Brazilian Journal of Biometrics, **42**, 59-67 (2024)
doi:10.28951/bjb.v42i1.651

ARTICLE

Genetic dissimilarity between sugarcane genotypes at different harvest period for brown sugar production[1]

Patrícia Jesus de Melo[1], Alessandro Dal'Col Lúcio[1], Elisangela Marques Jeronimo Torres [2], Maria Inês Diel[3], Tiago Olivoto[4], Marcos Guimarães de Andrade Landell[2], Darlei Michalski Lambrecht *[1], André Luís Tischler[1] and Francieli de Lima Tartaglia[1]

[1] Department of Plant Sciences, Federal University of Santa Maria, Santa Maria - RS, Brazil
[2] São Paulo Agribusiness Technology Agency - Apta Pólo Centro Oeste, Bauru - SP, Brazil
[3] Federal University of Santa Maria, Campus Cachoeira do Sul , Cachoeira do Sul - RS, Brazil
[4] Department of Plant Sciences, Federal University of Santa Catarina, Florianópolis – SC, Brazil
*Corresponding author. Email: darleilambrecht@gmail.com

(Received: January 11, 2023; Revised: May 19, 2023; Accepted: June 30, 2023; Published: March 15, 2024)

Abstract

The purpose of this study was to characterize and identify the dissimilarity between sugarcane genotypes through technological and agronomic traits, in three harvest periods for brown sugar production. A randomized complete block design with four replications, using a split-plot treatment layout was used (IACSP 93-3046, RB 96-6928, IACSP 95-5094, IACSP 97-4039, SP 81-3250, IACSP 95-5000, RB 86-7515, IACSP 96-3060, IACSP 04-704, IACSP 04-656) and three harvest periods (15, 17 and 19 months of cultivation) with four replications. Technological traits related to quality parameters and agronomic traits related to the productive aspect were evaluated. According to the results, all genotypes showed a better response in the second harvest. The qualitative variables the apparent sucrose in sugarcane, total recoverable sugars and soluble solids in sugarcane showed differences between genotypes and seasons and are the ones that most contribute to the genetic divergence of brown sugar. For the production of dark colored sugar, the RB96-6928 genotype is recommended among all those evaluated, in the three growing seasons. As for the production of light-colored brown sugar, the cultivation of the genotypes IACSP 04-656 and IACSP95-5094 in season 1, the IACSP 97-4039 in season 2 and the IACSP95-5094 in season 3 is recommended.

Keywords: Multivariate Analysis; Clustering; Principal Components; Quality; *Saccharum* spp.

1. Introduction

Sugarcane (*Saccharum* spp.) belongs to the Poaceae family, originated from a tropical climate. Brazil leads the ranking of world production of sugarcane, harvesting a total of 642 million tons estimated in 2020/2021 crop season. The state of São Paulo is the largest producer of sugarcane, with an estimated harvest of 335 million tons in 2020/2021 (Conab, 2020). Brown sugar is produced by small and medium-sized properties for the purpose of own consumption or for

commercialization in a homemade way by the evaporation and concentration of the cane juice, thus forming the raw sugar (Jeronimo, 2020).

Characterized as a healthy food with nutritional value, higher than other industrially produced sugar, brown sugar is catching the attention of consumers who search for higher nutritional quality food (Asiskin *et al.*, 2016). It has higher concentrations of minerals, vitamins, and proteins, besides several positive effects like the strengthening of the immune system, cytoprotectors, anticancer properties, and reduces the occurrence of diabetes and hypertension (Santos *et al.*, 2018) and presenting antioxidant properties (Nayaka *et al.*, 2009; Okabe *et al.*, 2009).

Due to the economic and social importance of brown sugar, studies are needed indicating genotypes and the growing period, with the most favorable harvesting moment to obtain higher productivity and quality of brown sugar (Silva *et al.*, 2014). For this purpose, multivariate analysis techniques are useful to assist in decision making in genetic improvement research, selection and indication of genotypes for a given cultivation and production condition, reducing labor and financial resources, without losing the experimental precision and reliability of the results.

To study the genetic variability structure among the genotypes, one option is performing the cluster analysis based on the estimates of the genetic dissimilarity among them and also to use principal component analysis to visualize how the genotypes group to each other and to identify the traits that most and least contribute to this behavior (Cruz *et al.*, 2012).

Considering the economic and social importance of sugarcane in Brazil, and the increase in brown sugar consumption, studies that elucidate the production capacity, the quality of the sugar of the genotypes and the influence of the harvest time on the brown sugar production and mainly the genetic divergence between genotypes is welcome. Some authors, such as Ftwi *et al.* (2016) and Nardino *et al.* (2017) used multivariate analysis techniques as cluster analysis and principal component analysis to study the divergence between sugarcane genotypes, but not aimed at brown sugar production.

In this context, the objective was to characterize and quantify the dissimilarity between sugarcane genotypes based on technological and agronomic traits, study the relationships among the traits, and prospect promissory genotypes for brown sugar production across three harvest periods.

2. Materials and Methods

2.1 Conditions for cultivation and preparation of the study area

The experiment was carried out in the city of Jaú - São Paulo, Brazil, located at 22º17'S and 48º 34'W with 580 meters of altitude. Based on the Köppen's classification, the climate in this region, is Aw-dry type (Alvares *et al.*, 2013), temperature annual average of 21.6 ºC, annual rainfall average of 1.344 mm. Fertilization throughout the experiment followed soil analysis, with applications according to the technical recommendations for the crop. The planting was carried out in the first fortnight of April 2013, with planting carried out in furrows, was planted in the rows at a density of 18 buds per meter.

2.2 Experimental design

A randomized block design with four replications, using a split-plot treatment layout was used. The study consisted of ten sugarcane genotypes (allocated at the whole-plots), characterized in Table 1, with their parents, origin, maturation group, and acreage. Three harvests, name Season 1, Season 2 and Season 3 were proceeded, respectively, at 15, 17 and 19 months after planting. The experimental plot was composed of five 8 m length cropping rows spaced by 1.5 m, making a total useful area of 60 m^2 (2.400 m^2 of area experimental).

Table 1. Classification according to the maturation group of genotypes and their progenitors

Genotype	Code	Maturation group	Origin	Acreage in crop 2017/2018 in Brasil %	Acreage in crop 2018/2019 in state of SP %	Parents	
IACSP04-656	G1	WI	IAC	WI	WI	IACSP93-3046	SP77-5181
IACSP04-704	G2	WI	IAC	WI	WI	IACSP95-3028	SP77-5181
IACSP93-3046	G3	Medium/Late	IAC	WI	WI	SP79-1011	WI
IACSP95-5000	G4	WI	IAC	WI	1.8	SP84-2066	SP80-185
IACSP95-5094	G5	Medium	IAC	WI	WI	SP80-3280	WI
IACSP96-3060	G6	Medium/Late	IAC	WI	WI	SP82-6108	WI
IACSP97-4039	G7	Early	IAC	WI	WI	RB835486	RB855453
RB867515	G8	Medium/Late	RIDESA	26	18.3	RB72454	WI
RB966928	G9	Early	RIDESA	9.2	15.1	RB855156	RB815690
SP81-3250	G10	Medium	COPERSUCAR	5.7	2.1	CP70-1547	SP71-1279

*WI: Without information; IAC: Campinas Agronomic Institute, RIDESA: Interuniversity Network for the Development of the Sugar and Energy Sector, SP: Copersucar.

2.3 Measured traits

In each sub-plot (harvest period), a random sample of 62 stems was collected. This sample was split up into two subsamples. One, containing 12 stems was used to measure the biometric traits related to sugarcane and the other sub-sample, with 50 stems, used to measure broth- and sugar-related traits (Table 2). From the total volume of broth extracted from the 50 stems, eight liters were destined for the production of brown sugar, using an open aluminum pan, at atmospheric pressure, on a high-pressure semi-industrial gas stove. The temperature throughout the process was 95 ± 5ºC. At the end of the broth cooking process, the temperature was raised to 115ºC, it is removed from the fire at the moment called brown sugar obtaining, then the beating process was carried out to form brown sugar. These followed the information in the Manual for the Manufacture of Brown Sugar, Melado and Rapadura (Embrapa, 2014). For each day of analysis, four cultivars were analyzed, therefore, four batches of brown sugar were produced, each batch was produced from eight liters of broth. Each season was analyzed for 10 days and in total 40 lots of brown sugar were produced. In all, 120 lots of brown sugar were produced.

Table 2. Classification according to the maturation group of genotypes and their progenitors

Code	Description	Unit
	Sugarcane-related traits	
SH	stem height	cm
IN	internodes number	n
ASM	average stem mass	kg
ASC	apparent sucrose in sugarcane (pol cane)	%
TSSC	total soluble solids in sugarcane (ºbrix cane)	%
CF	cane fiber	%
P	purity	%
TRS	total recoverable sugars	kg ton^{-1}
	Broth-related traits	
VB	volume of the broth	L
ASB	apparent sucrose of the broth (pol broth)	%
TSSB	total soluble solids of the broth (ºbrix cane)	%
	Sugar-related traits	
L*	Brightness	0-100
a*	Red/Green value	ratio
b*	Blue/Yellow value	ratio
Chroma	Chroma	Hue angle
BSTS	brown sugar per tone of sugarcane	kg
BS100	mass of brown sugar obtained in 100 liters of broth	kg
ASS	apparent sucrose of the sugar (pol sugar)	%

The soluble solids content was measured in automatic bench refractometer, brand Reichert, model I300 and the readings were carried out in Polarimeter, brand Anton Paar, model MCP 200.

Purity and fiber content were determined according to the CONSECANA manual (Consecana, 2006). The total soluble solids were measured using a refractometer.

The sugar samples were analyzed for instrumental color, using Minolta portable colorimeter, model CR400, scale CIELAB. The device measures the coordinates L*, which represents the brightness on a scale of zero (black) a hundred (White); a*, which represents a scale of shades of red (0+a) to green (0-a); and b* which represents a scale of shades of yellow (0+b) the blue one (0-b). The Chroma trait (Hue angle) was measured by converting the values of a* and b* obtained, through this occurs the definition of color intensity, (0) in the center and increases according to the distance.

2.4 Statistical analysis

To test the effect of genotypes, seasons and their interactions in the measured traits, each trait was analyzed using the following linear mixed-effect model eq. 1:

$$y_{ijk} = \mu + \beta_k + \alpha_i + \varepsilon^w_{i(k)} + \tau_j + (\alpha\tau)_{ij} + \varepsilon^s_{j(ki)} \quad (1)$$

where y_{ijk} is the response trait observed in the k the block of the j the harvest (subplot) of the i the genotype (whole plot), μ is the grand mean, β_k is the effect of the k the block assumed to be random with $\beta_k\ i.i.d \sim N(0, \sigma^2_\beta)$, α_i is the effect of the i the genotype (fixed effect), $\varepsilon^w_{i(k)}$is the whole-plot random error $\varepsilon^w_{i(k)}\ i.i.d \sim N(0, \varepsilon^2_w)$, τ_j is the effect of the j the harvest (fixed effect), $(\alpha\tau)_{ij}$ is the interaction effect between the i the genotype and j the harvest (fixed effect), and $\varepsilon^s_{j(ki)}\ \varepsilon^s_{j(ki)}$ is the effect of split-plots and random noises $\varepsilon^s_{j(ki)}\ i.i.d \sim N(0, \varepsilon^2_s)$. The significance of fixed effects was tested by the Kenward-Roger's F test (Kenward & Roger, 1997). These procedures were carried out using the R packages lmer (Bates *et al.*, 2015) and lmerTest (Kuznetsova *et al.*, 2017)

For each growing season, a two-way table (genotypes in rows and traits in columns) was created using the scaled marginal estimates of the above-mentioned mixed-effect model. This table was further used in a principal component analysis (PCA) using the correlation matrix to explore the relationships among traits, the contribution of each one of them in the total variance as well as the magnitude of these traits concerning the genotypes. Biplots (Gabriel, 1971) were confectioned with the factoextra R package (Kassambara & Mundt, 2020).

Additionally, a cluster analysis was also computed within each season using the Unweighted Pairs-Groups by Arithmetic Average (UPGMA) based on the Euclidean distance matrix estimated with the traits that made the greatest contribution in the PCA. The cut-point was chosen following the method of Mojena (Mojena, 1977). The association between the cophenetic matrix and the original matrix was determined by the cophenetic correlation coefficient (CCC) (Sokal & Rohlf, 1962). To verify the coincidence among the distance matrices, a pairwise Mantel Test between distance matrices of the three seasons was performed. This analysis was carried out using the function *clustering* and *pairs_mantel* of the metan R package (Olivoto & Lúcio, 2020). All statistical analysis were performed using R software (R Core Team, 2020).

3. Results and Discussion

3.1 Principal components analysis

The traits CF and BSTS were excluded from the PCA due to the low contribution to explaining the variation in the three seasons (Supplementary material - Figure S2). IN and ASS, although they also have a small contribution, remained due to the more assertive recommendations of the genotypes that these enable and are based, among other quality attributes, on the important trait of ASS productivity. The first two principal components explained 65.4%, 60.3%, and 71.8% of the total variation for season 1, season 2, and season 3, respectively (Figure 1; Supplementary material – Figure S4).

For season 1, the L*, b* and Chroma that are related to the color pattern, were negatively related with genotypes G8 and G9, for both PCs, which indicate the lowest values of these traits

for these genotypes. On the other hand, these same genotypes presented high values for the ASM and SH (Figure 1a). Such an observed response indicates that longer stems and with greater weight present less yellow and less shiny sugars, which may influence the acceptance of the product in the consumer market. The genotype G6 has similar behavior as G8 and G9 when considering PC1.

The traits ASC, TSSC, ASB, TSSB, and TRS are associated with the presence of sucrose in sugar cane and were positively related according to PC1 (in all seasons), and both PCs in seasons 1 and 2 (Figure 1). The highest positive related magnitudes among these traits, according to PC1 were observed for the G2, G7 and G10 genotypes (Season 1), G1, G2, G6, and G7 (Seasons 2), and G2 and G6 (Seasons 3). The genotypes G1 and G2 were also positively related to Chroma, L* and b* based onPC1, in all seasons.

For season 2 (Figure 1 b), SH, ASM, and a* present greater positive related magnitude with the G8 and G9, and negative related with Chroma, L*, b*, diverging of G7. As L* indicates brightness, the lower this value, the darker the sugar. The color of the sugar produced by the G8 and G9 genotypes showed a dark color, whereas, for the G7, a light color was observed for the brown sugar. G9 has shown the same pattern of behavior with L*, b* and chroma in all seasons, as well as G8. In season 2, the traits TSSB, TSSC, ASB, ASC and TRS showed greater magnitude for genotypes G2, G3, and G6, according to both PCs, and are also positively related with Chroma, L* and b*, considering PC1.

For season 3, considering both PCs, the G8 showed greater magnitudes for SH, VB and ASM and smaller values of L*, b*, Chroma (Figure 1 c, Figure S4). Genotypes G4, G7, and G10 showed a negative related whit trait a*, and b*, indicating positioning patterns with a hue close to the green and blue, respectively. According to PC2, for the L*, b* and Chroma presented greater magnitude for G1, indicating that it produced sugar with intense yellow color. In PC2, genotypes G2, G5, and G6 showed positive related with ASS, TRS, BS100, ASC, and a*.

In all harvest times, ASC, ASB, ASS, TSSB, TSSC, TRS were positively related with PC1 (Figure 1). These traits are related to the quality and quantity of the sugar that will be produced. Regarding the studied genotypes, it is observed that G2 (clone IACSP04-704) showed stability of sugar production in the three seasons, thus precocity prevailing and inferring that stable genotypes in TSS and TRS in different harvest times as in this case can be indicated for harvest at 15 months.

In the three harvest periods the traits Chroma, L*, b* were positively related among them and inversely related with ASM, SH, VB, and IN in high magnitude for seasons 1 and 2 and in low magnitude for season 3. This suggests that the increase of the weight results in sugars of yellow coloration and with lower brightness, which may influence the acceptance of the product in the consumer market. The coloring of brown sugar is an important quality trait and is an indicative of consumer preference, which may or may not is affected by its color (Guerra & Mujica, 2010). The brown sugar is obtained when the cooking temperature is high, causing, for example, Maillard reactions and caramelization. Besides that, the presence of phenols and flavonoids in cane stem may promote differences in the formation of dark pigments in brown sugar and may vary according to the cultivar (Asikin *et al.*, 2016).

The apparent sucrose in both cane broth and brown sugar is directly related to the amount of sugar that will be obtained. Studies with sugar production revealed that the higher amounts of total soluble solids (which are desired) are observed in later harvests, with ~14 months of cultivation (Ahmed *et al.*, 2016). However, there is a trend to decrease as the sugarcane remains in the field for longer periods, e.g., 16, 18 months (Hagos *et al.*, 2014).

The accumulation of sucrose occurs increasingly, until the peak of sucrose in the stem (Chandra *et al.*, 2014). The amount of sucrose contained in cane broth is related to the amount of sugar that will be obtained, since sucrose is the crystallizable sugar, unlike glucose and fructose, which does not have the crystallization property and which may negatively interfere with the crystallization of brown sugar if they are at higher levels (Wang *et al.*, 2017).

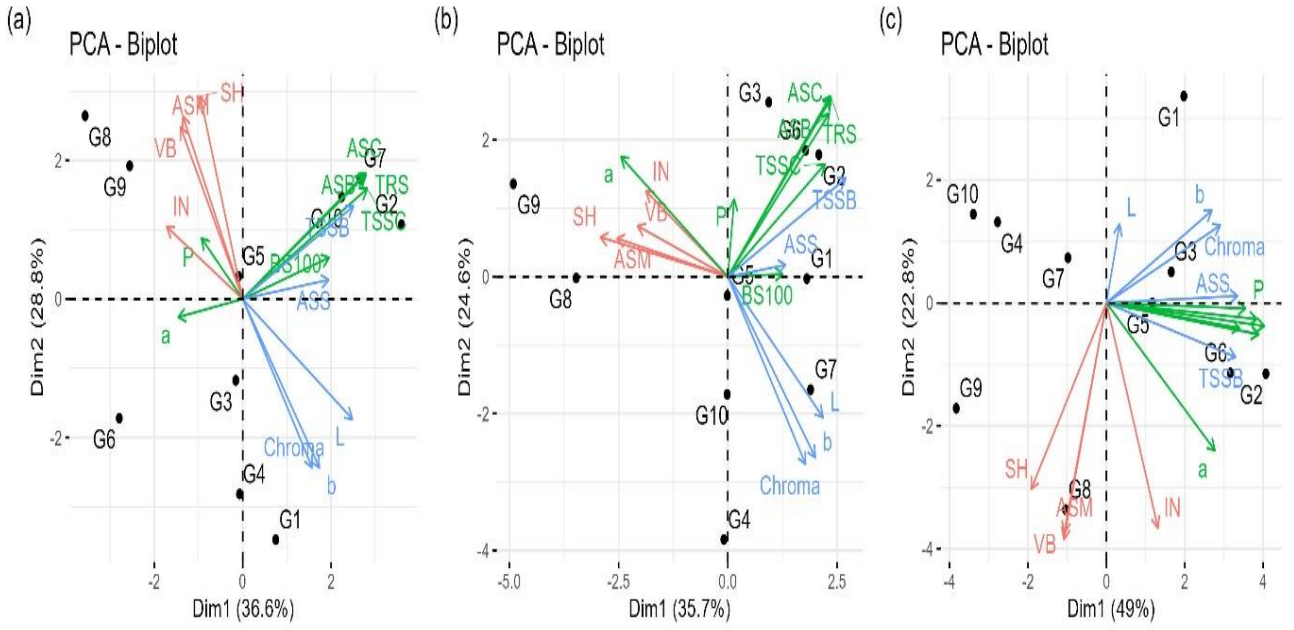

Figure 1. Biplot of principal component analysis, for the first (a), second (b) and third (c) harvests, considering the traits: stem height (SH), mass of brown sugar obtained in 100 liters of broth (BS100), total recoverable sugars (TRS), a*, b*, Chroma, L*, apparent sucrose in broth (ASB), apparent sucrose in sugarcane (ASC), average stem mass (ASM), soluble solids content of broth (TSSB), soluble solids in sugarcane (TSSC), number of internodes (IN), purity (P), broth volume (VB) and apparent sucrose in brown sugar (ASS).

3.2 Clustering analysis

Clustering analysis showed a cophenetic correlation of r = 0.80, r = 0.83, and r = 0.65 for the seasons 1, 2, and 3, respectively (Supplementary material). According to the Mantel's test, the distance matrices presented a low to intermediate correlation estimates across the harvest periods (Supplementary material, Figure S6). For the three harvests, two groups of genotypes were obtained (Figure 2). This number of groups is less than that found by Fwti *et al.* (2016) and by Nardino *et al.* (2017) studying the production of sugarcane, which may be explained due to the higher number of genotypes tested. Values close to the average were observed for ASB and for the P, showing little variation between the three seasons. For the other traits, there were variations according to the harvest period (Supplementary material - Figure S7).

In this way it is possible to verify the genetic dissimilarity among the genotypes within each harvest period, suggesting the genotypes that can be used for crosses in future breeding programs with the sugar cane crop destined for the production of brown sugar. Therefore, the use of multivariate methodology allows the discovery of the genotypes with the greatest genetic divergence and thus directs them to a genetic breeding program.

The obtained dendrogram for season 1 (Figure 2a) shows one group formed by genotypes G8 and G9, presenting mean values of SH and ASM higher than the other group for seasons 1 and 2 (Supplemental material – Figure S7), but with lower sugar production compared to group 2. This was probably observed because the peak of sucrose synthesis did not occur. The endogenous synthesis of sucrose can be derived from the expression of Susy genes (Thirugnanasambandam *et al.*, 2019). The peak accumulation of sucrose can occur from the endogenous synthesis of reducing sugars (Castro *et al.*, 2001).

The results of Chroma, b* and L* presented low values for cluster 1 in the seasons 1 and 2, which was inverse in the season 3. This corroborates the results also evidenced in the principal component analysis (Figure 2, Supplementary material – Figure S7). Such results may come from intrinsic characteristics of the genotypes such as the appropriate maturation cycle, as well as favorable environmental conditions such as precipitation and temperature (Silva *et al.*, 2014).

For season 3, cluster 2 (G9, G4, G10, and G8), shows on average a darker sugar coloration than

seasons 1 and 2, which is inferred by the lower values of L* in this season (supplementary material Figure - S7). Lower L* values may occur due to the decrease in the amount of sucrose, according to the maturation curve of these genotypes, where reducing (fructose and glucose) and non-crystallizable sugars tend to predominate (Rook *et al.*, 2006).

The sugarcane crop is characterized as semi-perennial, whose harvest occurs at the end of its growth and maturation (accumulation of sucrose) when the maximum productivity and accumulation of TRS occurs. The reduction of the TRS amounts occurs due to the natural decrease of the maturation curve and consequently the conversion of sucrose into reducing sugars (fructose and glucose) (Conab, 2020).

The traits that most contributed to the variability between genotypes were apparent sucrose from sugarcane, total recoverable sugars and soluble solids content of sugarcane. For each crop season, different groups of genotypes were formed. For most genotypes, the largest brown sugar production occurred after 17 months of cultivation. The colour of brown sugar varied according to the time of harvest and the cultivar used to produce it.

The results obtained in this manuscript point to the importance of studies capable of identifying the differences between sugarcane genotypes in each harvest period, since this influences the yield and quality of the brown sugar that will be produced. Thus, management strategies such as planning the planting and harvesting times, combined with the genotype that expresses the characteristics of interest to the producer can bring greater benefits, increasing the yield and quality of brown sugar.

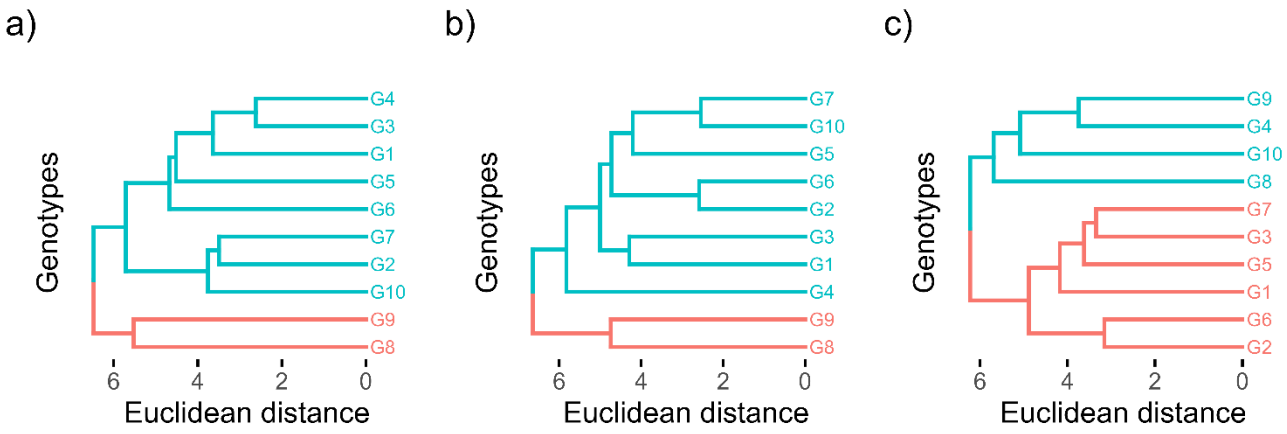

Figure 2. Dendrograms obtained by the unweighted pair group method using an arithmetic average (UPGMA), utilizing the Euclidean distance estimated between the pairs of ten genotypes (G1, G2, G3, G4, G5, G6, G7, G8, G9, G10) for each harvest period, (a) the first harvest; (b) the second harvest; (c) the third harvest.

4. Conclusions

The variables ASC, TRS and TSSC, Chroma, b* and L* showed differences between genotypes and seasons and are the ones that most contribute to the genetic divergence of brown sugar.

The RB96-6928 genotype can be recommended for the production of brown sugar in the three seasons, whereas the genotypes IACSP 04-656 and IACSP95-5094 in season 1, the IACSP 97-4039 in season 2 and the IACSP95-5094 in season 3 can be recommended for a lighter brown sugar color in the final product.

Acknowledgments

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for fellowships and grants to the authors and an APTA (Agência Paulista de Tecnologia do Agronegócio) for the availability of the area and experimental conduction. Also, we thank reviewers and editor for their comments.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Ahmed, A. Z., El-Bakry, A. & Sakina, R. A. Assessment of the optimum age for harvesting some promising sugarcane varieties. *Minia Journal of Agricultural Research and Development* **36**, 635-651, (2016).
2. Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L de M. & Sparovek, G. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* **22**, 711-728 (2013). https://doi.org/10.1127/0941-2948/2013/0507
3. Asikin, Y., Hirose, N., Tamaki, H., Ito, S., Oku, H. & Wada, K. Effects of different drying e solidi fi cation processes on physical properties, volatile fraction, and antioxidant activity of non-centrifugal cane brown sugar. *Food Science and Technology* **66**, 340-347 (2016). https://doi.org/10.1016/j.lwt.2015.10.039
4. Bates, D., Machler, M., Bolker B. & Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* **67**, 1–48 (2015). https://doi.org/10.18637/jss.v067.i01.
5. Castro, P. R. C., Miyasaki, J. M., Bemardi, M., Marengo, D. & Nogueira, M. C. S. Efeito do ethephon na maturação e produtividade da cana-de-açúcar. *Brazilian Journal of Agriculture* **76**, 277-290, (2001).
6. Chandra, A., Verma, P. K., Islam, M. N., Grisham, M. P., Jain, R., Sharma, A. & Solomon, S. Expression analysis of genes associated with sucrose accumulation in sugarcane (*Saccharum* spp. hybrids) varieties differing in content and time of peak sucrose storage. *Plant Biology* **17**, 608-617 (2014). https://doi.org/10.1111/plb.12276
7. Conab, C. N. de A. *Acompanhamento da safra brasileira: cana-de-açúcar: segundo levantamento*. (Conab, 2020).
8. Consecana. *Instruction manual*. (Piracicaba-SP: Consecana, 2006.
9. Cruz, C. D., Regazzi, A. J. & Carneiro, P. C. S. *Modelos biometricos aplicados ao melhoramento genético*. 4ª ed. (Viçosa, Editora UFV, 2012).
10. Embrapa. *Manual CCP Fabricação de Açúcar Mascavo Melado e Rapadura*. (EMBRAPA, 2014). Available at: http://www.iicabr.iica.org.br/wp-content/uploads/2015/11/Manual-CCP-Fabricação-de-Açúcar-Mascavo-Melado-e-Rapadura.pdf
11. Ftwi, M., Mekbib, F. & Abraha, E. Multivariate analysis of sugar yield contributing traits in Sugarcane (*Saccharum officinarum* L.), in Ethiopia. *African Journal of Plant Science* **10**, 145-156, (2016). https://doi.org/10.5897/AJPS2016.1419
12. Gabriel, K.R. The biplot graphic display of matrices with application to principal component analysis. *Biometrika* **58**, 453–467, (1971). https://doi.org/10.2307/2334381.
13. Guerra, M. J. & Mujica, M. V. Physical and chemical properties of granulated cane sugar "panelas". *Sociedade Brasileira de Ciência e Tecnologia de Alimentos* **30**, 250-257, (2010). https://doi.org/10.1590/S0101-20612010005000012
14. Hagos, H., Mengistu, L. & Mequanint, Y. Determining optimum harvest age of sugarcane varieties on the newly establishing sugar project in the tropical areas of tendaho, Ethiopia. *Advances in Crop Science and Technology* **2**, 2-5, (2014). https://doi.org/10.4172/2329-8863.1000156.
15. Jeronimo, E. M., Pinotti, R, N., Arruda, M, C., Sodario Cruz, J, C., Henrique, C, M., Prat, P., Verdi, A, R., Pazinato, B, C., Beraldo, M, A, P. & Ishicava, S, M. *Produção artesanal de derivados de cana-de-açúcar: Açúcar Mascavo – Melado – Rapadura.* (Campinas, CDRS, 2020). Available at https://www.cdrs.sp.gov.br/portal/themes/unify/arquivos/produtos-e-servicos/acervo-tecnico/cana-de-acucar-2020.pdf. (accessed 11 November 2020).

16. Kassambara, A. & F. Mundt. *factoextra: Extract and Visualize the Results of Multivariate Data Analyses.* R Package Version 1.0.7. (2020). https://cran.r-project.org/package=factoextra.
17. Kenward, M.G. & J.H. Roger. Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood. *Biometrics* **53**, 983–997, (1997). https://doi.org/10.2307/2533558.
18. Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* **82,** 1-26, (2017).
19. Mojena, R. Hierarchical grouping methods and stopping rules: an evaluation. *The Computer Journal* **20**, 359–363, 1977. https://doi.org/10.1093/comjnl/20.4.359
20. Nardino, M., Carvalho, I. R., Ferrari, M., Pelegrin, A. J., Szareski, V. J., Follmann, D. N. & Souza, V. Q. Indução de mutação e mensuração da variabilidade genética em genótipos de cana-de-açúcar. *Revista de Ciências Agroveterinárias* **16**, 247-255, (2017).
21. Nayaka, M. A. H., Sathisha, U. V., Manohar, M. P., Chandrashekar, K. B. & Dharmesh, S. M. Cytoprotective and antioxidant activity studies of jaggery sugar. *Food Chemistry* **115**, 113-118, (2009). https://doi.org/10.1016/j.foodchem.2008.11.067.
22. Okabe, T., Toda, T., Inafuku, M., Wada, K., Iwasaki, H. & Oku, H. (2009). Antiatherosclerotic Function of Kokuto, Okinawan Noncentrifugal Cane Sugar. *Journal of Agricultura and Food Chemistry* **57**, 69-75, (2009). https://doi.org/10.1021/jf802796m
23. Olivoto, T. & Lúcio, A.D.C. Metan: An R package for multi-environment trial analysis. *Methods in Ecology and Evolution* **11**, 783–789, (2020). DOI: 10.1111/2041-210X.13384.
24. R Core Team. *A language and environment for statistical computing*. Vienna, Austria. (2020). Retrieved from https://www.r-project.org/. (accessed 10 July 2020).
25. Rook, F., Hadingham, S. A., Li, Y. & Bevan, M. W. Sugar and ABA response pathways and the control of gene expression. *Plant, Cell & Environment* **29**, 426-434, (2006). https://doi.org/10.1111/j.1365-3040.2005.01477.x
26. Santos, J. M., Quináia, S. P. & Felsner, M. L. Fast and direct analysis of Cr, Cd and Pb in brown sugar by GF AAS. *Food Chemistry* **260**, 19-26, (2018). https://doi.org/10.1016/j.foodchem.2018.03.106
27. Sokal, R. R. & Rohlf, F. J. The comparison of dendrograms by objective methods. *Taxon* **11**, 33–40, (1962). https://www.jstor.org/stable/1217208
28. Thirugnanasambandam, P. P., Mason, P. J., Hoang, N. V., Furtado, A., Botha, F. C. & Henry, R. J. Analysis of the diversity and tissue specificity of sucrose synthase genes in the long read transcriptome of sugarcane. *BMC Plant Biology* **19**, 1-14, (2019). https://doi.org/10.1186/s12870-019-1733-y
29. Wang, J., Zhao, T., Yang, B. & Zhang, S. Sucrose metabolism and regulation in sugarcane. *Journal of Plant Physiology e Pathology* **5**, 1-6, (2017). https://doi.org/10.4172/2329-955X.1000167