Phenotypic and molecular diversity of local popcorn populations in Southern Brazil

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Abstract

The aim of this study was to characterize the phenotypic and genetic diversity, of popcorn populations from Far West region of Santa Catarina state, Southern Brazil, and generate subsidies for strategies of participatory genetic breeding. Thus, ten local popcorn populations were evaluated phenotypically, in three environments, and genotypically, using six molecular markers. From the results, the genetic and phenotypic diversity, and the agronomic potential of the populations were estimated. The 574A population stood out for its high popping expansion (36.33 mL g^{-1}), whereas 283A, 319E, and 880A were distinguished by their high grain yield, which ranged from 1,669.3 kg ha^{-1} to 1,905.7 kg ha^{-1}. Diversity amongst the ten populations was measurable and significant, both by phenotypic evaluations and by means of microsatellite markers. However, there was a low correlation between the dendrograms generated based on phenotypic and molecular data, and for outlining the strategies of breeding, this study recommends molecular analysis as a basis for genetic divergence, complementing with the information of morpho-agronomic traits. Populations 884B, 574A, 880A, 66A, 977A, 857C, 283A and 319E resulted promising parents for diallel crosses, and the populations 66A, 574A, and 880A for intrapopulation recurrent selection.

Abbreviations

A - total number of alleles per locus
ccc - cophenetic correlation coefficient
DTA - degrees-days to anthesis
f - fixation index
FWSC - Far West of Santa Catarina State
GY - grain yield
Ĥe - expected mean heterozygosity or genetic diversity
Ĥo - observed mean heterozygosity
IDV - Variety Identification Code
Na - mean number of alleles per locus
NBT - number of branches in the tassel
Ne - effective number of alleles per polymorphic locus
NEABio - Agrobiodiversity Study Center
NLC - number of leaves above the main cob
PCA - principal components analysis
PE - popping expansion
PH – Plant height
PIC - polymorphic information content
PRL - cob prolificacy
RPC - relative position of the insertion of the main cob in the plant
STC - stem diameter in the internode just below the main cob
UFSC - Universidade Federal de Santa Catarina
UPGMA - Unweighted Pair-Group Method using Arithmetic Averages

Introduction

The Far West of Santa Catarina State (FWSC), located in the Southern Brazil, is recognized by the wide diversity of local varieties (Costa *et al.* 2016; Silva *et al.* 2016a; Silva *et al.* 2016b) and wild relatives of the *Zea* genus (Silva *et al.* 2015a). Therefore, FWSC has been indicated as a micro center of diversity of *Zea mays* L., as it is a geographic microregion comprising a genetic reserve of maize gene pool, in a diversification process, arising from a regular human activity, associated with socio-cultural aspects (Ogliari 2019). A significant part of the diversity of local populations identified along 558 km^2 on this region was due to the high number of fields cultivated with varieties of popcorn (1,078 of a total of 1,513 fields), among which almost 13% corresponded to the white grain popcorn populations (Costa *et al.* 2016).

Despite Brazil's position as the second largest popcorn

Table 1 - Information about local populations of popcorn (origin, kernel shape, growing time) from Far West of Santa Catarina, Southern Brazil, evaluated on the present research as vegetable material.

IDV	Community / Municipality of origin of the collection	Kernel shape	Growing time (years)
574A	Liso Baixo / GBA	Pointed	8
283A	Guataparalto / GBA	Rounded	80
319E	Guataparema / GBA	Pointed	Wi
66A	Barra da Traíra / GBA	Pointed	3
2360A	Cordilheira / ANC	Pointed	Wi
884B	São João / GBA	Pointed	2
977A	São Vicente / GBA	Pointed	37
244A	Flores da Cunha / GBA	Very pointed	20
857C	São Cristovão / GBA	Pointed	3
880A	São Domingos / GBA	Very Pointed	6

IDV: Variety Identification Code. Wi: Without information. GBA – Guaraciaba municipality; ANC – Anchieta municipality.

producer worldwide and with a dynamic and continuously growing market (Paula *et al.* 2010; Rangel *et al.* 2011), several authors highlight many weaknesses factors in popcorn cultivation in the country. Among them, the limited number of improved cultivars recorded in the National Cultivar Register, with regard to the growing demand for the product (Paula *et al.* 2010; Silva *et al.* 2015b).

Knowing the diversity potential which is conserved in situ on farm is one of the alternatives to increase the competitiveness and genetic variability of national genetic breeding programs (Humphries *et al.* 2015; Dwivedi *et al.* 2016). For this purpose, genetic resources conserved in situ on farm are crucial for both farmers and breeders, as they are the primary raw material for developing a wide range of products and for meeting the demands imposed by climate change and by the incidence of pests and diseases (Fenzi *et al.* 2015; Ficiciyan *et al.* 2018).

Against a backdrop of limited studies on the national diversity of local varieties of popcorn, this study aimed at characterizing i) the phenotypic and genetic diversity, and ii) the agronomic and culinary potential of ten local varieties (landraces) from the FWSC, with the goal of generating subsidies for the definition of participatory genetic breeding strategies

Material and methods

Plant material

Ten local populations of popcorn from the municipalities of Anchieta and Guaraciaba, in FWSC, Brazil, were chosen for this study (Table 1), based on a previous study of popping expansion comprising 85 local varieties (Silva *et al.* 2016b). Belonging to the morphological group of predominantly white popcorn, these varieties comprise part of the core collection composed of 147 accessions of popcorn from the Gene Bank supervised by the Agrobiodiversity Study Center (NEABio) of *Universidade Federal de Santa Catarina* (UFSC).

The term "population" has been used herein to refer to each local variety conserved by a farming family in their production unit under in situ on farm conservation, and each population was registered as one accession and identified by the Variety Identification Code (IDV).

Phenotypic characterization

Those ten populations were evaluated in three municipalities (environments), in two regions of Santa Catarina state, Brazil. Florianópolis is located in the coastal area (latitude 27°41'08" S, longitude 48°32'38" W, 5 m altitude), while the municipalities of Anchieta (latitude 26°32'01" S, longitude 53°19'17" W, 745 m altitude) and Guaraciaba (latitude 26°34'59" S, longitude 53°31'12" W, 720 m altitude) are at the Far West of the State.

Trials were carried out as a randomized complete blocks design, with three replications and plots composed of double rows of 5.0 meters long, spaced 0.8 meters between each other, and plants spaced 0.20 meters apart in the row at a density of 62,500 plants ha^{-1}.

Nine quantitative traits were evaluated, as follows: number of branches in the tassel – NBT; number of leaves above the main cob – NLC; plant height – PH (in m); relative position of the insertion of the main cob in the plant – RPC (cob height/PH); stem diameter in the internode just below the main cob – STC (in mm); degrees-days to anthesis – DTA; cob prolificacy – PRL; popping expansion – PE (in mL g^{-1}); and grain yield – GY (in kg ha^{-1}).

Table 2 - Averages of the morphoagronomic variables evaluated in ten local populations of popcorn and three locations (Anchieta, Guaraciaba and Florianópolis), Southern Brazil, growing season 2014/2015.

Population	PH (m)	RPC	STC (mm)	NBT	NLC	DTA	PRL	PE (ml g^{-1})	GY (kg ha^{-1})
574A	1.47	0.59	13.2	17.6	5.18	1,063.84	0.79	36.33	1,416.4
283A	1.55	0.53	15.0	17.7	5.64	1,027.20	0.92	21.08	1,905.7
319E	1.46	0.54	12.8	19.5	4.98	974.21	0.73	21.00	1,669.3
66A	1.52	0.58	13.8	18.9	4.76	1,025.85	0.76	24.33	1,494.7
2360A	1.70	0.57	13.6	18.7	4.90	1,060.42	0.79	25.22	1,584.4
884B	1.80	0.62	14.3	25.1	5.31	1,090.76	0.62	28.50	1,266.3
244A	1.49	0.55	13.6	16.5	5.04	1,051.20	0.64	22.50	1,153.2
857C	1.76	0.60	14.3	15.0	5.20	1,035.82	0.58	28.33	1,203.6
880A	1.68	0.62	14.5	19.1	5.16	1,063.43	0.96	30.72	1,889.6
977A	1.57	0.55	15.5	19.9	5.44	1,109.47	0.64	28.28	1,121.4
LSD $_{(\alpha=5\%)}$[1]	0.10	0.03	1.02	2.97	0.25	30.15	0.11	1.56	270.69
Mean[2]	1.60	0.58	14.06	18.80	5.16	1,050.2	0.74	26.63	1,470.46
Int. $GxA_{(p)}$[3]	0.073	0.060	0.708	0.662	0.536	0.141	0.062	0.005	0.0396
$Location_{(p)}$[3]	0.605	0.117	0.001	0.044	0.003	0.000	0.002	0.058	0.0000
$Genotype_{(p)}$[3]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0006
MSE[4]	0.018	0.002	1.89	16.03	0.114	1,648.4	0.024	4.429	132850
CV(%)[5]	8.83	7.71	9.77	21.29	6.54	3.86	20.96	7.90	24.78

Plant height (PH), Relative cob position (RPC), Stem diameter in the internode just below the main cob (STC), Number of branches in the tassel (NBT), Number of leaves above the main cob (NLC), Degrees-days to anthesis (DTA), Cob prolificacy (PRL), Popping expansion (PE); e Grain yield (GY), [1]Last Significant Difference (base on t table), [2]Average of three replications, [3]Significance's effect, [4]Mean Square Error, [5]Experimental coefficient of variation

Principal Component Analysis (PCA)

A Biplot graph of the principal components (PCA) was generated based on phenotypic data, with the objective of understanding population dispersion and the effect of variables on variation between populations. The calculation of the Euclidean Distance and the PCA were carried out using the R program, by means of the "factoextra" (Kassambara and Mundt, 2016) and "FactoMineR" (Lê *et al.* 2018) packages, on the basis of the standardized variable mean:

$$X_{ij} = X_{ij}/S(X_j),$$

in which X_{ij} corresponds to the mean of the i-th treatment for the j-th variable, divided by the standard deviation of the j-th variable. The PE and GY variables were not included in the diversity calculations, because this could approximate the genotypes with the greatest potential for these traits, hindering finding contrasting genotypes with high potential for PE and GY.
Analysis of variance was performed for each variable, according to the following model:

$$y_{ijk} = \mu + b/l_{k(j)} + g_i + l_j + g_{lij} + e_{ijk},$$

in which yijk is the observation of the i-th treatment, the j-th location, and the k-th block; μ equals the overall mean of the locations; "g_i" is the fixed treatment effect; l_j is the fixed site effect; $b/l_{k(j)}$ is the random effect of blocks within sites; g_{lij} is the effect of genotype x location interaction; and e_{ijk} is the experimental error effect.
The means were submitted to the Student t test at the same level of significance, when significant differences were observed in the effects of treatment or genotype x location interaction by the F test ($p \leq 0.05$). The software used for the analysis of variances was the SAS (SAS Institute Inc. 2018).

Characterization with microsatellite molecular marker DNA extraction

DNA was extracted from the leaf tissue of plants sown in polystyrene trays, in sheltered screened conditions and collected at 15 days post-emergence. The DNA extraction procedure was based on the CTAB (cetyltrimethylammonium bromide) methodology, described by Doyle and Doyle (1990). DNA was extracted from 50 plants in each population, for a total of 500 plants from ten varieties. DNA quality and quantity were verified by electrophoresis agarose gel and NanoDrop spectrophotometer (Thermo Scientific).

Analysis with microsatellite markers

All individuals (500 plants) were analyzed with six microsatellite primers (umc1071/bin 1.01, phi090/bin

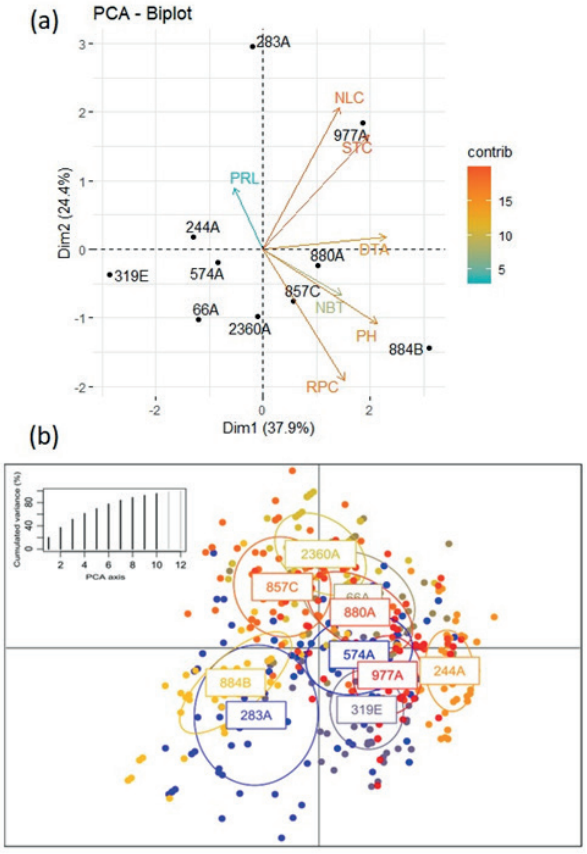

Fig. 1 - PCA Biplot with traits and genotypes (a) and based on six microsatellite loci for the ten local populations of popcorn (b) from Far West Santa Catarina, Southern Brazil.

2.08, phi053/bin 3.05, bnlg565/bin 5.02, umc1653/bin 6.07 and bnlg669/bin 8.03), which were identified by the MaizeGDB website (http://www.maizegdb.org). PCR reactions were performed on a T100 ThermalCycler thermocycler (BioRad), and conditions were adjusted to a final volume of 10 µl, containing 25 ng of genomic DNA, 0.2 mM of each dNTP, 1 U Taq DNA polymerase (Invitrogen), 1x reaction buffer (50 mM KCl, 1.5 mM MgCl2, 100 mM Tris-HCl, pH 9, and 0.1% Triton X-100), and variable primer concentrations (0.165 µM: bnlg565 and bnlg669; 0.33 µM: umc1071, umc1653, phi090 and phi053) according to the optimizing protocol described by Ogliari *et al.* (2000). The amplification conditions consisted of an initial denaturation step of 95°C for 5 minutes, followed by 38 amplification cycles (94°C/30 seconds, 57-67°C/45 seconds, 72°C/30 seconds), and a final extension of 72°C for 7 minutes.

The amplification products were separated by means of 3% agarose gel electrophoresis (AMRESCO's Agarose RA™), with a constant voltage of 80 volts during a 1.5-hour period. The visualization of the gel was made using the Fusion FX6 XT photo documentator (VilberLourmat) and images were analyzed by the FUSION CAPT software.

Statistical analysis of molecular traits

For each locus studied, the number of amplified alleles in each population was quantified, together with the absolute and relative frequencies of heterozygous and homozygous genotypes. Estimates of allele frequencies and descriptive statistics, such as total number of alleles per locus (*A*), mean number of alleles per locus (*Na*), effective number of alleles per polymorphic locus (*Ne*) (Kimura and Crow, 1964), as well as observed mean heterozygosity ($\hat{H}_o$) and expected mean heterozygosity or genetic diversity ($\hat{H}_e$) (Nei 1978), were made by the

$$p = \sum_{i=1}^{a} \frac{nii}{N} + \frac{1}{2} \sum_{(i \neq j)=1} \frac{nij}{N}$$

program GeneAlEx 6.5 (Peakall and Smouse 2012). The polymorphic information content (PIC) was manually calculated using the following formula:

in which *nii* and *nij* are the number of occurrences of the homozygous and heterozygous genotype, respectively, and *N*, the number of samples.

The fixation index (*f*) or inbreeding coefficient, which measures the probability of an individual within the population being an homozygous by ancestry, as well as the Wright F Statistics (*FIS*, *FST*, and *FIT*) for subdivided populations, were calculated using the Fstat program through 1000 resampling (Goudet 2001), resulting in a significant 95% confidence interval.

Genetic and phenotypic distance

The calculation of phenotypic distance was made by the Euclidean Distance estimator and the genetic distances between populations were quantified using the Prevosti Genetic Distance (Prevosti *et al.* 1975). Both distances were calculated using R software through the means of "vegan" package (Oksanen *et al.* 2013) and "adegenet" package (Jombart 2008), respectively.

Dendrograms

From the phenotypic (Euclidian) and genetic (Prevosti) distance matrix, dendrograms were generated using the UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) clustering method and compared on the basis of entanglement between the dendrograms. The dendrograms were generated in the R software, by means of the "dendextend" package (Galili 2015).

Table 3 - Measures of genetic diversity of ten local populations of popcorn based on average data from six microsatellite primers.

Population	*Na*	*Ne*	$\hat{H}o$	$\hat{H}e$	*f*
574A	2.67	2.21	0.579	0.539	-0.075^{ns}
283A	2.50	2.05	0.414	0.563	0.106*
319E	2.83	2.16	0.384	0.498	0.230*
66A	2.83	2.35	0.571	0.567	-0.007^{ns}
2360A	2.67	1.94	0.470	0.466	-0.010ns
884B	2.67	2.29	0.427	0.494	0.137*
244A	2.67	2.10	0.328	0.424	0.230*
857C	2.67	1.98	0.382	0.464	0.179*
880A	2.67	2.37	0.606	0.572	-0.059^{ns}
977A	2.67	1.97	0.456	0.456	0.002^{ns}
Mean[1]	2.68	2.14	0.462	0.494	0.067
SE[2]	0.09	0.08	0.037	0.022	0.055

1Estimated means based on the 10 local populations of popcorn. 2SE: Standard error. Mean number of alleles per locus (*Na*), Effective number of alleles (*Ne*), Observed mean heterozygosity ($\hat{H}o$), Expected mean heterozygosity ($\hat{H}e$), Fixation index or inbreeding coefficient (*f*). Statistical significance (p=0.05): significant (*), not significant (ns).

Results and discussion

Phenotypic evaluations

The differences between genotypes were significant for all variables, showing the high genetic diversity among the accessions under evaluation. The effect of location was significant for almost all variables, with exception of PH, RPC, and PE, for which the probabilities (*p*) by the F test were 0.60, 0.11, and 0.06, respectively. Significant differences were found for the effect of the interaction between genotype x location only for the GY and PE traits, pointing to the requirement of local-specific evaluation for the variety recommendation in the different regions where the study takes place and for the maximization of the expression of two of the most important popcorn characters (Table 2).

PH, RPC, and STC traits, related to the plant architecture and, mainly, to lodging (Duvick 2005), displayed 1.60 m, 0.58, and 14.06 mm means, respectively. For PH, accessions 319E, 574A, 244A, 66A, and 283A showed means below 1.50 m, without differentiating significantly from each other. Between these genotypes with smaller PH, accession 283A stood out for presenting, at the same time, one of the smallest relative position of the insertion of the main cob in the plant, and one of the largest stem thicknesses.

Accession 884B presented the highest number of branches in the tassel (25.1), and accessions 283A and 977A displayed the greatest number of leaves above the cob. This last trait often relates to the plant cycle length, i.e., plants with more leaves tend to have a longer vegetative cycle (Bennetzen and Hake, 2008), such as observed for 977A.

In general, the accessions analyzed demonstrated in mean a long cycle (974 to 1,109 degrees-days to anthesis) according to the Ritchie *et al.* (2003) classification, low prolificacy (0.6 to 0.9 cob plant^{-1}), and, in some cases, infertile plants were found in the plot.

Some genotypes had high genetic potential for the two most relevant traits of popcorn (PE and GY), even without any prior formal breeding. Accession 574A distinguished itself for PE (36.33 mL g^{-1}), and the accessions 283A, 880A and 319E stood out for GY, whose values were 1,905.7, 1,889.6 and 1,669.3 kg ha^{-1}, respectively. Comparing it with a recent study using landrace populations of popcorn in the South of Brazil, these means can be considered satisfactory (Zulkadir *et al.* 2021)

When analyzing the principal components, Figure 1(a), 62.3% of the total phenotypic variation could be grouped in the first two dimensions (components). There is a greater contribution of the STC and NLC variables to the variation between the genotypes and a greater dispersion of them in dimension 1, which encompasses 37.9% of the total variation, having as greater weight the DTA trait. On the basis of these variables, genotypes 319E, 283A, 977A, and 884B are further apart from the others.

Diversity analysis with microsatellite markers

For the six microsatellite loci, a total of 18 alleles were identified, with a mean of 2.68 alleles per polymorphic locus, assuming a DNA sample of 500 plants from ten popcorn populations. The mean PIC value for all six loci was 0.514, and it ranged from 0.375 (phi090 and phi053) to 0.724 (bnlg669). Taking the total mean value into account, the degree of polymorphism detected by

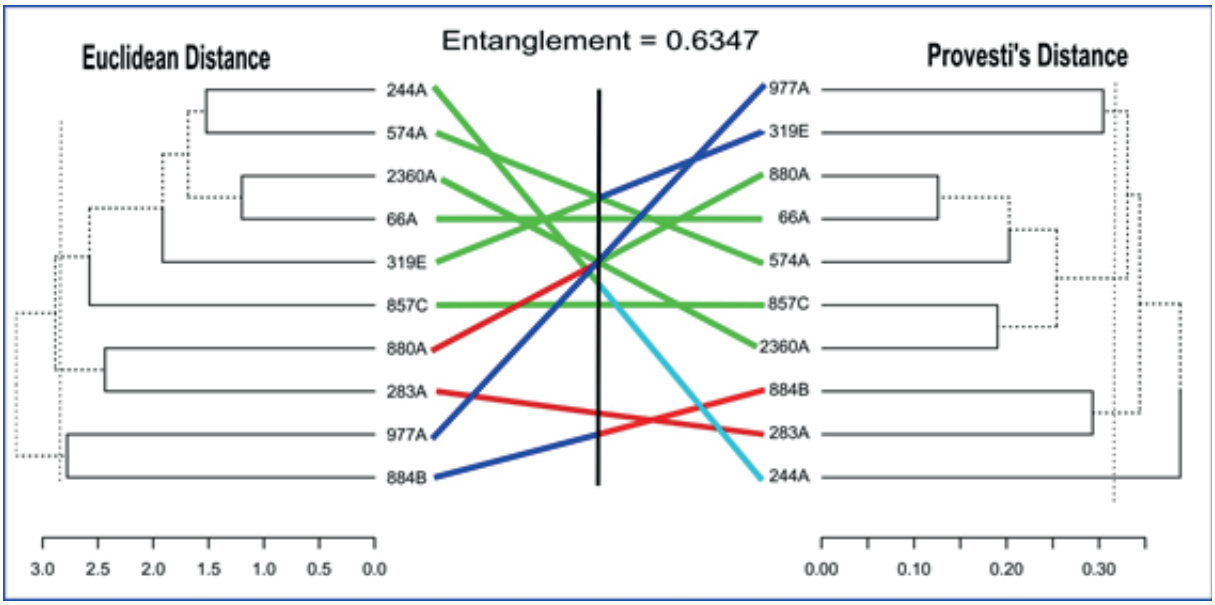

Fig. 2 - Dendrogram based on seven phenotypic variables (ccc=0.823) on the left, and molecular markers (ccc=0.764) on the right. Both were evaluated in 10 local populations of popcorn from Far West of Santa Catarina, Southern Brazil. Ligation of the same color represent the same group of each dendrogram. In cases of ligation with two colors, it corresponds to discrepancy between the dendrograms.

the six microsatellite loci can be considered as moderate. Loci microsatellite identification, annealing temperature, number of alleles and PIC value for each one is provided in Supplementary Table S1. PIC values found herein ranged from 0.37 to 0.72, with a mean value of 0.51, demonstrating a population profile composed of heterozygous genotypes and, mainly, heterogeneous in terms of allele frequencies, independently of the low number of alleles by locus, in relation to other maize landraces.

The estimated values of Na (2.68) for this study are slightly lower than the ones achieved by Pena *et al.* (2016), evaluating 47 superior genotypes of popcorn (Na=2.88); however, they obtained a greater number of total alleles (A= 72), probably because of the greater number of populations and loci of microsatellites studied. Superior values of Na were achieved by Carvalho *et al.* (2013) in a study performed with eight varieties of open pollination of popcorn using 15 loci of microsatellites (Na=4.06). Studies of landraces and native races have reported much higher values of variability. Bracco *et al.* (2009) identified 65 alleles, with 7.22 mean alleles per locus, using nine loci of microsatellites to evaluate six populations of popcorn landraces maintained in indigenous settlements from Northeastern and Northwestern Argentina.

With respect to the individual populations (Table 3), the estimates of the mean heterozygosity observed ($\hat{H}o$) of the six loci ranged from 0.328 to 0.606, indicating a total mean of 0.46. The highest relative frequencies of heterozygous genotypes were reported for populations 880A (H_o = 0.606), 574A (H_o = 0.579), and 66A (H_o = 0.571); the smallest, in turn, were presented by populations 244A (H_o = 0.328), 857C (H_o = 0.382), and 319E (H_o = 0.384). The mean values of the observed and expected heterozygosities, estimated in this study from the ten populations, were similar (H_o = 0.46; He = 0.49), which may indicate, initially, a trend of the population group toward panmixia. It should, however, be taken into account that, individually, some populations have significant deviations from the values H_o and H_e, by which the existence of general levels of equilibrium cannot be completely asserted.

Five populations presented effective allele values above the total mean (Ne = 2.14), with populations 880A, 66A, and 884B standing out from the largest to the smallest for this parameter. The estimated mean fixation index for each population was relatively low, as well as in the mean of the populations (f = 0.067). Four populations (574A, 66A, 2360A, and 880A) showed negative f values; the other ones displayed positive f estimates, which ranged from 0.002 (977A) to 0.230 (319E and 244A). Half of the populations (283A, 319E, 884B, 244A, 857C) presented significant deviations from the genotypic frequencies in relation to the Hardy-Weinberg equilibrium, while the remaining five (574A, 66A, 2360A, 880A, 977A) did not deviate significantly from this condition (Table 3). The low mean fixation value seen for all ten populations (f = 0.067) suggests the lack of strong inbreeding processes in these materials.

Both values are similar to the ones found by Bracco *et al.* (2009), who obtained heterozygosity estimates (H_o = 0.33 and H_e = 0.37) consistent with those expected in the equilibrium condition and fixation estimates, which ranged from 0.008 to 0.28. On the other hand, Pineda *et al.* (2013), using 20 microsatellite primers, detected moderate fixation values (f = 0.17) for a set of 28 populations of maize landraces in Sinaloa state, Mexico. Within these populations, a popcorn accession, known as "Reventador", was evaluated. This accession presented a value He of 0.58 and a fixation index (f) of 0.54. The Wright's F-statistics for each locus are depicted in Table 4. Regarding genetic structure, the study evidenced a moderately high and significant level of differentiation between populations (FST = 0.195), with little differentiation values recorded for umc1653 (FST = 0.067), bnlg565 (FST = 0.074), and umc1071 (FST = 0.086) loci, up to high differentiation values recorded for the phi053 locus (FST = 0.398). Within the populations, the fixation index was relatively low (FIS=0.066), with no statistical significance, basically because of the excess of heterozygotes registered in the umc1071 and umc1653 loci. This parameter (FIS) is related to panmixia deviations resulting from the reproductive system. Differently, the increase in FST homozygosity relates to spatial structuring (subdivision into varieties), forcing matings between individuals with the most relatedness due to finite size sampling; as such, it is not related to the reproductive system. On the other hand, the total fixation index (FIT) encompasses the effects of inbreeding produced by the subdivision and by the reproductive system. This parameter had a relatively higher mean estimate (FIT = 0.249), but it was not significant in relation to the set of populations.

The PCA generated based on molecular information showed a low explanatory power, close to 40%. Nevertheless, it is possible to verify the tendency of isolation of genotype 244A and genotypes 283A and 884B, Figure 1(b), confirming once again that the genotypes 283A and 884B are also further apart from the others by molecular analysis, as observed previously by phenotypic analysis.

Molecular versus phenotypic diversity

Based on the Euclidean Distance for the ten populations from seven phenotypic variables, with a cut in the 2.75 distance, the UPGMA clustering grouped those ten populations into three clusters. The first and largest one, composed by populations 244A, 574A, 2360A, 66A, 319E, and 857C; the second cluster comprised populations 880A and 283A; and the third by populations 977A and 884B (Figure 2). The cophenetic correlation coefficient (ccc) was high (0.823), which demonstrates an excellent correspondence between the distance matrix and the dendrogram (James 1969).

On the basis of the calculation of the Prevosti Genetic Distance coefficients for the ten populations from allele frequencies of six microsatellite primers, UPGMA clustering exhibited the formation of four clusters, with the cutoff point established at 0.32 distance (Figure 2). The first cluster is composed of population 244A; the second, populations 283A and 884B; the third one, populations 2360A, 857C, 574A, 66A, 880A; lastly, the fourth cluster comprises populations 977A and 319E. The ccc was 0.764, which corresponds to a good representation of the distance matrix by the dendrogram. The entanglement calculated was 0.6347, which suggests a low correspondence between the two dendrograms as well as the need to consider both molecular analysis and phenotypic/quantitative evaluations to indicate genetically heterotic groups.

Even though this study provides analyses that may assist in breeding strategies, the low correspondence between phenotypic and molecular distances, represented by dendrograms (Figure 2), indicates that it is necessary to be cautious when they are observed separately. As, in theory, the molecular information is not susceptible to environmental variation and corresponds directly to genetic variation (Schulman 2007), this study recommends, whenever possible, inclusion of this tool as a basis for genetic divergence studies, although complementing with the information of morpho-agronomic characters. However, it is appropriate to bear in mind that diversity information from microsatellite markers is associated with the amplification of non-expressed regions of the genome and that it is also more conservative with regard to variations in DNA (Zhu *et al.* 2005).

In terms of individual diversity values, populations 880A, 574A, and 66A displayed significant potential for developing intrapopulation selection programs as a first breeding strategy, aiming at improving the population *per se*, initially as a result of the high estimates of heterozygosity found in this study (H_o = 0.60; 0.57; and 0.57, respectively). Moreover, these same populations had promising mean values for grain yield (880A, 66A) and expansion capacity, excluding unpopped kernels (880A, 574A). In particular, population 880A differs from others for having expressive means for the two traits of greatest interest in the genetic breeding of popcorn, that is, the expansion capacity and yield. The relevance of these populations in obtaining genetic progress derives from the high performance for attributes of interest in popcorn and from the significant variability of genetic nature to be explored in cyclic schemes of recurrent selection.

In accordance with a second breeding strategy, the performance of studies on genetic divergence is fundamental for the definition of crosses between genetically divergent parents and for obtaining a greater heterotic effect, or even greater genetic variability in segregated generations (Melchinger and Gumber, 1998; Acquaah 2007), in expectation of occurrence of transgressive genotypes. To that end, we suggest a study on the genetic bases of the main attributes of popcorn in order to identify distinct heterotic groups with high specific combining ability and populations with a high general combining ability by means of experiments conducted in the complete diallel scheme. To achieve this first goal, we propose evaluating the progeny resulting from the crosses between accessions 880A, 857C, 66A, 574A 977A, 884B, 283A and 319E. Considering the high phenotypic potential for the GY and PE traits of each group, together with the levels of population divergence and genetic variability within populations, estimated herein, populations 884B (intermediary GY and PE), 574A (highest PE and intermediary GY), 880A (high GY and PE) , 66A (intermediary GY and PE), 977A (high PE), 857C (high PE and intermediary GY), 283A (highest GY), and 319E (high GY) would be interesting materials to include in a breeding program.

Conclusions

The evaluated popcorn landraces showed potential for a breeding program, regarding GY and PE. As criteria for outlining the strategies of breeding, this study recommends molecular analysis as a basis for genetic divergence, complementing with the information of morpho-agronomic traits, due to the low correspondence between phenotypic and molecular distances presented in this work, and considering that, in theory, the molecular information is not susceptible to environmental variation.

From the molecular information, the findings point to the existence of clearly defined heterotic groups and potential genetic diversity for a breeding program. With the help of phenotypic information to determine which populations have the greatest genetic potential and based on molecular information, it was possible to suggest at least two strategies for genetic breeding of popcorn landraces from FWSC based on a participatory approach. Regarding the context of the agricultural ecosystems in this region of the country and on the basis of the results from this study, we suggest, as next steps, the development of inter-varietal hybrids and of open-pollinate improved varieties as two integrated and participatory approaches for the management and use of local diversity by traditional farming communities. Production of inter-varietal hybrids and improved local variety breeding have been strategies frequently demanded by farmers in FWSC and, therefore, it has been included by researchers as good practices of diversity community management within this Brazilian region.

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Conflict of interest

No conflict exists for any author and disclosure of potential conflicts of interest

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