

ASSESSMENT OF THE GENETIC DIVERSITY OF NEW SUGARCANE HYBRIDS USING PRINCIPAL COMPONENT AND CLUSTER ANALYSES

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ABSTRACT

With a cultivated area of approximately 800 thousand hectares distributed in 15 states, sugarcane (*Saccharum* spp.) is one of the main crops in Mexico. Despite its socioeconomic importance, the genetic base of this crop has been reduced in recent decades, making the production system vulnerable to both biotic and abiotic stresses. For this reason, the Colegio de Postgraduados Campus Córdoba initiated a varietal selection program in 2009. In this study, morphological and agronomic attributes of 20 advanced hybrids were analyzed during the 2020-2021 and 2021-2022 growing seasons to determine genetic variability based on principal component analysis (PCA) and cluster analysis. Commercial cultivars widely planted in the area of influence of the Córdoba Campus (CP 72-2086, Mex 79-431, Mex 69-290 and Mex 05-204) were used as reference samples. In terms of agronomic indicators, most of the new hybrids showed higher averages than the commercial control cultivars. The hybrids COLPOSCCMex 09-50 and COLPOSCCMex 09-66 showed better germination capacity. The average height of the tallest milling stems was presented by the hybrids COLPOSCCMex 09-341 (318.1 cm) and COLPOSCCMex 09-93 (311.85 cm). The hybrid COLPOSCCMex 09-289 showed outstanding values for five variables: diameter (36.43 mm), °Brix (21.89 %), stem height (149.33 cm), leaf width (5.36 cm) and number of leaves (11.15). Morphological characterization was carried out according to 54 descriptors, combining categorical and continuous traits that were easy to observe, with a high degree of differentiation and low environmental influence. Principal component and cluster analyses showed the distribution of hybrids into five groups. These results show promising sugarcane lines with high yields, which can contribute to genetic diversity in agroecological zones where they can be adapted.

Keywords: Poaceae, genetic variability, promising lines, descriptors, agroecological zones.

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INTRODUCTION

Sugarcane (*Saccharum* spp.) is the largest plant source of table sugar and one of the most important crops for the generation of green energy worldwide. Currently, 80 % of the sugar consumed in the world comes from this grass (FAO, 2023). The sugar industry has positioned itself as one of Mexico's main economic activities, generating income through agricultural, processing and marketing activities. The total area of Mexico is 198 million hectares, of which only 27.5 million hectares are used for agriculture (INEGI, 2014; Torres-Torres and Rojas-Martínez, 2018). Of these, about 800,000 hectares are planted with sugarcane annually (CONADESUCA, 2021).

Increasing agricultural productivity in the sugar industry has become more important in recent years, aiming for a more competitive level in global markets. In the 2020-2021 growing season, the national average yield was 64.92 Mg ha⁻¹, which was considered low compared to previous harvests. The highest national yield was recorded in the 2012-2013 harvest, with a value that reached 78.74 Mg ha⁻¹ (CONADESUCA, 2021). These variations are closely related to the need for innovation in sugarcane production, since adaptation to climate change, efficient use of water, fertilizers, biofertilizers and manures, resistance and tolerance to biotic and abiotic stress factors, increased yields and the generation of cultivars for different uses are among the main demands to be considered to improve the primary sector of this agribusiness (Gómez-Merino *et al.*, 2014; 2017).

With respect to genetic variability, the sugar industry in Mexico mainly relies on four commercial cultivars: CP 72-2086, Mex 69-290, Mex 79-431 and ITV 92-1424, with percentages of land planted of 31, 26, 8 and 6 %, respectively (CONADESUCA, 2016). This highlights the need to increase the genetic base of the crop, as it is exposed to the severe effects of climate change and the attack of new pests and diseases. Prioritizing the main problems of the sugar industry in Mexico, in 2009 the Colegio de Postgraduados implemented a program for the selection and exchange of sugarcane cultivars (Lavín-Castañeda *et al.*, 2020). The main objective of this program has been to increase the productivity and profitability of the sugar agroindustry through the selection of new outstanding sugarcane genotypes. Among other elements requiring improvement, there is a need for a greater ability to adapt to the different agroecological zones where sugarcane is grown in Mexico, higher sucrose yields than current commercial cultivars, greater resistance to pests and diseases, and agronomic characteristics that facilitate its cultivation and management.

The correct selection of a new genotype to be planted in one of Mexico's six agroecological zones is a fundamental requirement to exploit its maximum productivity. This is why the analysis of genetic diversity has become one of the main tools for studying the relationships that exist among sugarcane genotypes. Biotechnology has provided breeders with a large number of DNA-based markers to analyze the genetic diversity of sugarcane (Junior *et al.*, 2020; Emegha *et al.*, 2021). Phenotypic traits to estimate this type of diversity are now widely used because, compared to molecular markers, they are simple, easy to identify and collect, inexpensive, effective and practical (Wu *et al.*, 2021).

Among the different characteristics that define a genotype, morphological, agronomic and agro-industrial traits are used to identify a new sugarcane cultivar. Several statistical approaches are used to study genetic diversity, depending on the amount and nature of data collected. Multivariate data analysis techniques and the use of morphological data are frequently employed for the understanding of genetic diversity. Cluster analysis and principal component analysis (PCA) use agronomic and quality characteristics to investigate the nature of genetic divergence among different genotypes (Seema *et al.*, 2017). These analyses also help to quantify the variability available in the gene pool, as well as to identify groups of genetically similar or different plants (Baloch *et al.*, 2017).

The objective of this study was to analyze the genetic diversity of a group of 20 hybrids in advanced selection processes and four commercial cultivars (controls) based on analysis of variance (ANOVA), multivariate cluster analysis and principal component analysis of morphological and agronomic variables. The multivariate analysis was based on the clustering technique to form groups of observations (hybrids) with common characteristics through Euclidean distance. On the other hand, the principal component analysis showed the formation of small groups of uncorrelated variables (morphological and agronomic) that can facilitate the interpretation of the genetic diversity of the hybrids through small groups based on the original variables.

MATERIALS AND METHODS

Plant material and experimental design

The study involved 20 new sugarcane hybrids (Table 1) selected on the basis of their performance during previous phases, compared to three control cultivars with the largest area planted in Mexico: CP 72-2086 of early maturity, Mex 79-431 of intermediate maturity and Mex 69-290 of late maturity. As an additional control, an outstanding cultivar selected by the Colegio de Postgraduados Campus Tabasco was evaluated: COLPOSCTMEX 05-204 (Mex 05-204), also early maturing.

Genotypes were evaluated in experimental plots at the Colegio de Postgraduados Campus Córdoba, located in the municipality of Amatlán de los Reyes, Veracruz, Mexico (18° 51' N, 96° 51' E; 650 m elevation), during the 2020-2021 and 2021-2022 growing seasons. The climate of the area is tropical humid, with rainfall in summer and an average annual temperature of 20 °C, a maximum temperature of 35 °C and a minimum of 10 °C. Average annual precipitation is 1807 mm (Soto-Esparza and García, 1989). The main soil types of this experimental center are vertisols and feozems (CONABIO, 2011).

The 24 genotypes (20 hybrids and four commercial controls) were planted in a randomized block design in plots with dimensions of 6 m long x 6 m wide and 2 m between each plot, with 1.2 m row spacing. For the characterization of the genotypes, nine traits of interest were recorded 2 m linearly from the center of each plot: stem height (cm); stem diameter (mm); leaf number, length (cm) and width (cm); SPAD indirect chlorophyll index (ICC); °Brix and sprouting of the sown buds (%). The entries

Table 1. Sugarcane genotypes (*Saccharum* spp.) evaluated during the Semi-commercial Stage of the genetic improvement program of the Colegio de Postgraduados Campus Córdoba.

No.	Cross	Parents		Genotype
		Female	Male	
01	222	LCP 81-10	× Gloria 57	COLPOSCCMex 09-29
02	255	CP 52-68	× CP 70-1527	COLPOSCCMex 09-50
03	257	CP 92-1401	× CP 81-1384	COLPOSCCMex 09-66
04	258	CP 81-10	× CP 70-133	COLPOSCCMex 09-93
05	258	CP 81-10	× CP 70-133	COLPOSCCMex 09-95
06	264	Tue 72-9	× CP 80-1827	COLPOSCCMex 09-97
07	264	Tue 72-9	× CP 80-1827	COLPOSCCMex 09-125
08	294	CC 93-3826	× CP 62-378	COLPOSCCMex 09-132
09	CMI V	LTMex 92-52	× ¿?	COLPOSCCMex 09-208
10	CMI V	LTMex 92-52	× ¿?	COLPOSCCMex 09-212
11	CMI V	LTMex 92-52	× ¿?	COLPOSCCMex 09-217
12	CMI V	LTMex 92-52	× ¿?	COLPOSCCMex 09-222
13	774	PR 62-632	× CP 80-1743	COLPOSCCMex 09-273
14	707	Mex 79-431	× CP 89-2377	COLPOSCCMex 09-289
15	707	Mex 79-431	× CP 89-2377	COLPOSCCMex 09-290
16	529	ITV 92-1424	× B 45-181	COLPOSCCMex 09-312
17	529	ITV 92-1424	× B 45-181	COLPOSCCMex 09-321
18	527	ITV 92-1424	× CP 81-1384	COLPOSCCMex 09-333
19	523	CP 87-1490	× Mex 79-341	COLPOSCCMex 09-341
20	523	CP 87-1490	× Mex 79-341	COLPOSCCMex 09-348
21	Control 1	CP 62-374	× CP 63-588	CP 72-2086
22	Control 2	Co 421	× Mex 57-473	Mex 79-431
23	Control 3	Mex 56-476	× Mex 53-142	Mex 69-290
24	Control 4	CC 92-2198	× CC 93-4206	Mex 05-204

were made every month throughout the crop cycle, except for °Brix, which was not evaluated until cane maturity, i.e., 12 months after planting.

The hybrids were characterized by 50 descriptors, using morphological and agronomic characteristics based on the methodologies described in the UPOV (2005) and Gómez-Merino and Senties-Herrera (2015). The traits were identified in fully developed sugarcane plants from the useful section of each experimental plot in the 24 genotypes. The descriptors evaluated were: growth habit, stem diameter, stem height, internode arrangement, longitudinal aspect of internodes, internode length, color of sun-exposed internodes, color of non-sun-exposed internodes, wax on internodes, bud canal, length of bud canal, narrow fissures or grooves, stem cracks, root band shape, bud position at node, bud shape and color, pubescence and bud position, node color, bud size and type, bud lala width, leaf width, leaf blade conformation, leaf blade color, leaf edge pubescence, amount of sheath wax, sheath color, sheath pubescence, length of sheath hairs, auricle shape, ligule shape, ligule width, collar color, collar shape, sheath adherence, crown conformation, crown size, inflorescence, presence of

lals, number of water suckers, spreading, pith, hollowness, °Brix, number of milling stems, and growth ring thickness on bud side and opposite bud side.

Statistical analyses

The data obtained were processed using Minitab statistical software version 19.2020 (Minitab; State College, PA, USA). An analysis of variance (ANOVA) was performed for each agronomic variable studied and a comparison of means was applied using Tukey's test ($p \leq 0.05$). Multivariate analyses were carried out considering agronomic and morphological variables, using cluster analysis by similarity of observations with the complete linkage method, with which a dendrogram was constructed. A principal component analysis was also performed for the same group of variables.

RESULTS AND DISCUSSION

Some of the hybrids evaluated showed a faster emergence than the controls. At 13 days after planting, up to six shoots were found on some hybrids, compared to controls that showed three shoots on average (data not shown). The results revealed differences in the development of the hybrids (Figure 1). One month after planting, the hybrids

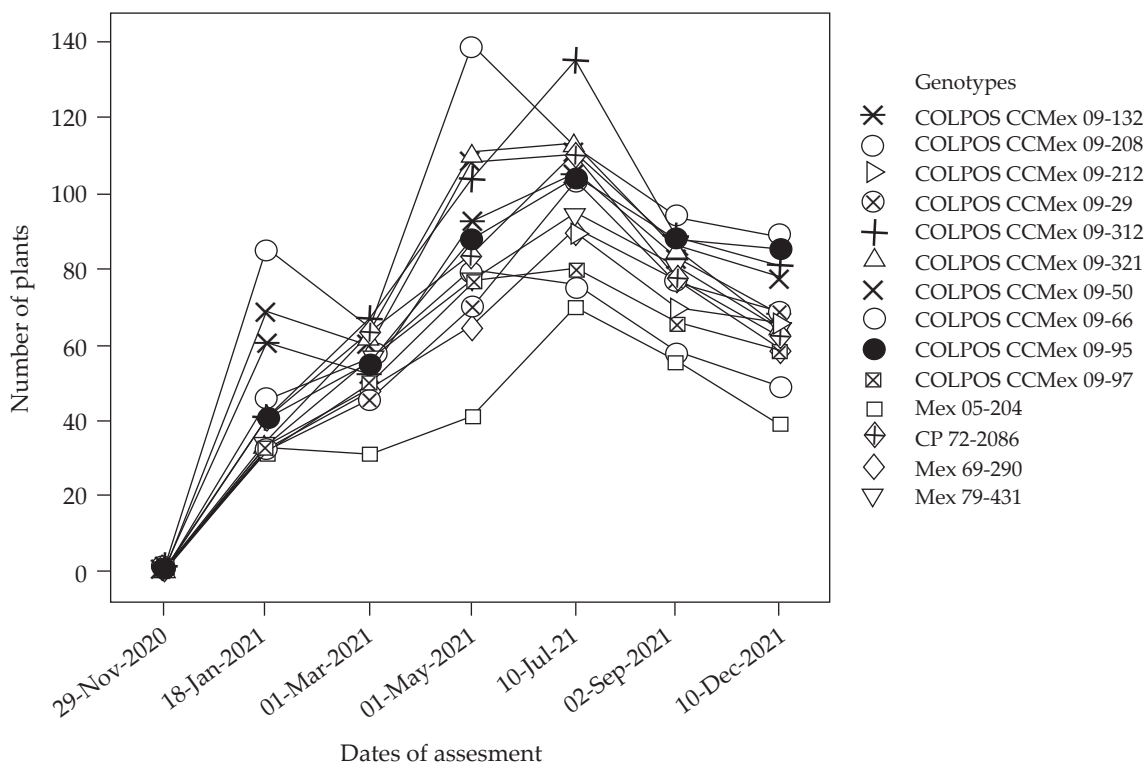


Figure 1. Comparison of the number of plants in hybrids under selection and commercial control cultivars of sugarcane (*Saccharum* spp.) in the Semi-commercial Stage, grown at the Experimental Field of the Colegio de Postgraduados Campus Córdoba, 2020-2021 and 2021-2022 growing seasons.

that showed the best development were COLPOSCCMex 09-66 and COLPOSCCMex 09-50, with average values of 85.33 and 68.67 shoots emitted, respectively, higher than those observed in the control cultivars. Just recently, Lesmes-Vesga *et al.* (2023) demonstrated that lalas may produce more tillers and higher shoot dry biomass than pop-eyes and ungerminated buds, while both lalas and pop-eyes produced higher root dry biomass than ungerminated buds.

This greater initial development was maintained throughout the crop cycle since these same hybrids presented the highest number of milling stems in May and June of the first cycle, which coincides with the tillering phase, with means of 138.83 and 108.50 stems, respectively. At this evaluation stage, COLPOSCCMex 09-312 outperformed the aforementioned hybrids with an average of 162.3 shoots in June, followed by COLPOSCCMex 09-321 with an average of 120.7 shoots (data not shown in the graph). Others such as COLPOSCCMex 09-29, COLPOSCCMex 09-95, COLPOSCCMex 09-132 and COLPOSCCMex 09-212 reached their highest value during this same month, with averages of 98.8, 93.3, 89.0 and 92.2 stems emitted, respectively.

During the high growth phase, from June to September, the hybrids exhibited a significant decrease in the number of milling stems, a negative characteristic that should be avoided in new commercial cultivars, since the number of stems is closely related to the final yields obtained in the field. Finally, the hybrids that presented the highest number of stems at the final stage of the harvest cycle were COLPOSCCMex 09-66, COLPOSCCMex 09-29 and COLPOSCCMex 09-312, with averages of 88, 84.8 and 80.8 stems, respectively.

The germination percentage is a critical characteristic that determines the productive potential of the cultivars to be established (Ganapathy and Purushothaman, 2017). The first factor determining germination percentage is genetic (Patil and Patel, 2017). Furthermore, the age of the buds used in planting and their position on the stalk also affect germination indicators, which can be increased when using buds from the middle and upper part of the sugarcane (Samiullah *et al.*, 2015), due to the greater availability of reserves in these parts. Temperature, humidity and soil conditions are also determinants of germination. Temperatures close to 35 °C and irrigation immediately after planting increase germination, as well as planting with a 6-10 cm layer of soil (CINCAE, 2004). In a study on integrated selection criteria in sugarcane breeding programs using discriminant function analysis (DFA), Abu-Ellail *et al.* (2020) determined that the most important attributes are stalk weight, stalk height, purity, °Brix and milling stalk yield.

In this study, there were significant differences ($p \leq 0.05$) among the hybrids evaluated for the characteristics plant height, stem diameter, SPAD index and °Brix (Table 2).

The greatest height was observed in the hybrids COLPOSCCMex 09-93, with 311.8 cm, and COLPOSCCMex 09-341, with 318.1 cm, while the lowest heights were obtained with COLPOSCCMex 09-222 and COLPOSCCMex 09-333 with values of 236.9 cm and 225.31 cm, respectively. In general, most hybrids responded significantly to this variable when compared to the control cultivars. The length of milling stalks is one

Table 2. Average performance with respect to stalk height, stalk diameter, SPAD index and °Brix in hybrids under selection and commercial cultivars of sugarcane (*Saccharum* spp.) in the Semi-commercial Stage, grown at the Experimental Field of the Colegio de Postgraduados Campus Córdoba, 2020-2021 and 2021-2022 growing seasons.

Genotype	Stem height (cm)	Stem diameter (mm)	SPAD Index (ICC)	°Brix (%)
CP 72-2086	283.43±6.56 ^m	29.11±0.63 ^{ghijkl}	29.43±1.14 ^{hijk}	22.42±0.17^a
Mex 79-431	265.46±4.01 ^{ghij}	32.13±0.66 ^{cdef}	36.70±1.59 ^{hijk}	20.58±0.21 ^{bcdefghi}
Mex 69-290	301.24±5.11 ^{gh}	34.95±0.56 ^{cde}	28.30±1.66 ^{ghij}	19.45±0.24 ^{ghi}
Mex 05-204	272.31±4.61 ^{cdef}	26.7±0.56 ^l	45.52±2.86^{def}	16.64±0.56 ^j
COLPOSCCMex 09-29	277.06±4.83 ^{fgh}	25.25±0.47 ^{kl}	29.05±1.96 ^{ijklm}	19.21±0.27 ⁱ
COLPOSCCMex 09-50	292.59±3.74 ^m	27.59±0.48 ^{lm}	12.96±0.69 ^m	19.79±0.32 ^{fghi}
COLPOSCCMex 09-66	299.31±4.62 ^{bcde}	27.73±0.53 ^{hijkl}	18.66±1.48 ^{klm}	19.33±0.17 ^{hi}
COLPOSCCMex 09-93	311.85±6.68^a	30.01±0.77 ^{ijkl}	36.51±1.79 ^{bc}	20.26±0.24 ^{efghi}
COLPOSCCMex 09-95	285.63±5.68 ^{kl}	25.12±0.42 ⁿ	27.16±1.76 ^{efgh}	20.30±0.43 ^{defghi}
COLPOSCCMex 09-97	292.31±4.58 ^{jk}	26.59±0.43 ^{mn}	22.96±1.15 ^{klm}	22.16±0.14^{ab}
COLPOSCCMex 09-125	310.93±4.18 ^{ghijkl}	28.8±0.47 ^{kl}	22.92±1.34 ^{lm}	20.31±0.24 ^{defghi}
COLPOSCCMex 09-132	293.96±5.67 ^{ijk}	30.24±0.40 ^{ijkl}	32.0±1.27 ^{ijkl}	20.45±0.31 ^{cdefghi}
COLPOSCCMex 09-208	276.30±7.58 ^{bcd}	35.17±0.68^{bc}	40.90±1.83^{cde}	20.60±0.21^{bcdefghi}
COLPOSCCMex 09-212	245.00±3.76 ^{hijk}	29.85±0.46 ^{bcd}	44.13±1.77^{cd}	20.20±0.28 ^{efghi}
COLPOSCCMex 09-217	256.39±4.84 ^{bcd}	28.85±0.52 ^{fghi}	31.26±1.52 ^{fghi}	21.52±0.33 ^{abcde}
COLPOSCCMex 09-222	236.94±6.19 ^{ghijkl}	29.05±0.44 ^{efgh}	25.37±0.91 ^{hijk}	20.97±0.38 ^{abcdefg}
COLPOSCCMex 09-273	282.22±4.24 ^{lm}	30.29±0.53 ^{ghij}	20.01±0.98 ^{klm}	20.98±0.38 ^{abcdefg}
COLPOSCCMex 09-289	268.59±4.37 ^{efg}	36.43±0.76^a	28.71±1.17 ^{defg}	21.89±0.15^{abcd}
COLPOSCCMex 09-290	274.63±6.29 ^{gh}	30.29±0.71 ^{bcd}	41.12±2.07^{def}	20.09±0.26 ^{efghi}
COLPOSCCMex 09-312	286.98±6.00 ^{ghi}	31.06±0.54 ^{cdef}	52.33±3.81^a	17.19±0.52 ^j
COLPOSCCMex 09-321	298.06±6.44 ^{bc}	29.99±0.36 ^{ghijk}	51.76±2.06 ^b	20.93±0.26 ^{abcdefgh}
COLPOSCCMex 09-333	225.31±5.35 ^m	29.43±0.39 ^{defg}	22.65±1.06 ^{hijk}	22.04±0.42^{abc}
COLPOSCCMex 09-341	318.09±6.77^b	33.45±0.56 ^{cdef}	22.54±1.09 ^{lm}	20.77±0.34 ^{bcdefghi}
COLPOSCCMex 09-348	309.33±6.84 ^{defg}	32.98±0.88 ^b	39.69±2.29 ^{cd}	21.17±0.22 ^{abcdef}

Means± standard error. Means with different letters indicate statistical differences (Tukey, $p \leq 0.05$). Averages of measurements taken twelve months after planting are shown.

of the characteristics that can indicate the tolerance of sugarcane to drought stress, in addition to having a wide heritability, so it can be used as a selection criterion in breeding programs (Hartati and Yuniyati, 2022). In addition to the genotype factor, the significant differences found in stem height may be related to the climatic conditions and soil characteristics in the area where the study was conducted since the driest period coincides with the formative phase of sugarcane, a limiting factor for the normal development of the hybrids (Zhao *et al.*, 2020).

The largest stem diameter was found in the hybrid COLPOSCCMex 09-289, with 36.43 mm, and COLPOSCCMex 09-208, with 35.17 mm. The latter genotype also showed high values for the SPAD and °Brix index traits, with values of 40.90 ICC and 20.60 %, respectively. Hybrids Mex 05 204, COLPOSCCMex 09-212 and COLPOSCCMex 09-290 showed the highest values for the SPAD index, with averages of 45.52, 44.13 and 41.12 ICC, respectively.

The hybrids showed significant differences in soluble solids content in the juice extracted from their stems. However, none exceeded the control cultivar CP 72-2086, which reached 22.42 °Brix. The hybrids that most closely resembled this value were COLPOSCCMEX 09-97, COLPOSCCMEX 09-333, COLPOSCCMEX 09-289, COLPOSCCMEX 09-217 and COLPOSCCMEX 09-348, with values of 22.2, 22.1, 21.9, 21.5 and 21.2 %, respectively (Table 2). The absence of significant differences observed among the hybrids evaluated with respect to the °Brix variable could be due to the uniform expression of genes controlling this attribute in the new sugarcane hybrids, rather than to environmental or management conditions (Muhammad *et al.*, 2014). Regarding the means of the variables leaf length, leaf width and number of leaves (Table 3), the hybrids Mex 79 431 and COLPOSCCMex 09-29 presented the longest leaf length, with 163.65 and 163.37 cm, respectively. However, leaf width was narrower for the hybrid COLPOSCCMex 09-29 (3.82 cm) compared to the other hybrids and control

Table 3. Agronomic response of phenotypic traits in control cultivars and hybrids of sugarcane in the Semi-commercial Stage, grown at the Experimental Field of the Colegio de Postgraduados Campus Córdoba, 2020-2021 and 2021-2022 growing seasons.

Genotype	Leaf length (cm)	Leaf width (cm)	Number of leaves
CP 72-2086	149.28±2.52 ^{ik}	4.24±0.12 ^{ijk}	11.39±0.38^{cdefg}
Mex 79-431	163.65±2.21^{cde}	4.46±0.12 ^{fg hij}	11.0±0.31 ^{bcd}
Mex 69-290	161.39±2.69^{defgh}	5.08±0.11^{efghi}	10.91±0.31 ^{defg}
Mex 05-204	157.91±2.53 ^{bcd}	4.93±0.14 ^{bcdef}	10.15±0.31 ⁱ
COLPOSCCMex 09-29	163.37±2.97^a	3.82±0.12 ^{hijk}	9.91±0.27 ^{ab}
COLPOSCCMex 09-50	137.91±1.56 ^{kl}	4.58±0.11 ^{ijk}	10.56±0.29 ^{bc}
COLPOSCCMex 09-66	139.04±2.92 ^{fgh}	4.57±0.14 ^{ghij}	9.21±0.28 ^{efghi}
COLPOSCCMex 09-93	142.61±3.09 ^{hij}	4.64±0.11 ^{ghijk}	10.68±0.33 ^{ab}
COLPOSCCMex 09-95	141.31±2.04 ^{kl}	4.68±0.13 ^{jk}	11.52±0.36^{cde}
COLPOSCCMex 09-97	148.11±1.91 ^{ijk}	3.95±0.12 ^k	10.33±0.31 ^{fghi}
COLPOSCCMex 09-125	132.50±2.55 ^l	4.94±0.15 ^{cdefg}	11.29±0.39^{defg}
COLPOSCCMex 09-132	150.91±3.03^{gh}	4.99±0.16 ^{defgh}	10.29±0.28 ^{hi}
COLPOSCCMex 09-208	142.78±2.52 ^{cdef}	5.56±0.17^{abcde}	10.59±0.29 ^{efgh}
COLPOSCCMex 09-212	139.98±2.12 ^{efgh}	5.85±0.14^a	10.37±0.29 ^{ghi}
COLPOSCCMex 09-217	138.13±2.18 ^{cdefg}	4.87±0.12 ^{defghi}	10.67±0.35 ^{defg}
COLPOSCCMex 09-222	135.63±1.62 ^{ghi}	4.73±0.13 ^{abc}	10.61±0.42 ^{ab}
COLPOSCCMex 09-273	135.46±1.56 ^m	4.87±0.12 ^{fg hij}	10.68±0.36 ^{efgh}
COLPOSCCMex 09-289	149.33±2.39^a	5.36±0.15^{abcd}	11.15±0.34^a
COLPOSCCMex 09-290	129.81±2.18 ^l	5.93±0.15^a	11.13±0.33^{ab}
COLPOSCCMex 09-312	149.98±2.6 ^{bc}	5.23±0.13 ^{abcde}	9.71±0.31 ^{ghi}
COLPOSCCMex 09-321	142.02±3.95 ^{fgh}	5.81±0.13^{ab}	10.24±0.42 ^{cdefg}
COLPOSCCMex 09-333	127.80±3.08 ^m	5.53±0.14^a	10.52±0.33 ^{ghi}
COLPOSCCMex 09-341	159.11±2.65^{ab}	4.92±0.12 ^{hijk}	10.15±0.31 ^{defg}
COLPOSCCMex 09-348	151.06±2.67^{cdef}	4.65±0.12 ^{fg hij}	10.96±0.34 ^{cdef}

Means± standard error. Means with different letters indicate statistical differences (Tukey, *p* ≤ 0.05). Averages of measurements taken twelve months after planting are shown.

cultivars evaluated. The hybrid COLPOSCCMex 09-289 was the one that obtained high values for the variables stem diameter, °Brix, width and number of leaves. The hybrid COLPOSCCMex 09-290 showed significant values for leaf width (5.93 cm) and number of leaves (11.13).

For the leaf length variable, the control cultivar Mex 79-431 and the hybrid COLPOSCCMex 09-29 showed the highest averages, with 163.65 and 163.37 cm, respectively. The hybrid COLPOSCCMex 09-290 had the shortest leaf length (129.81 cm), although the value for leaf width was outstanding (5.93 cm). Furthermore, the latter genotype showed a higher number of leaves. The COLPOSCCMex 09-95 hybrid presented the highest mean for leaf number, which was 11.52 (Table 3). Leaf area is an important trait associated with plant growth rate and yield. Sugarcane stands out for being a C4 plant, which allows a high photosynthetic rate and a great capacity for carbohydrate synthesis (Rincón-Castillo and Becerra-Campiño, 2020).

Morphological characterization

After agglomerative hierarchical grouping, the hybrids and control commercial cultivars were grouped into five clusters (Figure 2). The first one (cluster I) consisted of nine hybrids and two control cultivars (Mex 69-290 and Mex 05-204) based on their similarity. These genotypes were characterized by erect and semiprostrate growth habit, thick stems and long internodes, no bud canal, absence of pubescence on leaf structures, abundant stem wax and scarce sheath wax, large crown size and representative leaf length. The control commercial cultivar Mex 79-431 conformed to cluster II, with a more erect growth habit, medium height, yellowish-green nodes with abundant wax, a narrow bud canal and a tender green arched leaf blade and a brownish-green ligular collar.

Cluster III had five hybrids, which were characterized by an inclined growth habit and curved internodes of medium diameter, medium stem height, medium leaf length and width, and color variation from pale green to tender green. They had an open cup conformation, medium sheath adherence and lateral lalas. Cluster IV consisted of seven hybrids and the control cultivar CP 72-2086. These genotypes presented a semi-erect growth habit, medium height and stem diameter with zigzag internodes, stem color among yellow, green and purple with scarce wax, absence of bud canal, smooth bark and some with cracks in their stems, large buds of bulky and erect forms, with a curved apex of the leaf blade with a profuse inflorescence. Moreover, sheath adherence was medium and presented significant values for the number of leaves.

Cluster V was formed by the hybrids COLPOSCCMex 09-66 and COLPOSCCMex 09-222. The most representative features of these genotypes were their basket growth habit, medium stem diameter, long internodes and zigzag internode arrangement. The color of the nodes did not vary and presented a purplish-greenish coloration and a large number of stems in two linear meters, abundant wax on their stems, a very profuse inflorescence, lateral lalas on their stems and abundant pith.

Chidambaram and Sivasubramaniam (2017) found different morphological profiles of ten sugarcane cultivars, which help in the identification of different genotypes in the

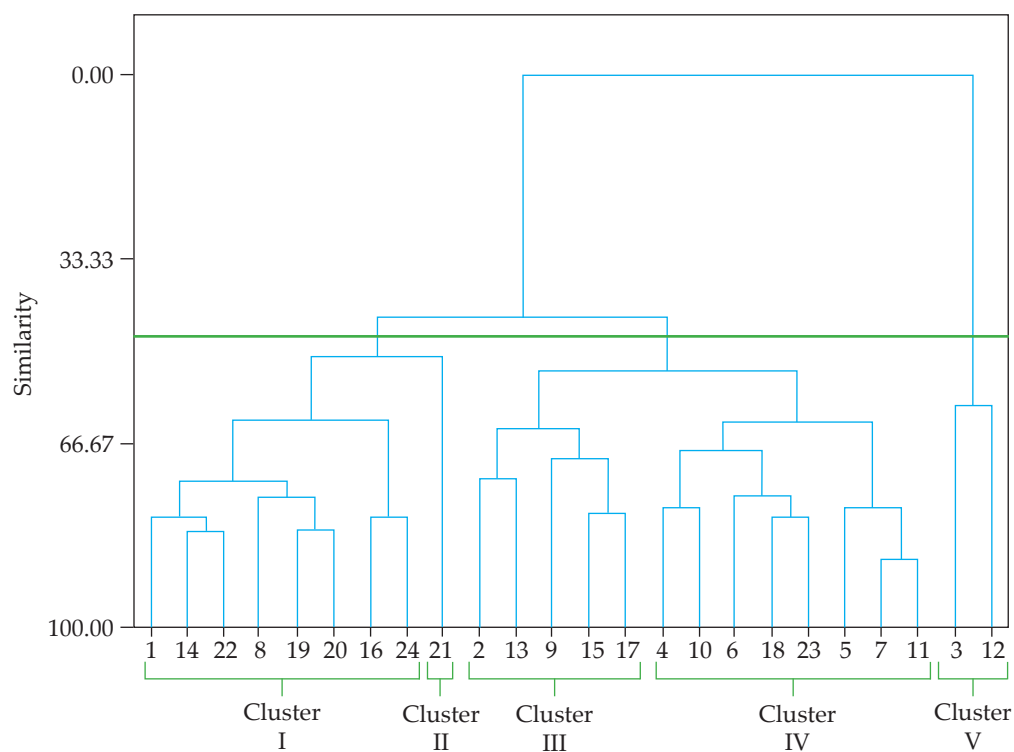


Figure 2. Dendrogram of genetic relationships among different sugarcane (*Saccharum* spp.) hybrids evaluated, based on morphological and agronomic traits. 1: COLPOSCCMex 09-29; 2: COLPOSCCMex 09-50; 3: COLPOSCCMex 09-66; 4: COLPOSCCMex 09-93; 5: COLPOSCCMex 09-95; 6: COLPOSCCMex 09-97; 7: COLPOSCCMex 09-125; 8: COLPOSCCMex 09-132; 9: COLPOSCCMex 09-208; 10: COLPOSCCMex 09-212; 11: COLPOSCCMex 09-217; 12: COLPOSCCMex 09-222; 13: COLPOSCCMex 09-273; 14: COLPOSCCMex 09-289; 15: COLPOSCCMex 09-290; 16: COLPOSCCMex 09-312; 17: COLPOSCCMex 09-321; 18: COLPOSCCMex 09-333; 19: COLPOSCCMex 09-341; 20: COLPOSCCMex 09-348; 21: Mex 79-431; 22: Mex 69-290; 23: CP 72-2086; 24: Mex 05-204.

field. Characteristics such as plant growth habit, stem bark surface, auricle shape, leaf sheath, internode wax and growth ring were considered for their work. Knowledge of morphology is of vital importance for a clear and precise identification of clones, cultivars and species in the field.

Principal component analysis (PCA) was used to differentiate genotypes into discrete groups associated with morphological and agronomic variables, and thus facilitate the identification of the traits most responsible for the variations observed among a set of genotypes. The first principal component (PC1) accounted for 12.4 % of the total variation, followed by 12.1 % for PC2. The most outstanding factors of PC1 based on their loadings were: growth ring thickness on the opposite side of the bud (0.33), presence of lalas (0.28), growth ring thickness on the bud side (0.21), leaf blade

conformation (0.24), inflorescence (0.22) and pith (0.23). For PC2, the factors of highest relevance were the number of water suckers (0.33), leaf length (0.26), ligule length (0.26) and ligule width (0.26) (Figure 3).

The principal component analysis (PCA) is a statistical method that simplifies the complexity of data, since a large number of variables can be reduced to just a couple of them. Nevertheless, this method has advantages and disadvantages. Among the pros, the PCA prevents overfitting, removes correlated features, speeds up other machine

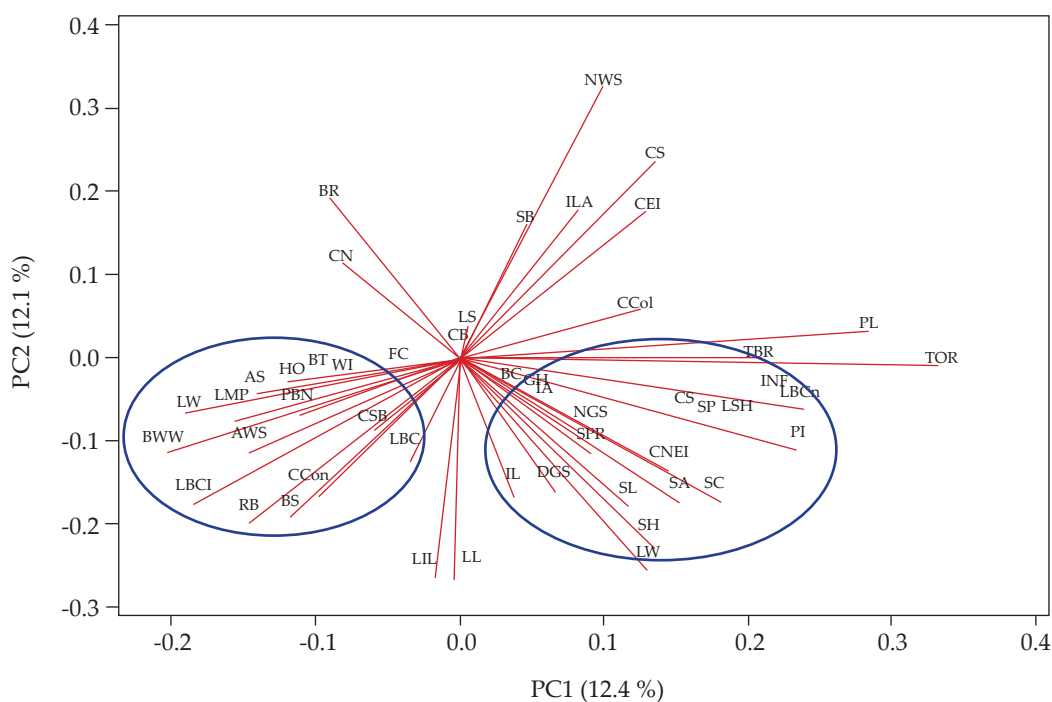


Figure 3. Loading biplot of 50 morphological and agronomic characteristics of 20 new hybrids and four control cultivars of sugarcane (*Saccharum* spp.) on principal components PC1 and PC2. GH: growth habit; DGS: diameter of ground stems (mm); IA: internode arrangement; SH: stem height (cm); ILA: internode longitudinal aspect; IL: internode length (cm); CEI: color of exposed internodes; CNEI: color of non-exposed internodes; NGS: number of ground stems; WI: wax on internodes; BC: bud canal; LBC: length of bud channel; FC: fissures corked; CSB: cracks in stem bark; TBR: thickness of bud side growth ring; TOR: thickness of opposite bud growth ring; RB: root band; PBN: position of bud at node; SB: shape of bud; CB: color of bud; CN: color of node; BS: bud size; BT: bud type; BWW: bud wing width; LL: leaf length (cm); LW: leaf width (cm); LBCn: leaf blade conformation; LBCl: leaf blade color; LMP: leaf margin pubescence; AWS: amount of wax in the sheath; SC: sheath color; SP: sheath pubescence; LSH: length of sheath hairs (mm); AS: auricle shape; LS: ligule shape; LW: ligule width (mm); LiL: ligule length (cm); CCol: collar color; CS: collar shape; SA: sheath adherence; SL: sheath length (cm); CCon: crown conformation; CS: crown size (cm); INF: inflorescence; PL: presence of lalas; NWS: number of water suckers; SPR: spreading; BR: °Brix; PI: pith; HO: hollowness.

learning algorithms, and improves visualization. On the other hand, using the PCA method can also have some disadvantages: all the variables should have a mean of 0 and a standard deviation of 1, which standardizes data; the PCA can lead to some loss of information, and therefore, interpretation of the outputs may result complicated. Indeed, Elhaik (2022) stated that PCA-based findings in population genetic studies are highly biased and must be reevaluated. Therefore, attention should be paid when implementing the PCA procedure and results must be deeply analyzed and correctly interpreted.

CONCLUSION

Based on the results of the statistical analysis, the hybrids COLPOSCCMex 09-312 and COLPOSCCMex 09-321 presented desirable agronomic characteristics, such as the number of milling stems, with values of 162.33 and 120.67, respectively. The hybrids COLPOSCCMex 09-341 and COLPOSCCMex 09-93 showed the longest stem length, with means of 318.1 and 311.85 cm, respectively.

Thanks to multivariate analyses, and through the study of morphological traits, it was possible to learn more about the genetic profile of this population. The classification system concentrated the hybrids in clusters, relating the hybrids COLPOSCCMex 09-93, COLPOSCCMex 09-289, COLPOSCCMex 09-312, COLPOSCCMex 09-321 and COLPOSCCMex 09-341, which could be selected based on traits of interest, such as growth habit, stem height and diameter, internode size, amount of wax, leaf length and width, and leaf blade size. It is suggested that the most representative sugarcane genotypes in this work should be further tested in the different potential growing areas, in order to identify the best genotypes according to the agroecological zone where they are planted and grown.

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