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Population structure and genetic diversity of Brazilian popcorn germplasm inferred by microsatellite markers

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ABSTRACT

Background: The genetic diversity and structure of 31 popcorn accessions of the germplasm bank of the State University of Maringá were assessed using 30 microsatellite primers.
Results: 127 alleles were identified from 30 evaluated loci. The number of alleles per locus ranged from two to eight. The overall mean of the polymorphic loci averaged 79.89%. The primers UMC1549 and UMC1072 detected polymorphism in all accessions analyzed. The mean observed heterozygosity ranged from 0.07 to 0.30 and the highest proportion of heterozygous plants was observed in accession BOZM 260 (Ho = 0.30). The analysis of molecular variance revealed that 60% of the total genetic variation was found within accessions and 40% was found between accessions. The Bayesian clustering approach grouped the 31 accessions into two genetically differentiated clusters. The dendrogram revealed that accessions TATU 2 and ARZM 05 083 are genetically less similar than the others.
Conclusions: The analysis allowed to identify microsatellite loci with high levels of heterozygosity (UMC1549 and UMC1072). These loci can be indicated as promising for detecting polymorphisms in popcorn accessions and in the monitoring of genetic improvement programs. Moreover, allowed to identify heterozygous accessions (BOZM 260), this accession showed allelic variation at all analyzed microsatellite loci and can be recommended for crosses with plants that have desirable agronomic characteristics, with a view to the broadening of the genetic base of popcorn accessions and developing new cultivars.

1. Introduction

The objective of a breeding program is to develop popcorn cultivars that unite good agronomic characteristics with a high popping expansion rate [1,2]. Lima et al. [3] stated that the success of a breeding program depends on the indication of the most promising population for the development of lines and on the choice of contrasting lines for hybrid formation, which can be done by establishing heterotic groups [4].

Thus, genetic divergence studies have become essential as an orientation for the selection of potentially promising parents to generate populations with high variability and adaptation capacity, which, when crossed, increase the chances of obtaining superior genotypes in segregating generations. This allows a concentrated breeding effort on a smaller number of hybrids obtained from the most divergent genotypes [5,6].

The genetic characterization of popcorn populations destined for improvement can be based on agronomic traits and/or molecular markers. Different types of molecular markers have been used to estimates of genetic variability; these markers indicate the similarity between genotypes based on a direct analysis of the genome [7].

The technique of SSR analysis (Simple Sequence Repeats of genomic DNA) has been considered appropriate to estimate the genetic diversity of popcorn accessions [1,8,9,10,11,12]. In spite of these studies, the number of researches addressing the genetic diversity of popcorn in Brazil is still comparably small in view of the number of varieties potentially available to be exploited in breeding programs. The SSR loci in popcorn have been analyzed to a limited extent, based on a restricted number of samples of accessions and plants per accession, and with a little more than a dozen primers. Therefore, the purpose of this study was to analyze 30 SSR loci in 31 popcorn accessions from the germplasm bank of the State University of Maringá, in order to: a) select and relate the promising SSR loci to estimates of genetic

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diversity and to monitor the improvement program, b) analyze the population structures, c) analyze the genetic variability of populations, and d) select promising accessions to guide our breeding program.

2. Materials and methods

2.1. Genetic material and DNA extraction

In this study, we used SSR markers to assess the genetic diversity and structure of 31 popcorn accessions of the germplasm bank of the State University of Maringá (1 — CHZM 13 0134; 2 — ARZM 13 050; 3 — URUG 298; 4 — ARZM 05 083; 5 — PARA 172; 6 — ARZM 07 049; 7 — BOZM 260; 8 — BOYA 462; 9 — IAC-125; 10 — IAC-112; 11 — JADE; 12-Zélia; 13 — ARGENTINA; 14 — SE 013; 15 — Viçosa; 16 — RS-20; 17 — BEIJA-FLOR; 18 — UNB-2UC1; 19 — UNB-2UC2; 20 — UENFV-Explosivo C4; 21 — UNB-2UC3; 22 — PA 091; 23 — PR-023; 24 — BRS ANGELA; 25 — SAM; 26 — PARA 170; 27 — COLOMBIANA; 28 — TATU 1; 29 — UNB-2U C0; 30 — UFVM2-Barão Viçosa; 31 — TATU 2), all popcorn accessions analyzed are hybrids (four accessions, 9, 10, 11 and 12) or open pollination populations (twenty-seven accessions). We analyzed 15 plants of each accession. The DNA was extracted from each plant individually, from leaf tissue by the method described by Hoisington et al. [13], with minor modifications and was quantified with a Qubit™ fluorometer using the Quant-iT assay kit (Invitrogen) and the DNA samples were diluted to a concentration of 10 ng/μL^{-1}, for use in amplification reactions.

2.2. SSR amplification

We tested 159 pairs of SSR primers mapped from common corn, of which 42 were polymorphic; of these, 30 were selected to study popcorn populations (Table 1). All microsatellites were obtained from the website Maize DB at http://www.maizegdb.org/ssr.php.

The PCR amplification was performed with the program "Touchdown" PCR [14] in a total volume of 20 μL containing 25 ng of DNA, with 2.0 μL of 10× reaction buffer, 2.5 mM $MgCl_2$, 0.8 mM of each dNTP, 1 U Taq-DNA-Polymerase (Invitrogen), and 0.4 μM of the specific primers F and R. After amplification, a total of 20 μL of each sample (465 total samples) were separated by electrophoresis on a 4% agarose gel (50% agarose and 50% agarose Metaphor (CAMBREX) containing 0.5× TBE buffer (44.5 mM Tris, 44.5 mM boric acid, and 1 mM EDTA)). All 465 samples amplified for each SSR primer were run at 60 V for 4 h. A 100 pb ladder (Invitrogen) was used as the weight molecular marker. Gels were stained using SYBR® Safe DNA gel stain, and the image was captured using Ultraviolet Transilluminator High Performance-Edas 290 using the Kodak 1D 3.5 program. The allele numbers per locus were determined based on their relative position in the gel.

Table 1
Microsatellite loci; replication block; location of each primer; mean observed H_o; Nei's mean expected H_e; PIC; number of alleles observed in 30 microsatellite loci (Na); and percentage of the most frequent allele per locus.

Loci	Replication	Bin	Ho	He	PIC	Na	% allele Most frequent
Umc2401	(TACGA)5	1.01	0.04	0.39	0.48	3.00	0.48
Umc2108	(ACG)4	1.01	0.03	0.39	0.55	3.00	0.43
Umc1125	(AG)30	1.04	0.31	0.30	0.36	2.00	0.63
Mmc0271	(GAGCA)4	1.11	0.22	0.29	0.51	6.00	0.47
Umc2246	(CCTCCT)4	2.00	0.12	0.35	0.58	5.00	0.42
Bnlg1175	(AG)38	2.04	0.21	0.49	0.79	8.00	0.31
Umc1755	(GAAGG)4	2.05	0.03	0.35	0.61	5.00	0.41
Umc2166	(ACA)17	2.05	0.12	0.43	0.70	5.00	0.30
Umc1118	(CACGAG)4	2.06	0.10	0.32	0.54	5.00	0.61
Umc1065	(GA)39	2.07	0.28	0.50	0.76	6.00	0.27
Umc1642	(CTCTCTCTCT)4	2.07	0.04	0.37	0.59	5.00	0.56
Umc2118	(CTTT)4	3.00	0.07	0.28	0.54	3.00	0.47
Umc1137	(CTGCA)4	3.00	0.22	0.22	0.26	4.00	0.84
Umc2059	(GCCTC)4	3.05	0.21	0.33	0.49	3.00	0.46
Umc1635	(CGC)6	4.07	0.27	0.42	0.64	5.00	0.38
Umc1071	(AGAAAGAA)4	5.04	0.33	0.45	0.64	4.00	0.46
Umc1415	(CGGC)4	5.05	0.06	0.05	0.06	4.00	0.97
Umc1336	(GGACTG)8	5.06	0.21	0.29	0.37	2.00	0.58
Umc1363	(GGA)10	5.07	0.15	0.32	0.45	3.00	0.58
Umc2205	(TTC)12	6.07	0.34	0.48	0.67	6.00	0.37
Umc1549	(GAAA)24	6.07	0.77	0.46	0.48	4.00	0.61
Bnlg2295	(CAG)8	6.08	0.23	0.32	0.41	4.00	0.73
Umc2302	(GCTA)6	7.00	0.09	0.23	0.40	3.00	0.70
Umc1072	(GCCTCT)4	7.02	0.99	0.56	0.55	4.00	0.47
Umc2165	(CTCG)5	7.04	0.14	0.33	0.57	3.00	0.45
Umc1847	(GAC)10	8.03	0.04	0.21	0.32	2.00	0.73
Umc1653	(CTCTCT)4	8.05	0.27	0.38	0.63	8.00	0.53
Mmc0501	(CT)15	9.08	0.19	0.42	0.68	4.00	0.33
Umc1524	(GA)36	10.02	0.34	0.47	0.66	4.00	0.42
Umc2164	(ACCAG)4	10.03	0.10	0.40	0.63	4.00	0.39
Mean			0.22	0.36	0.53	4.23	

2.3. Genetic diversity and population structure analysis

To analyze the genetic diversity in popcorn populations, each amplified DNA segment, identified as a band in the gel, was considered a distinct phenotype and independent of the others, determining the alleles of each SSR locus. Basic statistics were calculated using the software GENALEX 6.1 [15] to determine the allele frequencies, the mean observed heterozygosity (H_o) and Nei's expected heterozygosity (H_e), the number of alleles at each SSR loci, and the percentage of polymorphic alleles for each population. The polymorphic information content (PIC), and the allele with the highest frequency were calculated using PowerMarker 3.25 [16]. The analysis of molecular variance (AMOVA) and the principal component analysis (PCA) were also performed using the software GENALEX 6.1 The genetic population structure of the 31 popcorn accessions was analyzed by the program STRUCTURE [17], which assigns individuals to a number K of genetically homogeneous groups, based on the Bayesian estimate in accordance to the expected Hardy–Weinberg equilibrium and absence of linkage disequilibrium between the loci analyzed in each population. For the analyses with the program STRUCTURE, a burn-in period of 50,000 and a posterior number of Markov Chain Monte Carlo (MCMC) of 100,000 permutations was used. Fifteen replications (runs) were performed for each possible value of K (K = 1 to K = 10). An admixture and allele frequencies correlated model was used.

Two different approaches were used to detect the most likely K value: the first was the proposed by Pritchard et al. [17] based on the rate of change of LnP(D) for each K between 1 and 10 and the second was the criterion proposed by Evanno et al. [18], which is based on the second order rate of change of the likelihood function with respect to K (ΔK) (the ad hoc ΔK test). The results from STRUCTURE were processed with the software STRUCTURE HARVESTER v.0.6.1 [19]. The convergence of the Gibbs chains was determined using the test proposed by Heidelberger and Welch [20], which was performed in the R program with the convergence diagnosis and output analysis (CODA) library. The program CLUMPP [21] was used to analyze the stability among the fifteen runs for the real value of K determined. The barplot of the probability of membership from the results of Q-matrix were visualized by DISTRUCT software [22]. In order to assign accessions into groups, accessions with probability of membership ≥0.70 were considered to belong to discrete groups, whereas accessions with probabilities <0.70 were considered as a mixture.

From the matrix of Rogers' genetic distance [23], calculated by the program TOOLS FOR POPULATION GENETIC ANALYSES, a dendrogram was constructed by UPGMA cluster analysis (Unweighted Pair — Group Method Using the Arithmetic Average) using the program MEGA v.5.05 [24].

3. Results

The 30 primers revealed 127 alleles. The number of alleles per locus per accession varied from two to eight, with a mean of 4.23 alleles/locus (Table 1).

For most loci, the mean observed H_o was lower than the mean expected H_e, except for the loci *Umc1125*, *Umc1415*, *Umc1549* and *Umc1072*. The lowest mean heterozygosity was observed at locus *Umc2108*, while the highest values for H_o and H_e were found at locus. The mean PIC was 0.53. The highest PIC was 0.79 for primer BNLG1175, and the lowest was 0.06, for primer UMC1415 (Table 1).

The mean proportion of polymorphic loci was 79.89%. The mean observed heterozygosity for accessions ranged from 0.07 to 0.30; accession BOZM 260 had the highest proportion of observed heterozygous plants ($H_o = 0.30$). Accession TATU 2 had the lowest mean observed and expected heterozygosity ($H_o = 0.07$; $H_e = 0.09$) (Table 2). To assess the overall distribution of diversity within and between accessions, an AMOVA was performed from the distance matrix. The AMOVA revealed that 60% of the genetic variance was found within accessions and 40% between accessions, showing significant difference genetic ($P < 0.0001$). The results for the determination of the optimal K value based on Pritchard et al. [17] and Evanno et al. [18] approaches clearly indicated that the accessions were grouped into two clusters (Fig. 1). The percentages of individuals in each population belonging to each cluster were calculated (Table 2). Twenty-eight of the 31 popcorn accessions showed values of probability of membership greater than or equal to 0.7 and were therefore classified as a member of a particular cluster, while three accessions were classified as a mixture of the two clusters (probability of membership < 0.7) (Table 2, Fig. 2A).

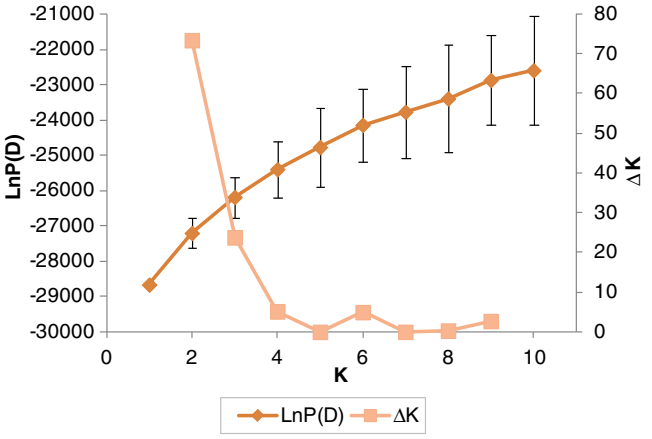

Fig. 1. Plot of two graphical methods allowing detection of the true value for K. Diamonds represent the mean of LnP(D)(±Var) over 15 runs for each K value analyzed. The black arrow indicates the break point in the increasing of the values of LnP(D) (Δ[LnP(D) K1–K2] = 1462.3; Δ[LnP(D) K2–K3] = 1017.6; Δ[LnP(D) K3–K4] = 789.6) and the increase of the variance associated to LnP(D) across different K values tested [17,22]. The squares represent the values of ΔK calculated, based on the methodology proposed by Evanno et al. [18].

Table 2
Origin of the popcorn accessions; percentage of polymorphic loci (%P); mean observed heterozygosity (H_o); mean expected heterozygosity (H_e); proportion of participation of each population in each of the two clusters obtained with the program STRUCTURE.

Genotype	Origin	%P	Ho	He	≥70%	Clusters	
						1	2
1-CHZM 13 0134	CIMMYT[a]	86.67	0.20	0.39	2	0.05	0.95
2-ARZM 13 050	CIMMYT	93.33	0.22	0.49	2	0.03	0.97
3-URUG 298	CIMMYT	93.33	0.26	0.44	2	0.22	0.78
4-ARZM 05 083	CIMMYT	56.67	0.12	0.23	2	0.11	0.89
5-PARA 172	CIMMYT	83.33	0.19	0.40	2	0.04	0.96
6-ARZM 07 049	CIMMYT	96.67	0.28	0.47	2	0.08	0.92
7-BOZM 260	CIMMYT	100.00	0.30	0.52	2	0.06	0.94
8-BOYA 462	CIMMYT	90.00	0.22	0.45	2	0.06	0.94
9-IAC-125	IAC[b]	73.33	0.28	0.29	1	0.94	0.07
10-IAC-112	IAC	70.00	0.27	0.28	1	0.98	0.02
11-JADE	Pioneer Hi-bred	50.00	0.14	0.17	1	0.98	0.02
12-Zélia	Pioneer Hi-bred	53.33	0.21	0.21	1	0.99	0.01
13-ARGENTINA	UEM[c]	53.33	0.13	0.21	1	0.96	0.04
14-SE 013	UEM	83.33	0.27	0.39	Mixture	0.47	0.53
15-Viçosa	UFV[d]	93.33	0.25	0.46	2	0.10	0.90
16-RS-20	IPAGRO/AGROESTE[e]	86.67	0.18	0.31	2	0.11	0.89
17-BEIJA-FLOR	UFV	90.00	0.20	0.37	2	0.14	0.86
18-UNB-2UC1	UENF[f]	96.67	0.23	0.47	2	0.13	0.87
19-UNB-2UC2	UENF	90.00	0.23	0.40	2	0.05	0.95
20-UENFV-Explosivo C4	UENF	93.33	0.29	0.45	2	0.17	0.83
21-UNB-2UC3	UENF	90.00	0.25	0.43	2	0.10	0.90
22-PA 091	UEM	86.67	0.25	0.40	2	0.05	0.95
23-PR-023	UEM	93.33	0.29	0.44	2	0.07	0.93
24-BRS ANGELA	EMBRAPA[g]	96.67	0.28	0.45	2	0.20	0.80
25-SAM	Sul-americana/EUA	73.33	0.17	0.28	Mixture	0.31	0.69
26-PARA 170	CIMMYT	73.33	0.19	0.35	2	0.08	0.92
27-COLOMBIANA	UEM	70.00	0.13	0.30	Mixture	0.53	0.47
28-TATU 1	UEM	56.67	0.12	0.23	2	0.02	0.98
29-UNB-2U C0	UENF	93.33	0.24	0.44	2	0.15	0.85
30-UFVM2-Barão Viçosa	UFV	83.33	0.25	0.37	2	0.10	0.90
31-TATU 2	UEM	26.67	0.07	0.09	2	0.07	0.93
Mean		79.89	0.22	0.36			

[a] CIMMYT: International Maize and Wheat Improvement Center.
[b] IAC: Agronomic Institute of Campinas.
[c] UEM: State University of Maringá.
[d] UFV: Federal University of Viçosa.
[e] IPAGRO: Agronomy Research Center.
[f] UENF: State University of North Fluminense.
[g] EMBRAPA: Brazilian Agricultural Research Corporation.

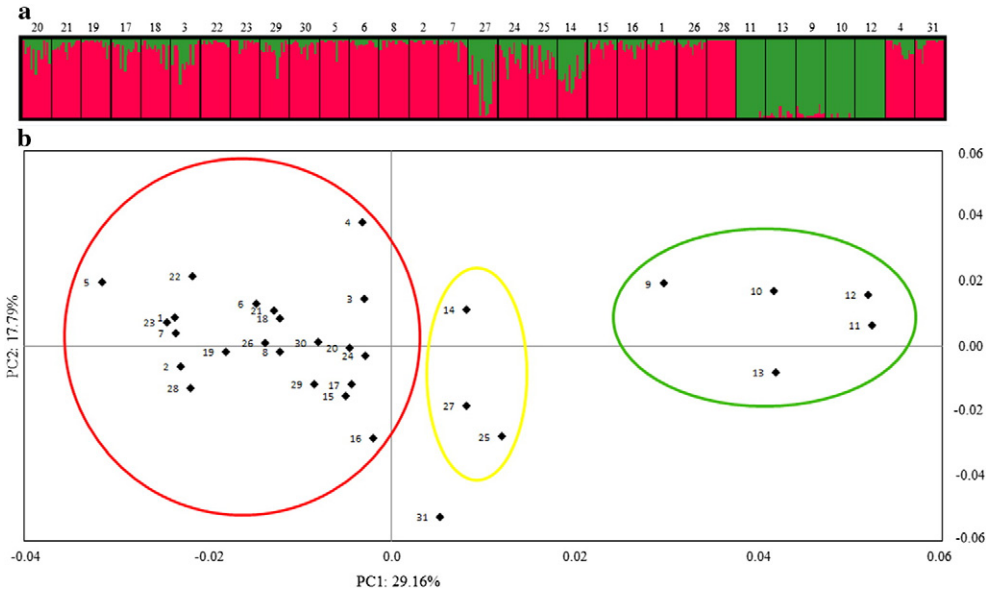

Fig. 2. Genetic stratification of 31 popcorn accessions based on the analysis of 30 microsatellites loci. (a) Output from STRUCTURE for the optimal value of K estimated (K2). The analysis was carried out using an admixture and allele frequencies correlated model. (b) Genetic clustering based on a PCA using the software GENALEX. The colors green and red are according to the STRUCTURE output in (A), and the yellow group correspond to Mixed group with probability of membership <0.7, according to Q matrix values.

The test of Heidelberg and Welch showed that the Gibbs chains of the FST values for each cluster were stable throughout iterations and these converged to specific values (means and Bayesian credible intervals of FST values for cluster 1 and cluster 2: 0.378 [0.314–0.447] and 0.0203 [0.002–0.046] respectively) (Fig. 3).

The PCA performed in GENALEX grouped the accessions into two clusters strongly differentiated, which are represented in red and green colors in the Fig. 2B. The eigenvalues obtained from the PCA showed that the two first components explained a 46.95% of the total variation (PC1: 29.16%; PC2: 17.79%). Accessions 14, 25, 27 and 31 showed not clear membership in any of the groups identified in the PCA. In addition, accessions 14, 25 and 27 showed the lowest membership coefficients based on Q-matrix values (Table 2), therefore these accessions were grouped in a different group called Mixed group (yellow color). Accession 31 was not considered within the Mixed group due to its high coefficient of membership and its high genetic divergence with the other accessions. This fact can be corroborated by comparison with the dendrogram (Fig. 4).

The dendrogram by the UPGMA method (Fig. 4) using the coefficient of Rogers' genetic distance [23], and adopting a cut off in the range of

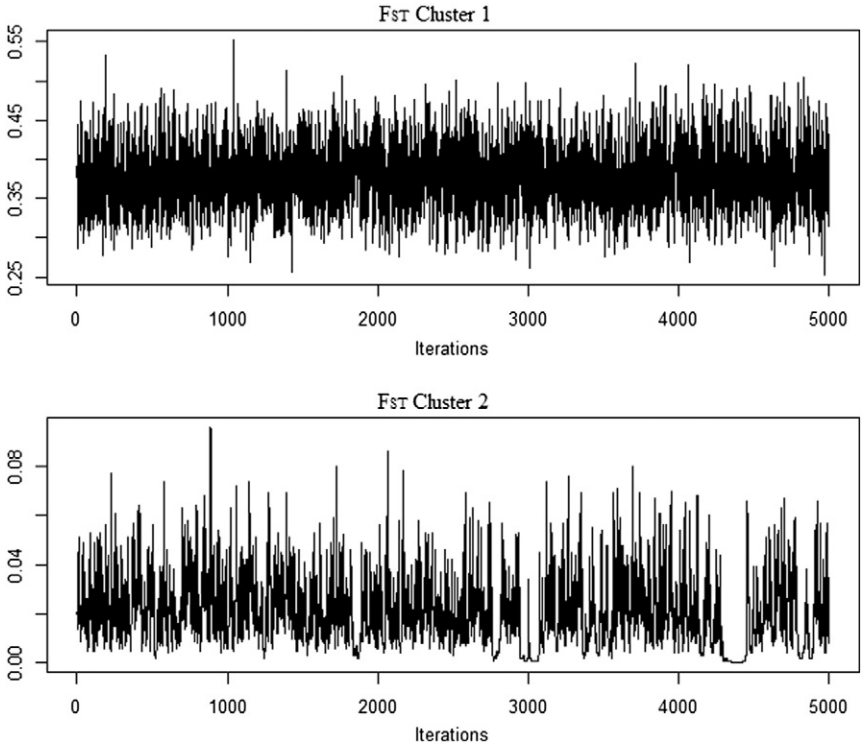

Fig. 3. Convergence of Gibbs chains for the FST values of the inferred clusters from STRUCTURE. The stationarity of chains was determined by the Heidelberg and Welch test using the CODA package in R v3.1.1.

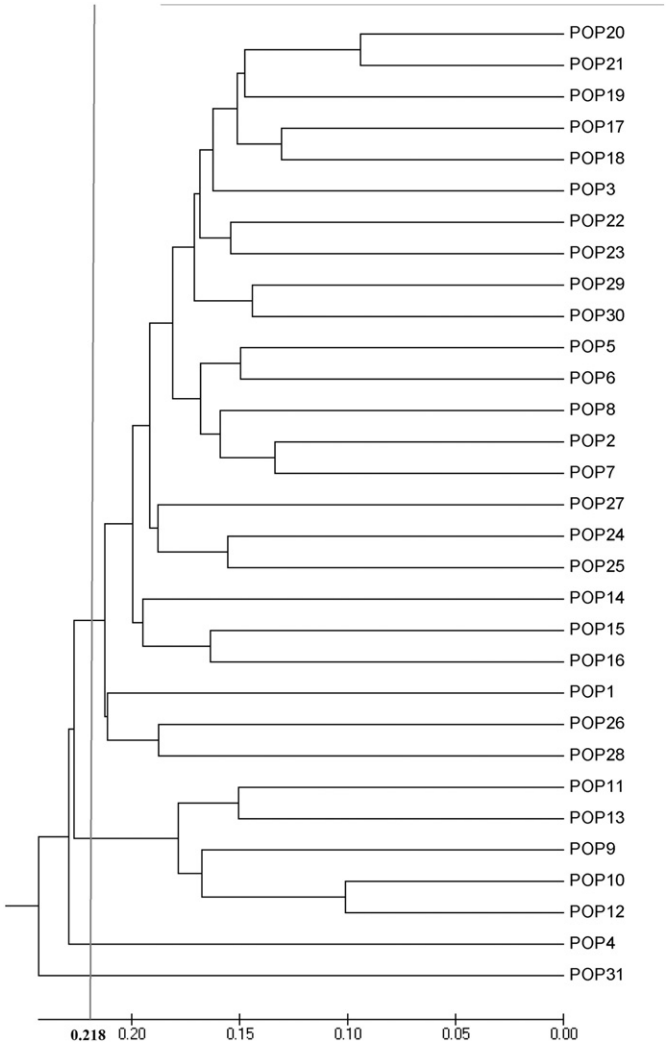

Fig. 4. Rogers' genetic distance among 31 popcorn accessions [23], estimated by the UPGMA method using program Mega 5.05.

distance of 0.218, revealed the presence of four groups. Based on the dissimilarity matrix obtained with the same coefficient, the most similar accessions were UENFV-Explosivo C4 and UNB-2U C3 (distance 0.19), and the most divergent were ARZM 05 083 and TATU 2 (distance 0.58). The cophenetic correlation coefficient obtained between Rogers' genetic distance [23] and the cophenetic distance matrix obtained from the dendrogram was high (0.837), confirming the consistency of the clusters.

4. Discussion

4.1. Population structure

The primers UMC1549 and UMC1072 showed polymorphism in all accessions studied with values of observed heterozygosity above 70% (0.77 and 0.99, respectively), and can therefore be indicated as effective and promising for detecting polymorphisms in popcorn accessions. The heterozygosity of a marker depends on the number of alleles and their frequency in the population. Heterozygosity values above 70% are considered more informative and considered a good indicator for consistent and accurate population studies [25]. The primer BNLG1175 had the highest PIC (0.79), eight alleles and the frequency of the most recurrent allele at this locus was 0.31. Romero-Severson et al. [26] explained that the PIC takes into account the number of alleles and the relative frequency of these alleles. Thus, low PIC values indicate a high frequency of one or two alleles, exactly as observed in our accessions of popcorn, where the lowest PIC (0.06) was evident in primer UMC1415, with four alleles, and the percentage of the most frequent allele was 96% (Table 1).

The AMOVA revealed that 60% of the molecular genetic variance was found within accessions and 40% between accessions, showing significant difference genetic ($P < 0.0001$). Although genetic variability among populations represents the small part of the total variability, we conclude that the 31 analyzed popcorn accessions are genetically structured populations. Similar results were found by Reif et al. [27] using SSR markers in populations of tropical maize, whose analysis of molecular variance revealed that 89.8% of the variation was found within populations and only 10.2% between populations. According to Loveless and Hamrick [28], the dispersal of pollen and seeds determines the patterns of genes dispersion within and among populations. Typically allogamous plants have high genetic variation within populations and low among them. Furthermore, it is characteristic of microsatellite loci reveal high heterozygosis within population, which produces low differentiation between them.

The genetic differentiation among the 31 accessions was observed by the presence of private alleles in some accessions. In accession ARZM 07 049, a private allele was detected with a frequency of 0.15 at locus *Mmc0271* and in accession BOZM 260 a private allele with frequency 0.03 at locus *Umc1415*.

Accession BOZM 260 showed allelic variation at all microsatellite loci analyzed, and can therefore be recommended for crosses with plants that have desirable agronomic characteristics, with a view to the broadening of the genetic base of popcorn accessions. The BOZM 260 had also the highest H_o (0.30) and can be considered promising for continuous progeny selection. The maintenance of genetic diversity during progeny formation is important for the progress of selection, because it increases the chances of selection in the medium and long term. Carvalho et al. [8] analyzed the genetic diversity of eight popcorn populations using microsatellite markers and found very similar results, since the accession BOZM 260 had the highest value of H_o. According to Allendorf and Luikart [29], high heterozygosity probably indicates high adaptive genetic variation of the population, preventing a uniform response and allowing it to escape the effects of a control agent.

4.2. Genetic distance and hierarchical clustering

The dendrogram (Fig. 4) showed a first cluster consisting of 24 accessions. The five accessions from the Universidade Estadual do Norte Fluminense (UENF) were grouped in this first group, namely: UNB-2U C1; UNB-2U C2; UENFV-Explosivo C4; UNB-2U C3 and UNB-2U C0. These results are in agreement with those of Amaral Junior et al. [30], who used ISSR molecular markers and accessions of common corn, popcorn, sweet corn, teosinte and *Tripsacum*, allocating these five accessions in group three of his dendrogram.

Of the nine accessions from CIMMYT, eight are part of group one, namely: ARZM 13 050; URUG 298; PARA 172; ARZM 07 049; BOZM 260; BOYA 462; CHZM 13 0134 and PARA 170. The accession ARZM 05083 was allocated alone in group three. Using the ISSR markers and 34 popcorn accessions, among them the nine accessions from CIMMYT used in our study, Amaral Junior et al. [30] showed that the nine populations were allocated in the groups as follows: group one URUG 298; BOYA 462, PARA 172 and CHZM 13 0134 in group two; ARZM 05 083 in group three; ARZM 07 049 and ARZM 13 050 in group four, and BOZM 260 and PARA 170 in group five. The difference may be due to the fact that the genetic basis of ISSR differs from that of SSR markers.

Group two contained the Brazilian commercial accessions IAC-125, IAC-112, Jade and Zélia, and accession Argentina. It is worth noting that this group corresponds exactly to group one obtained with the Bayesian clustering approach using STRUCTURE and the green marked group obtained by PCA (Fig. 2).

In line with our results, when evaluating the diversity among popcorn accessions by RAPD markers Munhoz et al. [31] reported the clustering of commercial genotypes developed in the country.

Silva et al. [12] used microsatellite markers to analyze the genetic variability of 25 popcorn genotypes, and revealed a dramatic situation: all commercial accessions developed in the country were classified into a single group by the Tocher method.

Similar results were presented by Santacruz-Varela et al. [32]. The authors evaluated the genetic distance of 56 popcorn accessions from 10 countries (including Brazil). The results were based on 29 morphological traits, 18 isozyme loci and 31 SSR primers. Three groups were identified: i) "Yellow Pearl Popcorn", in which the most important U.S. trade group accessions were allocated, ii) "North American Pointed Rice Popcorns", which probably originated from a combination of traditional popcorn races from Latin America and iii) "North American and North American early Popcorn", which are early maturing and closely related with flint corn types.

According to Amaral Junior et al. [30], the conjunction of the country's recommended cultivars in the same group, reinforced by other results with molecular markers and agronomic traits represents a warning for the few popcorn breeders in Brazil, emphasizing the urgency to increase the genetic diversity in the improvement programs of public and private institutions.

In breeding programs, well-founded knowledge about the genetic diversity is crucial for the early diagnosis of genetic narrowing and to outline efficient strategies for amplification [33].

Based on the hypothesis of association between heterosis and the frequency of heterozygous loci affecting the trait, Hallauer and Miranda-Filho [4] suggested the prediction of heterosis based on molecular markers. Rinald et al. [6] found a positive and significant correlation between heterosis and genetic diversity estimated by diallel crosses and RAPD markers for the traits grain weight, plant height, ear height and prolificacy, in research with eight popcorn populations. Reif et al. [34] genotyped 20 maize populations with 83 microsatellite markers. The values of genetic distances were correlated with data of heterosis obtained by diallel crosses between these populations. The correlations were positive and significant (0.18–0.56).

Thus, based on these results, one can assume that crosses between plants of the accessions ARZM 05083 and TATU 2, for example, are promising to broaden the genetic base of popcorn. Based on the previously discussed hypothesis of association between heterozygosity and heterosis, and the correlation between heterosis and genetic distance, these two accessions, separated by the greatest genetic distance, can be exploited in programs of reciprocal recurrent selection, destined for the synthesis of contrasting lines that can be intercrossed to generate superior hybrids.

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Appendix A. Supplementary data

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