *Crop Sci.* **1981**, *21*, 797. [CrossRef]
45. Asnaghi, C.; Roques, D.; Ruffel, S.; Kaye, C.; Hoarau, J.Y.; Telismart, H.; Girard, J.C.; Raboin, L.M.; Risterucci, A.M.; Grivet, L.; et al. Targeted mapping of a sugarcane rust resistance gene (*Bru*1) using bulked segregate analysis and AFLP markers. *Theor. Appl. Genet.* **2003**, *108*, 759–764. [CrossRef] [PubMed]
46. Costet, L.; Le Cunff, L.; Royaert, S.; Raboin, L.-M.; Hervouet, C.; Toubi, L.; Telismart, H.; Garsmeur, O.; Rousselle, Y.; Pauquet, J.; et al. Haplotype structure around *Bru*1 reveals a narrow genetic basis for brown rust resistance in modern sugarcane cultivars. *Theor. Appl. Genet.* **2012**, *125*, 825–836. [CrossRef] [PubMed]
47. Glynn, N.C.; LaBorde, C.; Davidson, R.W.; Irey, M.; Glaz, B.; D'Hont, A.; Comstock, J.C. Utilization of a major brown rust resistance gene in sugarcane breeding. *Mol. Breed.* **2012**, *31*, 323–331. [CrossRef]
48. Cordeiro, G.M.; Pan, Y.-B.; Henry, R.J. Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm. *Plant Sci.* **2003**, *165*, 181–189. [CrossRef]
49. Gilbert, R.A.; Comstock, J.C.; Glaz, B.; del Blanco, I.A.; Edmé, S.J.; Davidson, R.W.; Glynn, N.C.; Sood, S.; Zhao, D.; Miller, J.M.; et al. Registration of 'CP 03-1912' Sugarcane. *J. Plant Regist.* **2011**, *5*, 318–324. [CrossRef]
50. Davidson, W.R.; Sandhu, H.S.; McCord, P.; Comstock, C.; Edmé, S.J.; Zhao, D.; Glaz, B.; Sood, S.; Glynn, N.C.; Gilbert, R.A.; et al. Registration of 'CP 06-2042' Sugarcane. *J. Plant Regist.* **2017**, *11*, 121–128. [CrossRef]
51. Islam, S.; Sandhu, H.S.; Zhao, D.; Sood, S.; Momotaz, A.; Baltazar, M.; Arbelo, O.C.; Davidson, R.W.; Gordon, V.S. Registration of 'CP 13-4474' sugarcane for sand soils. *J. Plant Regist.* **2022**, *17*, 80–90. [CrossRef]
52. Gilbert, R.A.; Comstock, J.; Glaz, B.; Edmé, S.; Davidson, R.W.; Glynn, N.C.; Miller, J.M.; Tai, Y.P. Registration of 'CP 00-1101' Sugarcane. *J. Plant Regist.* **2008**, *2*, 95–101. [CrossRef]
53. Zhao, D.; Comstock, J.C.; Sandhu, H.S.; Glaz, B.; Edmé, S.J.; Davidson, R.W.; Sood, S.; Gilbert, R.A.; McCorkle, K.; Glynn, N.C. Registration of 'CP 06-2400' Sugarcane. *J. Plant Regist.* **2014**, *9*, 71–77. [CrossRef]
54. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 9.
55. Jackson, P.; McRae, T.; Hogarth, M. Selection of sugarcane families across variable environments I. Sources of variation and an optimal selection index. *Field Crop. Res.* **1995**, *43*, 109–118. [CrossRef]
56. Sengwayo, S.; Zhou, M.; Labuschagne, M. Location and crop-year effects on sugarcane genotype performance for the coastal short cycle breeding programmes in South Africa. *S. Afr. J. Plant Soil* **2017**, *35*, 79–87. [CrossRef]
57. Yan, W. *Crop Variety Trials: Data Management and Analysis*; John Wiley & Sons, Incorporated: New York, NY, USA, 2014; ProQuest Ebook Central; Available online: http://ebookcentral.proquest.com/lib/ufl/detail.action?docID=1650860 (accessed on 10 January 2020).
58. Zhou, M.; Shoko, M.D. Simultaneous selection for yield and ratooning ability in sugarcane genotypes using analysis of covariance. *S. Afr. J. Plant Soil* **2012**, *29*, 93–100. [CrossRef]

59. Deren, C.W.; Alvarez, J.; Glaz, B. Use of economic criteria for selecting clones in a sugarcane breeding program. *Proc. Int. Soc. Sugar Cane Technol.* **1995**, *21*, 437–447.
60. Burner, D.M.; Legendre, B.L.; Boykin, D.L.; Duet, M.J., Jr. A retrospective analysis of genetic advance in natural ripening of sugarcane. *Int. Sugar J.* **2015**, 370–377.
61. Gilbert, R.A.; Shine, J.M., Jr.; Rice, R.W. Sucrose accumulation and harvest schedule recommendations for CP sugarcane cultivars. *Crop. Manag.* **2004**, *3*, 1–7. [CrossRef]
62. Lingle, S.E.; Johnston, R.; Tew, T.; Viator, R. Changes in juice and sugarcane yield with recurrent selection for sucrose. *Field Crop. Res.* **2010**, *118*, 152–157. [CrossRef]
63. Irvine, J.E.; Richard, C.A. Production statistics as evidence of technological improvement: The Louisiana sugarcane industry as a case study. *Sugar Cane* **1983**, *1*, 13–18.
64. Yan, W.; Kang, M.S.; Ma, B.; Woods, S.; Cornelius, P.L. GGE biplot vs. AMMI analysis of genotype-by-environment data. *Crop Sci.* **2007**, *47*, 643–655. [CrossRef]
65. Zhao, D.; Irey, M.; LaBorde, C.; Hu, C.-J. Identifying Physiological and Yield-Related Traits in Sugarcane and Energy Cane. *Agron. J.* **2017**, *109*, 927–937. [CrossRef]
66. Yan, W.; Tinker, N.A. Biplot analysis of multi-environment trial data: Principles and applications. *Can. J. Plant Sci.* **2006**, *86*, 623–645. [CrossRef]
67. Zhao, Y.; Liu, J.; Huang, H.; Zhang, Y.; Zhao, P.; Zhao, J.; Deng, J.; Wu, C. Genetic improvement of sugarcane (*Saccharum* spp.) contributed to high sucrose content in China based on an analysis of newly developed varieties. *Agriculture* **2022**, *12*, 1789. [CrossRef]
68. Duma, S.W.; Shimelis, H.; Ramburan, S.; Shayanowako, A.I.T. Genotype-by-region interactions of released sugarcane varieties for cane yield in the South African sugar industry. *Agronomy* **2022**, *12*, 125. [CrossRef]
69. Guilly, S.; Dumont, T.; Thong-Chane, A.; Barau, L.; Hoarau, Y. Analysis of multi-environment trials (MET) in the sugarcane breeding program of Reunion Island. *Euphytica* **2017**, *213*, 213. [CrossRef]
70. Roach, B.T. Origin and Improvement of the genetic base of sugarcane. *Proc. Aust. Soc. Sugar Cane Technol.* **1989**, *11*, 34–47.