Molecular *versus* morphological markers to describe variability in sugar cane (*Saccharum officinarum*) for germplasm management and conservation

Marcadores moleculares y morfológicos para la descripción de variabilidad en caña de azúcar (*Saccharum officinarum*) con fines de manejo y conservación de germoplasma

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Abstract

Sugarcane is one of the most important industrial crops in tropical and subtropical regions. INTA (Argentina) administrates a Sugarcane Germplasm Bank and carries out a breeding program. The current study was designed to assess the phenotypic and genetic diversity among 65 sugarcane accessions selected from the INTA bank. Clustering and ordination methods based on quantitative and qualitative morphological traits and SSR data, were applied. Generalized Procrustes Analysis allowed evaluating the correlation between relationships established with both markers. A good fit between dendrograms and similarity matrices were revealed by high cophenetic coefficients ($r=0.82$, $p<0.0001$; $r=0.73$, $p<0.0001$; $r=0.82$, $p<0.0001$ for phenotypic quantitative, phenotypic qualitative and molecular data respectively). The presence of different reliable population structure was observed when considering different data sources. Procrustes allowed finding those accessions that should have been responsible for the low correlation found between the individual configurations (73%). Both morphologic and molecular markers resulted discriminative enough to differentiate among accessions. It was not possible, however, to correlate associations of markers with the origin of materials. Phenotypic and genetic distances based on morphology and molecular information serves to assist conservation and organization of collection of materials, and the choice of parent combinations for breeding purposes.

Keywords
multivariate analysis • morphological traits • SSR • sugarcane • genetic variability

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Resumen

La caña de azúcar es uno de los cultivos industriales más importantes de regiones tropicales y subtropicales. El INTA (Argentina) administra un Banco de Germoplasma de caña de azúcar y lleva a cabo un programa de mejora. El presente trabajo fue diseñado para estimar la variabilidad fenotípica y genética entre 65 accesiones de caña de azúcar seleccionadas del INTA. Se aplicaron métodos de clasificación y ordenamiento en el análisis de datos morfológicos y de SSR. EL Análisis de Procrustes Generalizado permitió evaluar la correlación entre las relaciones establecidas a partir de ambos tipos de marcadores. Un buen ajuste entre los dendrogramas y las matrices de similitud fue soportado por un alto coeficiente de correlación cofenética (r=0,82, p<0,0001; r=0,73, p<0,0001; r=0,82, p<0,0001 para datos cuantitativos, cualitativos y moleculares respectivamente). La presencia de una estructura poblacional fue reconocida cuando se consideraron los diferentes tipos de datos. El Procrustes permitió detectar aquellas accesiones que serían responsables de la baja correlación detectada entre configuraciones individuales (73%). Tanto los marcadores morfológicos como los moleculares resultaron lo suficientemente discriminativos para diferenciar accesiones. No obstante, no fue posible correlacionar las asociaciones establecidas por los marcadores con el origen de los materiales. Las distancias fenotípicas y genéticas basadas en información morfológica y molecular será de utilidad para asistir en la conservación y organización de los materiales de la colección y elegir combinaciones parentales con propósito de mejora.

Palabras claves

análisis multivariado • caracteres morfológicos • SSR • caña de azúcar • variabilidad genética

Introduction

Sugarcane is one of the most important industrial crops in tropical and subtropical regions. It is cultivated in more than 90 countries around the world, primarily for its ability to store high concentrations of carbohydrates to produce sugar and biofuel. INTA (Instituto Nacional de Tecnología Agropecuaria) administrates the main Sugarcane Germplasm Bank in Argentina and conducts a breeding program for this crop.

The germplasm bank fulfils aspects related to exploration, collection, evaluation, preservation and germplasm exchange. The core collection currently includes 429 sugarcane accessions and 120 clones from an annex collection with high Brix (total soluble solids) materials, an attribute related to potential sucrose yield. Some morphological traits have been measured to characterize these materials aiming at improving ttheir breeding value. However, these genetic markers have several limitations including low polymorphism, low heritability, late expression, and vulnerability to environmental influences. In addition, it is known that morphological traits do not always provide a sound measure of genetic values and may not accurately reveal the genetic variation in germplasm collections (13). Since germplasm provides the raw material for breeders to improve crop performance, knowledge on genetic variability should be an auxiliary tool for breeding and an important link between the conservation and use of sugarcane available genetic resources. Interesting

genetic resources for breeders include advanced material (*e.g.* pre-bred material, breeding lines, adapted varieties, elite materials) and research material (*e.g.* advanced core collections, mapping populations). However, researchers and other users may be interested in a wider range of materials. The conservation of genetic diversity in germplasm banks broadens the spectrum of materials targeted for storage (14). The usefulness of samples held in germplasm banks is dependent on the degree and quality of information connected to the samples (14). Morphological markers reflect variation of expressed regions of genome while molecular markers indicate variation of all genome including expressed and non-expressed regions. It has been reported that the patterns of allelic variation in a species may be very different for neutral markers compared with genes under selection. Based on a meta-analysis, Latta (2008) argued that variability at neutral and selected loci are not correlated because evolutionary forces act differently on them. Reed and Frankham (2003) showed only weak correlation between neutral molecular markers and morphological quantitative measures of variation. A joint analysis of morphological and molecular variability would undoubtedly increase the resolving power of the genetic diversity analysis of the sugarcane germplasm bank. It would also allow criteria for both, the choice of progenitor combinations to maximize the genetic variability of the progeny in the breeding program and to maintain variability of the germplasm collection. For those purposes, it is necessary to deal with a large number and different types of variables. The multivariate analysis has allowed the simultaneous evaluation of many traits by summarizing information in few synthetic variables. It has also permitted a better understanding of the structure of the sugar cane germplasm collection, helping to identify which variables are more relevant in order to identify relationships among accessions (3). The current study was designed to assess the phenotypic and genetic diversity of 65 sugarcane accessions selected from INTA's Germplasm Bank (Tucumán, Argentina), determining both the discriminating power and effectiveness of different SSR primers for sugarcane genotype identification and the optimal SSR primer combination to ensure unambiguous identification of a set of sugarcane genotypes. In addition, we also evaluated the correlation between the sugarcane accessions relationships established with both morphological and molecular data in order to provide guidance for future use of sugarcane accessions in the breeding programme and germplasm bank management.

MATERIALS AND METHODS

Sixty five sugarcane accessions from the INTA Germplasm Bank (Tucumán, Argentina) were included in this study (table 1, page 43). Most of these genotypes are of interest for breeding purposes in Argentina due to their adaptability to subtropical growing areas (short cycle and early maturity). Some of these materials are or were used as commercial varieties in Argentina and other countries.

Three basic materials (identified as US) were also included. Sugarcane accessions were grown in the greenhouse under controlled conditions.

Table 1. Sugarcane accessions included in the genetic variability analysis and Province-Country of origin (CO).

Tabla 1. Accesiones de caña de azúcar incluídas en el análisis de variabilidad genética y sus Provincias-Países de origen (CO).

Variety	Origin	Variety	Origin	Variety	Origin
LCP85-384	Louisiana, USA	NA84-3471	Salta, Argentina	TUC72-16	Tucumán, Argentina
LCP86-454	Louisiana, USA	NA63-90	Salta, Argentina	TUC74-6	Tucumán, Argentina
LCP85-376	Louisiana, USA	NA76-128	Salta, Argentina	TUC71-7	Tucumán, Argentina
HoCP85-845	Louisiana, USA	NA73-2596	Salta, Argentina	TUC68-18	Tucumán, Argentina
HoCP92-648	Louisiana, USA	NA88-948	Salta, Argentina	TUC67-24	Tucumán, Argentina
HoCP92-645	Louisiana, USA	NA73-1454	Salta, Argentina	TUC79-9	Tucumán, Argentina
HoCP92-624	Louisiana, USA	CP48-103	Louisiana, USA	TUCCP77-42	Tucumán, Argentina
HoCP89-888	Louisiana, USA	CP68-350	Louisiana, USA	TUC77-42b	Tucumán, Argentina
HoCP91-552	Louisiana, USA	CP70-1133	Louisiana, USA	TUC78-39	Tucumán, Argentina
HoCP92-631	Louisiana, USA	CP79-1380	Louisiana, USA	TUC72-4	Tucumán, Argentina
HoCP91-555	Louisiana, USA	NA84-3471	Salta, Argentina	TUC69-2	Tucumán, Argentina
HoCP88-739	Louisiana, USA	CP79-318	Louisiana, USA	L91-281	Louisiana, USA
HoCP90-941	Louisiana, USA	CP65-350	Louisiana, USA	RA89-686	Argentina
US74-1011	USA	CP57-603	Louisiana, USA	RA87-2	Argentina
US74-1015	USA	CP57-614	Louisiana, USA	RA91-209	Argentina
US72-1289	USA	CP72-2086	Louisiana, USA	RA93-154	Argentina
L75-33	Louisiana, USA	CP66-346	Louisiana, USA	CP88-1834	Louisiana, USA
TCP81-3067	Tucumán, Argentina	CP62-258	Louisiana, USA	F98-70	Tucumán, Argentina
TCP87-388	Tucumán, Argentina	FAM81-820	Tucumán, Argentina	F97-395	Tucumán, Argentina
NA84-3013	Salta, Argentina	FAM83-11	Tucumán, Argentina	F97-786	Tucumán, Argentina
NA78-724	Salta, Argentina	TUC80-7	Tucumán, Argentina	CP65-357	Louisiana, USA
				Nco310	Sud Africa

Morphological traits

A total of 59 morphological variables from stem and leaf were evaluated. From these, 43 correspond with sugarcane UPOV (*Union for the Protection of New Varieties of Plants*) descriptors, while 16 are descriptors defined by Wagih (2004). Morphological traits comprised both qualitative (43) and quantitative (16) attributes. Most of these attributes (48) are not subjected to selection in breeding programs; 4 of them, related to stem traits, are subjected to screening as primary conditioning requisites, while other 7 are subsidiary traits related to leaves and canopy (table 2, page 44-45).

The accessions were planted in 2017-2018 in single raw evaluation plots of 1 m length (50 cm spacing) at the experimental greenhouse of Universidad Nacional de Salta (24°43'22" S and 65°24'74" W). Irrigation was provided at appropriate time according to requirements. Data on measurable morphological characters, were recorded on year after planting. Quantitative traits were measured on five random stems for each accession and data were averaged.

Table 2. Qualitative and quantitative morphological markers assessed in 65 accessions of sugarcane. Name, abbreviation and categories or units is indicated for each variable.

Tabla 2. Marcadores morfológicos cualitativos y cuantitativos estudiados en 65 accesiones de caña de azúcar. Para cada variable se indica el nombre, abreviatura y la categoría o unidades.

Plant	Abbreviation	Category and/or Units
Stool growth habit	*PC*	erect/semierect/intermediate/ semipostrate/postrate
Leaf canopy	*F*	very sparse/sparse/medium/dense
Intensity of green color of leaf canopy	*ICV*	ligth/medium/dark
Depth of growth crack	*PRC*	absent/very shallow/shalow/medium/deep
Height of stalk	*TA*	Cm
Length of cane top	*LPSC*	Cm
Width of root band	*AZR*	Mm
Bud		
Shape of bud	*FY*	triangular-pointed/oval/obovate/ pentagonal/ rhomboid/round/ovate/rectangular/beaked
Hairs of budsor *Group 1* *Group 2* *Group 26* *Group 4* *Group 16* *Group 8* *Group 11* *Group 15* *Group 18* *Group 19* *Group 22* *Group 10*	 *P1* *P2* *P26* *P4* *P16* *P8* *P11* *P15* *P18* *P19* *P22* *P10*	 absent/present
Width of bud	*AY*	Mm
Width of bud wing	*AAY*	Mm
Bud groove	*CaY*	absent/present
Length of bud groove	*LCY*	short/medium/long
Depth of bud groove	*PCY*	very shallow/shallow/medium/deep
Position of bud tip in relation to growth ring	*PAY*	clearly below/intermediate/clearly above
Bud cushion (space between base of bud and leaf scar)	*CjY*	absent or very narrow/narrow/medium/wide
Internode		
Length of internode	*LE*	Cm
Diameter of internode	*DE*	Mm
Shape of internode	*FE*	cylindrical/tumescent/bobbin-shaped/conoidal/ obconoidal/concave-convex
Cross section of internode	*ST*	ovate/circular
Expression of zigzag alignment	*EZZ*	absent or very weak/weak/moderate/strong

Table 2 (cont.). Qualitative and quantitative morphological markers assessed in 65 accessions of sugarcane. Name, abbreviation and categories or units is indicated for each variable.

Tabla 2 (cont.). Marcadores morfológicos cualitativos y cuantitativos estudiados en 65 accesiones de caña de azúcar. Para cada variable se indica el nombre, abreviatura y la categoría o unidades.

Plant	Abbreviation	Category and/or Units
Wax ring	*AC*	Mm
Waxiness	*C*	absent or very weak/weak/moderate/strong
Leaf sheath		
Length of leaf sheath	*LV*	Cm
Distribution of hairs of leaf sheath	*DPV*	only dorsal/lateral and dorsal
Number of hairs: group 57	*P57*	absent or very few/few/medium/many/a lot
Number of hairs: group 60	*P60*	absent or very few/few/medium/many/a lot
Length of hairs: group 57	*LP57*	short/medium/long
Length of hairs: group 60	*LP60*	short/medium/long
Hairs around leaf sheath	*PAV*	absent/present
Length of hairs around leaf sheath	*LPAV*	absent/short/medium/long
Density of hairs around leaf sheath	*DPAV*	absent/scarce/medium/numerous
Adherence of leaf sheath	*AdV*	weak/medium/strong
Shape of underlapping auricle	*FASY*	transitional/deltoid/dentoid/unciform/ calcariform/ falcate/lanceolate
Shape of overlapping auricle	*FASP*	transitional/deltoid/dentoid/unciform/ calcariform/ falcate/lanceolate
Size of underlapping auricle	*TASY*	Mm
Size of overlapping auricle	*TASP*	Mm
Ligule		
Shape of ligule	*FL*	strap shaped/deltoid/crescent-shaped/bow-shaped/ asymmetrical, steeply sloping/asymmetrical horizontal
Ligule width	*Ali*	Mm
Density of ligule hairs: group 61	*DP61*	absent or very sparse/sparse/medium/dense/ very dense
Length of hairs: group 61	*LP61*	short/medium/long
Leaf blade		
Curvature	*CHL*	arched at base/curved/curved tips/arched/ straight
Width at the longitudinal mid-point	*AL*	Mm
Midrib width	*AN*	Mm
Ratio leaf blade width/midrib width	*L/N*	Mm
Length of leaf blade	*LL*	Cm
Pubescence on margin of leaf blade	*PBH*	absent or very sparse/sparse/medium/dense
Serration on margin of leaf blade	*ABH*	absent/present

All measures and observations were carried out in the greenhouse and laboratory by means of metric rule and calipter or under stereoscopic binocular loupe, by the same operators for each attribute, considered stable enough for the different genotypes.

SSR

Total genomic DNA was extracted from young leaves (+1 in Kuijper's denomination) (6) using a DNA Nucleospin II extraction kit following the manufacturer protocol. The quality and quantity of DNA was assessed using a NanoDrop ND-1000 (Thermo Fisher Scientific Inc., Waltham, USA) with 1 µl sample. Based on the consistency of band patterns obtained in a previous study, twenty SSR primers were evaluated (table 3). Polymerase chain reactions (PCRs) and electrophoresis and gel staining were carried out according to Pocoví *et al.* (2013) The resulting banding pattern was scored manually. Only consistent bands with strong intensity were considered for the analysis.

Table 3. Simple Sequence Repeat (SSR) primers used for genotyping 65 sugarcane accessions from the INTA Sugarcane Germplasm Bank (Tucumán, Argentina).

Tabla 3. Cebadores de Secuencias Repetitivas Simples (SSR) usados para el genotipado de 65 accesiones de caña de azúcar del Banco de Germoplasma de INTA (Tucumán, Argentina).

SSR	Repeat motif	Size range (bp)	Annealing T	Forward primer (5'-3') Reverse primer (5'-3')
NKS26	(TG)18	194-164	54	GTT CTC GAC ATG GGC CTA CT
				CTG CAC TTT CGG TCC TTT TT
mSSCIR19	(GA)23	130-160	48	GGT TCC AAA ATA CAC AAA
				CAA TCT TAT CTA CGC ACT T
NKS38	(AG)15	92-292	55	TGA ACT CGG CAA CAG TTT TT
				CCC ACC AAG TCG TTC TGA AT
NKS 23	(GA)18	113-498	54	TAA ACC CCC GAA AAA GAA CC
				TCC GGA GGT AGA TCC ATT TG
NKS34	(GT)18 (A)31	131-214	58	CGT CTT GTG GAT TGG ATT GG
				TGG ATT GCT CAG GTG TTT CA
mSSCIR16	(GA)18	130-300	54	TGG GGA GGG CTG ACT AGA
				GGC GGT ATA TAT GCT GTG
SMC703BS	(CA)12	186-229	62	GCC TTT CTC CAAACC AAT TAG T
				GTT GTT TAT GGA ATG GTG AGG A
mSSCIR3	(GT)28	171-187	60	AAT GCT CCC ACA CCA AAT GC
				GGA CTA CTC CAC AAT GAT GC
mSSCIR18	(GA)23	170-200	52	GGG TGT TCT GTT GAG CA
				GAG GTA GGA GGG AGT GTT
SMC766BS	(CA)20(GA)16	170-270	60	TTA CTC GGC TGG GTT TTG TTC
				TAA GAA TCG TTC GCT CCA GC
SMC7CUQ	(CA)10(C)4	160-170	60	GCC AAA GCA AGG GTC ACT AGA
				AGC TCT ATC AGT TGA AAC CGA
mSSCIR78	(GTT)6	150-310	48	TGCCTTAAC CGT GACATC
				GAGGACGAGGAGCAGAA
mSSCIR34	(GA)	130-300	56	ATCGCCTCCACTAAATAAT TTGTCTTTGCTTCCTCCTC

Despite being co-dominant, SSR markers were here considered as dominant markers, because in highly polyploid genomes such as that of sugarcane, the SSR markers difficulty distinguish the alleles of homologous chromosomes, making it difficult to determine heterozygosity or homozygosity at any particular locus. From this assumption, each band was treated as a unit locus and a binary system was considered scoring each individual for presence (1) or absence (0) of a band.

Statistical multivariate analysis

Clustering methods

For quantitative variables, phenotypic relationships between pairwise of sugarcane accessions were assessed using Euclidean distance calculated with their standardized means. To measure similarities between pairwise of genotypes on the basis of multistate qualitative traits, the Simple Matching Coefficient was used (25). For molecular data, relationships between pairwise of accessions were estimated using the Jaccard Coefficient. In the three cases, the accessions were then clustered by the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA). Cophenetics values matrices (25) of the UPGMA clustering were used to test goodness-of-fit of the clustering to the similarity matrix on which it was based, by means of computing the product-moment correlation (*r*) with 1000 permutations (Mantel, 1967). The relative support for the different groups and stability of the dendrograms were assessed by bootstrap analyses (1000 replicates). Bootstrap values exceeding a 50% cut-off are indicated above the corresponding clusters in the respective figures.

Ordination methods

A principal Component Analysis (PCA), using the canonical Euclidean distance from quantitative morphological data, was carried out. The ordination was visualized simultaneously by means of biplots where sugarcane genotypes and variables were represented in a common space. For qualitative morphological and molecular data, genetic similarities matrices were used to perform Principal Coordinate Analysis (PCoA). According to Cliff (Franco and Hidalgo, 2003), only those coordinates whose accumulated values accounted for 70% or more of the total variance were considered. To facilitate the understanding of the relationships sugarcane accessions, geometrical representations were obtained using Minimum Spanning Trees (MST).

In order to establish agreement or consensus between relationships among observations derived from morphological and molecular data, a Generalized Procrustes Analysis (GPA) was carried out.

Statical analyses were performed using Infostat v.2013 (9) and DARwin 6.0.0 software program (20).

Results and discussion

Phenotypic variability based on quantitative traits

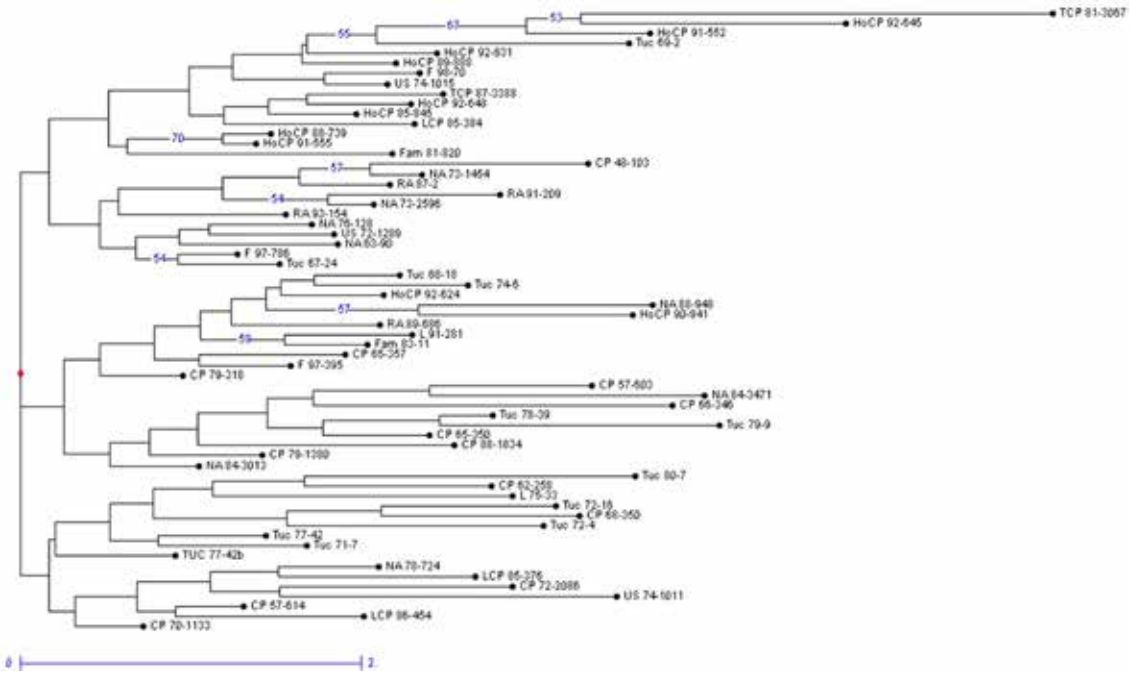
The highest distance value was estimated between the genotypes TUC79-9 and TCP81-3067 (10.79). In opposition, HoCP88-739 and HoCP91-555 were very close to each other showing the lowest Euclidean distance value (0.48). Non-Euclidean distance between pairs of accessions was zero meaning that quantitative traits included in this study were sufficiently discriminative to differentiate

unequivocally among all the accessions. The dendrogram generated with UPGMA cluster analysis of de Euclidean distance matrix, revealed nine clusters with more than 50% bootstrap values (figure 1). Probably, the small number of clusters supported by bootstrap can be explained due to many pair-wise genetic similarity coefficients with intermediate values, which allow several similar variants for dendrogram branching.

The cophenetic correlation between the dendrogram and the similarity matrix was significant ($r=0.82$; $p<0.0001$) revealing a high degree of fit.

Detail analysis of the cluster's composition does not show association patterns related to the origin or other agronomic characteristics of the materials. This result is interpreted because of the nature of the descriptors investigated, given that most of them are not associated with selection objectives of breeding. This fact can also explain the confusion of basic materials (US) with commercial ones.

The PCA analysis allowed reducing the set of correlated quantitative variables to a small number of linear combinations of these variables (principal components) such as expected (3).



Nine clusters showed in blue are those supports with more than 50% bootstrap values.
Los nueve grupos mostrados en azul son aquellos soportados por valores de bootstrap mayores a 50%.

Figure 1. Dendrogram (UPGMA) constructed with Euclidean distances based on quantitative morphological data.

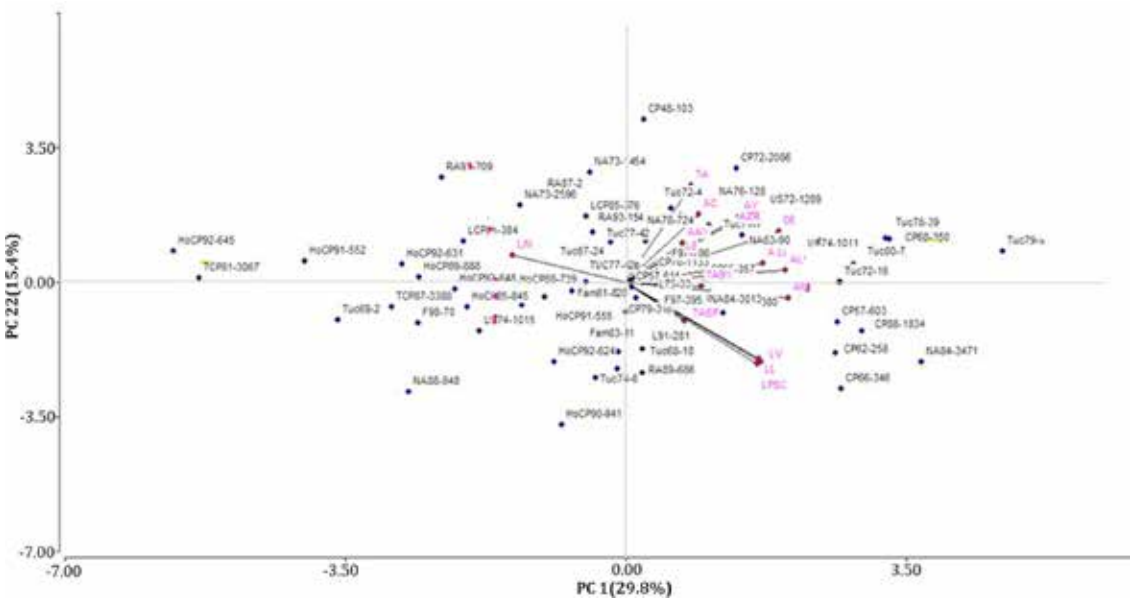
Figura 1. Dendrograma (UPGMA) basado en datos morfológicos cuantitativos construido a partir de distancias Euclídeas.

The first four principal components (PCs) had eigenvalues higher than one. The first and second synthetic variables (PC1 and PC2) explained 45% of the total variability. PC1, with an eigenvalue of 4.74, would contain equivalent information from at least four original quantitative variables. PC2, with an eigenvalue of 2.47, corresponded to two variables. According to Bhanupriya *et al.* (2014), characters with largest absolute value (eigenvectors) closer to unity within the first principal component, influence the clustering more than those with lower absolute value closer to zero. In the present study, differentiation of sugarcane accessions into different groups in PC1 can be explained because of the contribution of leaves descriptors (*Leaf sheath length, Ligule width, Midrib width, ratio Leaf blade width/Midrib width,* with eigenvalues of 0.30, 0.35, 0.30, 0.35, respectively) and cane traits (*Internode diameter, Bud width, Length of the cane top* with eigenvalues of 0.34, 0.25, 0.45, respectively) Except for diameter, the other descriptors influencing on PC1 are not primary but subsidiary traits for breeding. According to Gutiérrez-Miceli *et al.* (2002), the internode diameter is correlated with the sucrose content, so in the case of diameter it should be also considered that the range of the sample is strongly limited for being commercial type materials. These facts reinforce confounding associations discussed previously. According to Mohammadi and Prasanna (2003) when the total variation explained by the first two or three PCs is smaller than 25%, PCA provides faithful portrayal of the relationships between major groups of lines, but distances between closer genotypes are often distorted. In this study, PC1 and PC2 explained 45% of the original variation and allowed a better understanding on the structure of sugarcane genotypes. PC1 accounted for 30% of the morphological variation. Twenty of the 26 accessions (77%) classified in the first cluster (UPGMA) were grouped to the left of PC1, these genotypes would have greater *ratio Leaf blade width/Midrib width* than those on the right of CP1 (figure 2, page 50). According to Di Rienzo *et al.* (2013), the orthogonality of the principal components ensures that CP2 provides new information on variability compared to that provided by CP1. In this study, genotypes that could not be differentiated by leaf traits on PC1 could be identified by PC2, being stem height the main attribute associated to this component. Accession CP48-103 is the genotype with greater stem height.

Again, in this study, PCA analysis could not clearly differentiate materials according to their origin or nature (US) based on the morphological descriptors investigated.

Phenotypic variability based on qualitative traits

Morphological qualitative traits were also discriminative. Although some pairs of sugarcane accessions were phenotypically very close, with dissimilarities coefficients near zero (0.102), none of them showed a zero value. The histogram of pairwise dissimilarity from the qualitative data indicates a normal distribution. The dissimilarity coefficients ranged from 0.102 to 0.731. The fact that most of the dissimilarity coefficients ranged between 0.35 and 0.50 can probably explain that few internal branches (3) in the dendrogram (UPGMA) supported by bootstrapping (figure 3, page 51).



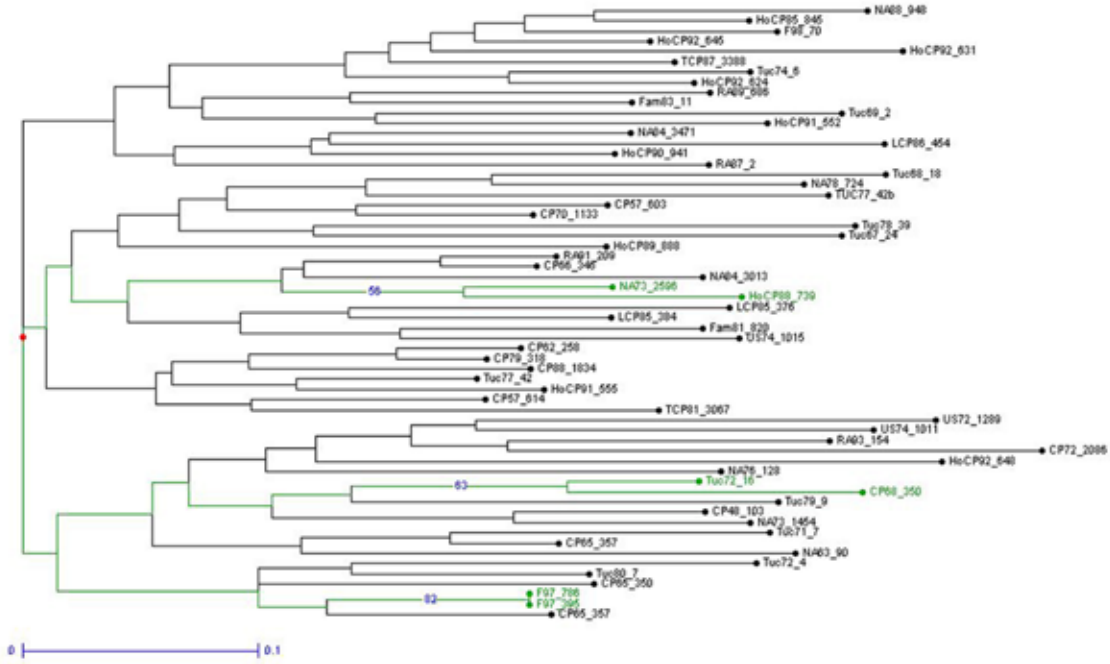
TA: Height of stalks; LPSC: Length of cane top; AZR: Width of root band; AY: Width of bud; AAY: Width of bud wing; LE: Length of internode; DE: Diameter of internode; AC: Wax ring; LV: Length of leaf sheath; TABY: Size of underlapping auricle; Ali: Ligule width; AN: Midrib width; L/N: Ratio leaf blade width/midrib width; LL: Length of leaf blade.

TA: Altura de tallo; LPSC: Longitud parte superior de la caña; AZR: Anchura de zona radicular; AY: Anchura de yema; AAY: Anchura del ala de la yema; LE: Longitud del entrenudo; DE: Diámetro del entrenudo; AC: Anillo ceroso; LV: Longitud de la vaina; TASY: Tamaño aurícula subyacente; Ali: Anchura de la lígula; AN: Anchura de la nervadura principal; L/N: Anchura del limbo/Anchura de la nervadura principal; LL: Longtud del limbo.

Figure 2. PCA biplot of 16 quantitative morphological traits with 65 sugarcane accessions and eigenvalue vectors for the traits.

Figura 2. Biplot PCA de 16 caracteres morfológicos cuantitativos con 65 accesiones y vectores de autovalores para los caracteres.

The cophenetic correlation between the dendrogram and the similarity matrix was significant ($r=0.73$; $p<0.0001$) indicating a good agreement between the graphical display of distances and the original matrix, supporting the visual inferences suggested in figure 3 (page 51). None of the sugarcane accession pairs with minimum and maximum distance values coincided with genotype pairs that appeared closer and more distant on the bases of quantitative data.



Numbers shown in clusters indicate those supported with more than 50% bootstrap values (clusters shown in green).

Los números mostrados en los grupos indican aquellos agrupamientos soportados por más de 50% de valores de bootstrap.

Figure 3. Dendrogram (UPGMA) constructed with Simple Matching Coefficients based on qualitative morphological data.

Figura 3. Dendrograma (UPGMA) basado en datos morfológicos cualitativos utilizando el Coeficiente de Simple Matching.

These differences could be explained because both types of quantitative and qualitative descriptors have different genetic bases and imply different genomic regions. It is expected that qualitative traits are mainly under monogenic or oligogenic control, conversely, quantitative traits have more complex genetic base as they are usually governed by multiple genes and their interactions (7). A much wider genomic area is expected to be considered when phenotypic relationships are estimated from quantitative data.

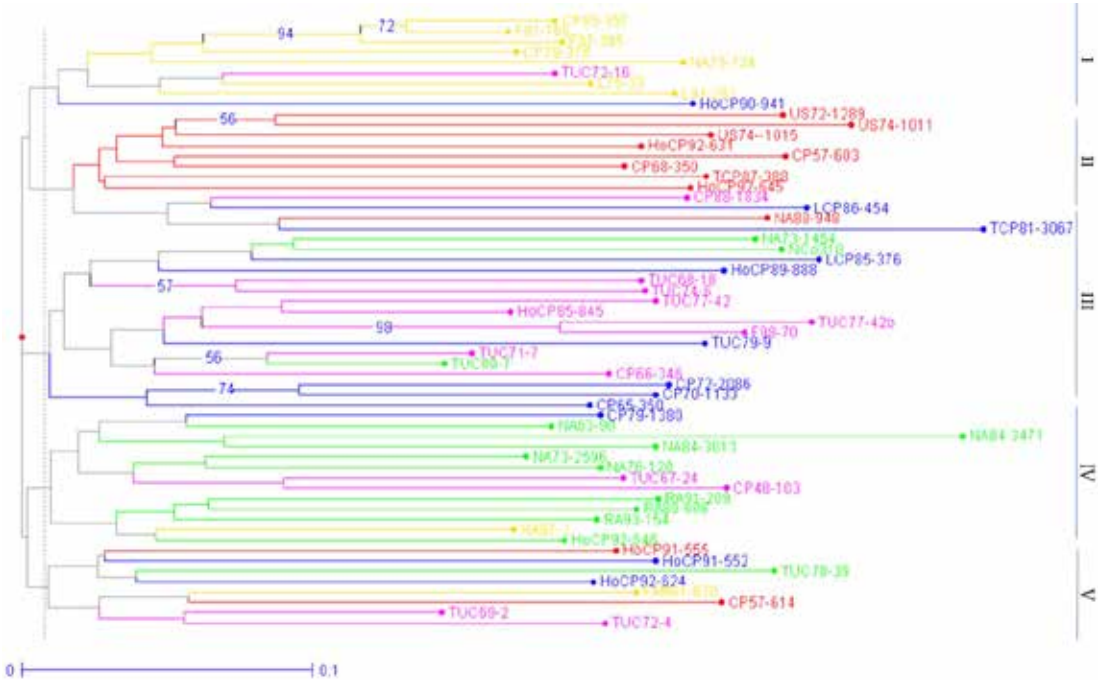
As in the case of the analysis based on quantitative traits, it was not possible to distinguish associations between qualitative based arrangements with the origin of materials. US 74-1011 and US72-1289 appear closely related and separated of commercial type accessions, while US74-1015 appears confounded with commercial types in a separate group.

PCA results based on qualitative data were not considered due to eigenvalues lower than 1, meaning that no PC explained even an original variable and small proportion of variance accounted for by the first two components (21%). ACP based on these qualitative data seemed to be inefficient to conglomerate defined sugarcane accessions groups.

Genotypic variability based on SSR

With 13 SSR primers, a total of 107 bands were detected of which, 94% were polymorphic among the studied sugarcane accessions.

The dissimilarity matrix, calculated from binary data, expressed the similarity pair to pair between sugarcane genotypes. The histogram of frequency distribution of the pairwise genetic distances fitted a normal distribution. Distance coefficients values among a total of 1711 pairs of genotypes showed an overall mean of 0.43. Of note, no dissimilarity value was zero, indicating that SSR included in this study were sufficiently discriminative for the sugarcane accessions. Most of the pairs of genotypes showed distances between 0.4 and 0.5, which allowed several similar variants for dendrogram branching and probably could explain the small number of clusters supported by bootstrap. Clustering percentage values above 50% for 1000 bootstrap cycles occurred in only seven groups (figure 4).



Numbers shown in clusters indicate those supported with more than 50% bootstrap values.
Los números de los clusters indican aquellos grupos con valores de bootstrap mayores a 50%.

Figure 4. Consensus dendrogram (UPGMA) constructed with dissimilarity genetic distances based on SSR data.

Figura 4. Dendrograma consenso (UPGMA) construido sobre la base de datos SSR utilizando medidas de disimilitud genética.

These dissimilarities values are like those reported by other authors in this species (18). According to the information indicated in table 4, we suggest that thirteen pairs of sugarcane accessions, with dissimilarities values higher than 0.65, might be considered as parental combinations accessions in the Breeding Programme, and thus, it could to some degree, benefit the broadening of the genetic basis in sugarcane hybridization. According to You *et al.* (2013), the innovation of parents with higher genetic diversity showed a positive role in sugarcane breeding programs in China. They suggested that more attention should be paid in the future to the selection of new parents in sugarcane hybrid breeding.

Differences were clear within clusters derived from quantitative morphological and molecular data. In both cases, cophenetic correlation coefficients were 0.82 indicating a high correlation between cophenetic distances and input distance matrices obtained from the data. Since cophenetic distance between two accessions is the distance at which two genotypes are first clustered together in a dendrogram from the bottom to the top (19), the cophenetic correlation coefficient, therefore, measures the relationships between the original pair wise distances between accessions (true distance) and pair wise distances predicted using dendrogram. In both cases, dendrograms corresponded graphically to 82% of the dissimilarity matrices. According to Odong *et al.* (2011) cophenetic correlation coefficient ≤ 0.8 is an indicator for strength of subgroup differentiation. Our results showed the presence of different reliable population structure in the studied sugarcane accessions when morphological and molecular data were considered. The phenotypic variation does not always follow the genetic pattern of variation and diversity of plant populations. The lack of congruence between morphological and genetic diversity has been reported in different plant species (1, 24).The different clustering can be explained due to a partial and insufficient genome representation when morphological data are used. Semang (2000) explained the lack of correspondence between molecular and morphological results, when stated that molecular markers cover a larger proportion of the genome, (including coding and noncoding regions), than the morphology ones. In addition, a large portion of the genetic variation detected by molecular markers is non-adaptive and, therefore, they are not subjected to either natural or artificial selection as many morphological traits.

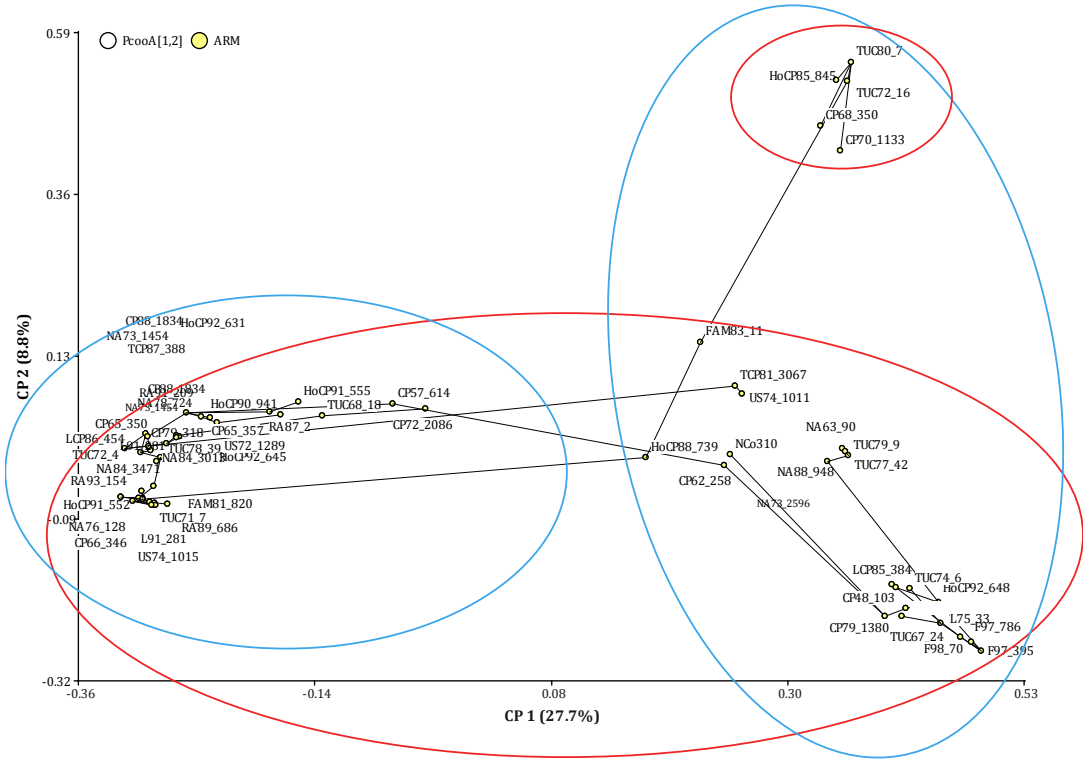
Table 4. Pairs of sugarcane accessions, with dissimilarities values higher than or equal to 0.60.

Tabla 4. Pares de accesiones de caña de azúcar con valores de disimilitud mayores o iguales a 0,60.

Pairs of sugarcane accessions		$D=1-S_{ij}$
F97-395	NA78-724	0.65
F97-395	L75-33	0.64
RA91-209	NA78-724	0.64
CP57-603	NCo310	0.63
RA91-209	L75-33	0.63
L75-33	HoCP91-555	0.62
TCP81-3067	LCP85-376	0.62
CP68-350	NA78-724	0.61
NA73-1454	NA78-724	0.61
TUC72-16	NA78-724	0.60
FAM83-11	L75-33	0.60
CP79-1380	L75-33	0.60
NA78-724	US74-1011	0.60

Differences in clustering can also be explained due to an absence of linkage between the loci that control the studied morphological characters and the evaluated SSR markers.

The first PCo summarized most of the variability present in the original data (28%) relative to all remaining PCos. The second PCo explained 9% of the variability and because PCos are orthogonal and independent to each other, they reveal different properties of the original data. According to Cliff criterion (10), the first 10 PCos explained 70% of cumulative variance, but only the first five showed eigenvalues equal to or greater than one. The minimum spanning tree (MST) imposed on the PCoA improved the representation of sugarcane relationships (figure 5).



The numbers in parenthesis refer to the proportion of the variance explained by the main coordinates. The blue and red circles indicate the different groups of PC1 and PC2, respectively. The colour of the accessions is related to their origin (blue: Louisian, USA; green: Salta, Argentina; Fuchsia: Tucumán, Argentina; red: USA).

Los números entre paréntesis indican la proporción de la varianza explicada por la coordenada principal. Los círculos azul y rojo muestran los diferentes grupos en PC1 y PC2 respectivamente. El color de las accesiones está relacionada con su origen (azul: Luisiana, USA; verde: Salta, Argentina; Fuccia: Tucumán, Argentina; rojo: USA).

Figure 5. Arrangement by Minimum Spanning Tree (MST) in the plane of the coordinates PC1 and PC2 of the 67 sugarcane accessions based on SSR data.

Figura 5. Árbol de recorrido mínimo (ARM) con proyección en el plano de las coordenadas PC1 y PC2 de las 67 accesiones de caña de azúcar basada en datos de SSR.

Although it was computed on the full dimension of data, the MST provided information about the quality of the projection on the low dimensional space, showing relationships that may have not been seen by inspection on the reduced space.

According to Balzarini *et al.* (2011) if many branches and segments cross each other, it suggests distortion problems in the projection which could bias regular interpretations. Even PCo2 explained only 9% of the variability; there is a group of accessions projected onto PCo2 that is clearly differentiated from the rest of the accessions (TUC 80-7; TUC72-16; CP68-350; CP70-1133 and HoCP85-845).

Qualitative morphological traits vs molecular markers

The distribution of values for morphological and genetic dissimilarity (calculated with qualitative traits and SSRs data) did not differ substantially. The distribution based on morphological data was slightly biased toward small values of distance (figure 6). Differences in the frequency distributions indicate that both types of markers detected a distinct pattern of association between sugarcane accessions. Consequently, complementary studies based on morphological and SSR will provide relevant information for establishing relationships among plant materials and a better description and interpretation of the available variability in germplasm banks and breeding programmes, as well as a foundation for promoting breeding and for germplasm conservation.

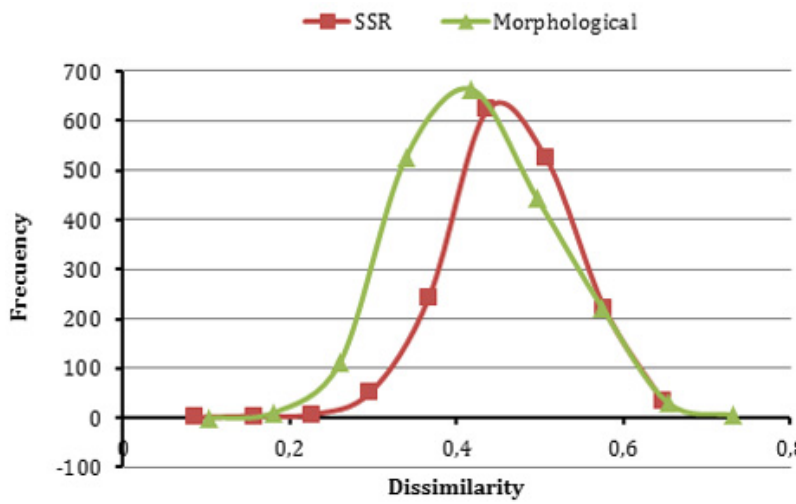


Figure 6. Frequency distribution of genetic dissimilarity among pairwise combinations of 65 sugarcane accessions based on morphological and SSR.

Figura 6. Distribución de frecuencias de disimilitudes genéticas entre pares de combinaciones de 65 accesiones de caña de azúcar basada en datos morfológicos y SSR.

Generalized Procrustes Analysis (GPA): Consensus between morphological and molecular data

GPA allowed a deeper study of the relationships among relative ordinations of the same sugarcane accessions under morphological and SSR data. Gower's (1975) recommended calculating an ANOVA to comparatively break down the total sums of squares into the between and within configurations.

Table 5. ANOVA Consensus between molecular and morphological ordinations. Accessions in bold showed the greatest discrepancy between the morphological and SSR data due to their higher relative values of Residual Sum of Squares (RSS).

Tabla 5. ANOVA Consenso entre ordenamientos basados en marcadores moleculares y morfológicos. Las accesiones en negrita mostraron las mayores discrepancias entre datos morfológicos y de SSR debido a sus valores de Sumas de Cuadrados Residuales (SCR) más altos.

	Consensus	**Residue**	**Total**	**Consensus proportion**
LCP85-384	0.014	0.007	0.021	0.673
LCP86-454	0.018	**0.012**	0.030	0.598
LCP85-376	0.031	**0.013**	0.044	0.706
HoCP85-845	0.014	0.007	0.021	0.673
HoCP92-648	0.022	**0.010**	0.031	0.684
HoCP92-645	0.022	0.009	0.031	0.701
HoCP92-624	0.011	0.007	0.018	0.628
HoCP89-888	0.016	**0.012**	0.027	0.576
HoCP91-552	0.018	0.008	0.026	0.675
HoCP92-631	0.024	0.004	0.028	0.852
HoCP91-555	0.012	**0.018**	0.030	0.406
HoCP88-739	0.014	0.009	0.023	0.604
HoCP90-941	0.040	0.009	0.050	0.816
US74-1011	0.050	0.006	0.056	0.890
US74--1015	0.030	0.007	0.037	0.800
US72-1289	0.030	0.008	0.038	0.784
L75-33	0.021	0.008	0.029	0.733
TCP81-3067	0.036	**0.013**	0.049	0.729
TCP87-388	0.023	0.006	0.029	0.781
NA84-3013	0.015	**0.010**	0.025	0.608
NA78-724	0.014	**0.016**	0.030	0.460
NA84-3471	0.026	0.008	0.034	0.763
NA63-90	0.028	0.008	0.036	0.778
NA76-128	0.028	0.006	0.034	0.816
NA73-2596	0.012	0.008	0.020	0.581
NA88-948	0.024	0.005	0.029	0.835
NA73-1454	0.032	**0.015**	0.047	0.680
CP48-103	0.037	0.009	0.046	0.814
CP68-350	0.023	0.007	0.030	0.755
CP70-1133	0.016	0.005	0.021	0.763

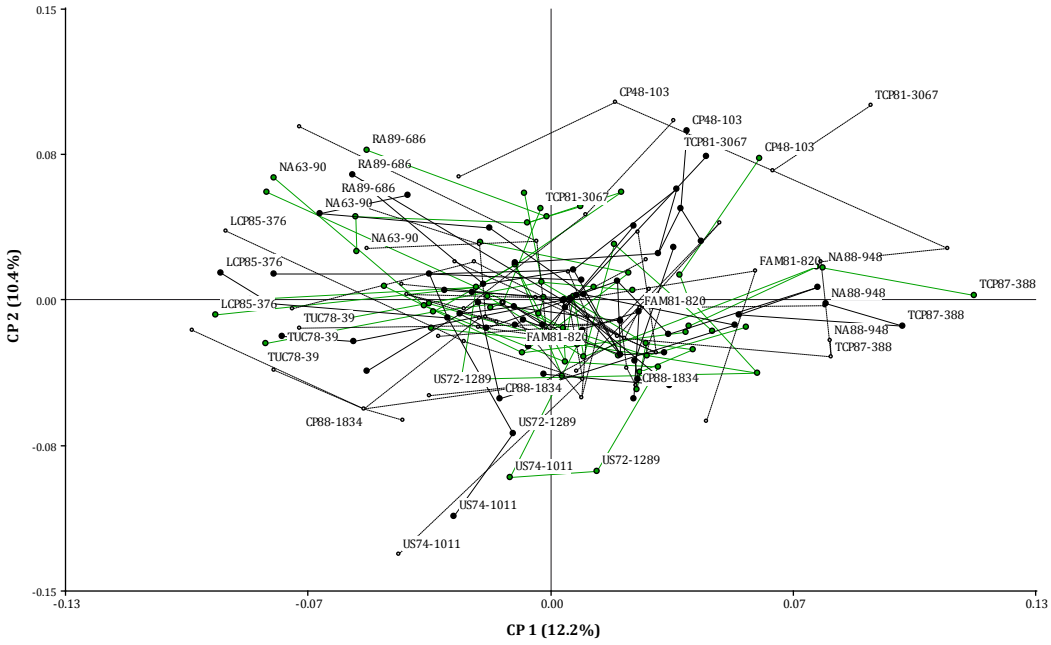
Table 5 (cont.). ANOVA Consensus between molecular and morphological ordinations. Accessions in bold showed the greatest discrepancy between the morphological and SSR data due to their higher relative values of Residual Sum of Squares (RSS).

Tabla 5 (cont.). ANOVA Consenso entre ordenamientos basados en marcadores moleculares y morfológicos. Las accesiones en negrita mostraron las mayores discrepancias entre datos morfológicos y de SSR debido a sus valores de Sumas de Cuadrados Residuales (SCR) más altos.

	Consensus	**Residue**	**Total**	**Consensus proportion**
CP79-1380	0.019	0.008	0.027	0.690
CP79-318	0.016	0.003	0.019	0.828
CP65-350	0.018	0.007	0.024	0.726
CP57-603	0.043	0.007	0.051	0.858
CP57-614	0.021	**0.011**	0.032	0.651
CP72-2086	0.034	0.007	0.040	0.834
CP66-346	0.026	**0.011**	0.037	0.706
CP62-258	0.034	**0.010**	0.044	0.769
FAM81-820	0.018	**0.012**	0.030	0.594
FAM83-11	0.049	0.005	0.055	0.900
TUC80-7	0.019	0.011	0.030	0.635
TUC72-16	0.033	0.010	0.043	0.761
TUC74-6	0.016	**0.018**	0.034	0.476
TUC71-7	0.018	**0.010**	0.027	0.649
TUC68-18	0.028	0.008	0.037	0.772
TUC67-24	0.024	**0.012**	0.036	0.661
TUC79-9	..0.020	0.006	0.026	0.769
TUC77-42	0.016	0.005	0.021	0.770
TUC78-39	0.026	0.008	0.034	0.775
TUC72-4	0.024	0.006	0.030	0.791
TUC69-2	0.014	0.008	0.023	0.629
L91-281	0.020	0.006	0.026	0.765
RA89-686	0.031	0.009	0.040	0.778
RA87-2	0.033	0.009	0.041	0.788
RA91-209	0.011	0.020	0.030	0.356
RA93-154	0.025	**0.010**	0.035	0.702
CP88-1834	0.0.18	0.006	0.024	0.757
F98-70	0.025	0.005	0.029	0.841
F97-395	0.014	0.003	0.017	0.798
F97-786	0.014	0.004	0.017	0.798
CP65-357	0.014	0.005	0.020	0.733
Total	1.459	0.541	2.000	0.730

According to Bramardi *et al.* (2005), the latter is broken into the consensus and the residual sum of squares. This residual sum of squares measures the divergence between the two points corresponding to the morphological and molecular characterization to the consensus one, respectively (table 5, page 56-57). The ratio between the consensus value (1.459) and the total sum of squares revealed a consensus of 73% between molecular and agronomic ordinations (2).

This percentage of consensus is an univariate measure of association between both groups of markers. According to table 5, accessions in bold letter are those that have shown a high discrepancy between morphological and molecular data, because they have grater residual sum of square values, therefore they should have been responsible for the low correlation found between the individual configurations.



The continuous green line indicates the MST based on morphological data and the black dotted line; the MST based on molecular data.

La línea verde indica el ARM obtenido con datos morfológicos y la línea discontinua negra, el ARM basado en datos moleculares.

Figure 7. Configuration of consensus matrix of GPA between morphological and molecular data with Minimum Spanning Tree (continuous black line).

Figura 7. Configuración consenso GPA con datos morfológicos y moleculares que incluye el Árbol de Recorrido Mínimo (ARM) en línea negra.

The consensus configuration of GPA with Minimum Spanning Tree (MST) is presented in figure 7 (page 58). The large number of accessions included in this study and the close genetic relationship among materials, hinders the identification of individuals in the consensus configuration.

In most of the references found for sugarcane, the assessment of the genetic variability is based, independently, on the analyses of morphological or molecular markers data.

Some papers estimate a correlation coefficient between distance matrices. According to Demey (2008), conclusions based only on correlation coefficient values can be inaccurate since the correlation is not only affected by the size of the compared samples but also because the configurations belong to the same reference system.

Conclusions

Based on results formerly presented and discussed, we propose the following general conclusions:

Both morphologic (quantitative and qualitative) and molecular markers included in this research resulted discriminative enough to differentiate among the studied accessions. It was not possible, however, to correlate associations of markers with the origin of materials.

The large number of pair-wise similarity coefficients with intermediate values determined a rather small number of nodes in clustering, which, on time, reflects the near genetic origin of most of the studied materials.

Diversity detected for morphological descriptors contributing to explain PC1 and PC2 (except for diameter), are expected since they are not usually subjected to selection in breeding.

Phenotypic and genetic distances based on morphology and molecular information serves to assist conservation and organization of collection of materials, and the choice of parent combinations for breeding purposes.

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