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Agro-morphological and molecular characterization of Argentine maize (*Zea mays* L.) landraces of 'Cristalino Colorado' race

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Abstract: Despite the high variability of Argentine maize (*Zea mays* L.) landraces, they are scarcely used by breeders due to the limited knowledge available about the genetic merit of these materials. In this study, we evaluated agro-morphological and molecular traits of 36 landraces of the 'Cristalino Colorado' race from Buenos Aires province, Argentina. Fifteen agro-morphological traits and five polymorphic microsatellite markers located on different chromosomes (48 alleles) were used. A principal component analysis was performed using average values of agro-morphological traits across two environments. Molecular markers were subjected to a principal coordinate analysis. A generalized procrustes analysis was used to evaluate agro-morphological and molecular traits together, showing seven groups. Distance between agro-morphological and molecular data had an average value of 0.24 and the range varied between 0.02 (ARZM01017) and 0.45 (ARZM01082). The results show that Argentine landraces of the 'Cristalino Colorado' race are a valuable source of new alleles for crop improvement. Studies of this type facilitate the selection of landraces for introduction in genetic breeding programmes and for the establishment of core collections.

Keywords: Genetic variability, Generalized Procrustes Analysis, SSR markers, agro-morphological traits, landraces.

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Introduction

Maize (*Zea mays L.*) landraces originated from long-term cultivation under natural and artificial selection in different environments and under different cultural management schemes (Xiang *et al*, 2010). These landraces maintain high genetic variation and good adaptation to the natural and anthropological environment where they have evolved (Lucchin *et al*, 2003; Di Pasquale *et al*, 2024). The mechanization of agriculture, the increase of urban areas, changes in consumption patterns and production systems has led to the replacement of landraces by improved varieties or hybrids (Pilling *et al*, 2020). Mechanization of agriculture and new market demands forced breeders to generate more uniform and productive crops with stable yield (Esquinas Alcázar, 2005). This homogeneity resulted in an irreversible loss of genetic variability, known as genetic erosion, with the consequent increase of the vulnerability of agricultural crops to future attack by biotic and abiotic stresses (Salhuana *et al*, 1998; Troyer *et al*, 1988; Esquinas Alcázar, 2005).

Genetic resources have long been an important source of new alleles for commercial plant breeding. However, high variability conserved in germplasm banks worldwide is poorly used because breeders prefer crosses among elite inbred lines for their improvement programmes (Vigouroux *et al*, 2008). Extensive exploitation of landraces is hampered by their high heterogeneity, low performance, seed underproduction and negative genetic load (Gorjanc *et al*, 2016).

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Using conserved germplasm in future plant breeding requires systematic evaluation of desired traits (Xiang *et al*, 2010; Balconi *et al*, 2024). For this purpose, there are many descriptors, such as agro-morphological traits and molecular markers, which contribute different and equally important information.

Germplasm characterization and evaluation must be complemented with appropriate statistical analyses to obtain a more complete description of the landraces and establish relations among them. Properly studied and catalogued plant genetic resources can be available for plant breeding programmes (Bramardi, 2023).

In Argentina, as in other countries of the Americas, there is a large diversity of maize types. Argentine maize landraces are classified into 44 races mainly based on specific traits related to ear and grain descriptors, such as shape, colour and texture, and use (Cámara Hernández and Miante Alzogaray, 2003; Solari, 2007). Argentina has a leading role in the production of export maize 'Plata', typical of the 'Cristalino Colorado' race, being currently the only producer of this type of maize worldwide. The kernel of 'Cristalino Colorado' maize is of intense orange colour; the endosperm is mostly hard and glassy in the periphery and floury in the centre, lacking indentation (Secretaría de Agricultura, Ganadería, Pesca y Alimentación, 1997). This kind of grain is widely used in dry milling processes for human consumption and poultry feeding. This grain contains higher carotenoid concentrations than dent corns (Chandler *et al*, 2013); when included in the diet of chicken, it gives a desirable colour to the skin and egg yolks, without the addition of synthetic pigments. Besides, true metabolizable energy values of 'Cristalino Colorado' maize are higher than those of the dent maize due to the higher concentration of oil in the grain. 'Cristalino Colorado' maize can supply the calories required by cattle and pigs, with no need for additional oil in their diet. A 'Cristalino Colorado' hybrid is available in the Argentine market; however, it is of lower quality than traditional genotypes (Paz, 2009).

Previous studies of maize landraces conserved in the Active Germplasm Bank at 'Instituto Nacional de Tecnología Agropecuaria' (INTA) Pergamino (BAP) revealed a high degree of molecular and agro-morphological variability (Salhuana *et al*, 1998; Defacio *et al*, 2005; Paz *et al*, 2005; Defacio, 2009; Paz, 2009; Defacio, 2017; Heck *et al*, 2020; Rivas *et al*, 2022), as well as in disease resistance (Presello *et al*, 1996; Presello *et al*, 2006; Iglesias, 2008; Defacio *et al*, 2018) and grain quality traits (López *et al*, 2005; Heck *et al*, 2019). The aim of the present study was to characterize the variability of 36 maize landraces of 'Cristalino Colorado' race collected from Buenos Aires province, Argentina, based on agro-morphological traits and SSR markers, and the relationship among them.

Materials and methods

Plant material

Thirty-six maize landraces of 'Cristalino Colorado' race conserved at BAP were evaluated. These landraces were collected in Buenos Aires province between 1951 and 1963 (Luna and Safont Lis, 1978). Landrace passport descriptors and races are presented in Table 1. Four synthetic open-pollinated (OP) varieties developed by the INTA Pergamino corn breeding programme, Payagua INTA, Candelaria INTA, SP1234 and BS13p, were included as checks. Payagua INTA and Candelaria INTA have semi-dent endosperm and SP1234 belongs to the 'Cristalino Colorado' race. BS13p has dent endosperm and was developed through recurrent selection applied to BS13.

Agro-morphological characterization

Evaluations were performed in two environments, Pergamino (33°53′01″ S, 60°34′01″ W) and Ferré (34°07'30" S, 61°08'27" W), Buenos Aires province, Argentina, during the 2004/2005 growing season. Both are characterized by typical Argiudol soil, (INTA, 1972). The climate is classified as humid temperate, characterized by annual average rainfall of 1,000mm and average temperature of 16–18 ºC. Figure 1 presents meteorological data recorded as long-term averages (1982–2005) and during the 2004/2005 growing season for the two environments. Minimum and maximum temperatures and long-term precipitation data were obtained from the NASA POWER package in R (Sparks, 2018), while precipitation during the 2004/2005 growing season was manually recorded in the field.

Both trials were conducted using a randomized complete block design with two replications. Each plot was planted in two 5m rows with a spacing of 70cm and 30 plant hills. Standard agronomic practices were followed for successful crop growth.

Fifteen quantitative traits, based on maize descriptor (CIMMYT/IBPGR, 1991) were evaluated: days to anthesis (GDU, growing degree units), days to silk (GDU), anthesis-silking interval (GDU), ear length (cm), ear diameter (mm), number of kernel rows (number), kernels per row (number), kernel width (mm), kernel length (mm), plant height (cm), ear height (cm), plant height/ear height ratio (index), 1,000-kernel weight (g), yield (kg/ha), and prolificacy (index). Phenological traits, 1000-kernel weight, yield and prolificacy were measured on the complete plot. Morphological traits were collected on ten plants per plot, randomly selected in each plot, using the average of the 10 units for performing the analyses. A principal component analysis (PCA) was performed using the standardized data matrix obtained from the arithmetic means of the agro-morphological quantitative variables corresponding to both environments and replications, in order to obtain an average characterization throughout the environments (Zuliani *et al*, 2018). Pearson correlation was computed to assess the relationship among traits prior to PCA.

Molecular characterization

Landraces were evaluated using a set of five public SSR markers (phi080, phi072, phi034, bnlg439 and phi96100) located on different chromosomes, with a high degree of polymorphism. Oligonucleotide sequences are publicly available at the Maize Data Bank (www.agron.missouri.edu/Coop/SSR-Probes/SSR1.html). DNA was extracted from young leaves of 25 plants per landrace, according to Kleinhofs *et al* (1993). PCR reactions were carried out in a MJ Research PTC-100 thermocycler (USA). The amplification products were visualized in 6% polyacrylamide gels and were

Table 1. Landrace passport descriptors and races.

Identifier	Location	Department	Altitude (masl)	Latitude	Longitude	Race
ARZM01001	Acevedo	Pergamino	70	33°46' S	60°27' W	Cristalino Colorado
ARZM01002	Rancagua	Pergamino	69	34°02' S	60°30' W	C. Colorado – C. Amarillo
ARZM01003	Rancagua	Pergamino	69	34°02' S	60°30' W	Cristalino Colorado
ARZM01005	Arroyo Dulce	Salto	75	34°06' S	60°24' W	Cristalino Colorado
ARZM01006	Tacuarí	Salto	69	34°13' S	60°19' W	C. Colorado – C. Amarillo
ARZM01007	Salto	Salto	51	34°18' S	60°15' W	Cristalino Colorado
ARZM01008	Salto	Salto	51	34°18' S	60°15' W	Cristalino Colorado
ARZM01012	Arenales	General Arenales	84	34°19' S	61°18' W	C. Colorado – C. Amarillo
ARZM01013	Rojas	Rojas	69	34°12' S	60°44' W	C. Colorado – C. Amarillo
ARZM01014	Chacabuco	Chacabuco	69	34°38' S	60°29' W	Cristalino Colorado
ARZM01015	Salto	Salto	51	34°18' S	60°15' W	Cristalino Colorado
ARZM01016	Arroyo Burgos	Bartolomé Mitre		34°04' S	60°07' W	Cristalino Colorado
ARZM01017	San Pedro	San Pedro	27	33°42' S	59°41' W	C. Colorado – C. Amarillo
ARZM01022	Ortiz Basualdo	Pergamino	64	34°03' S	60°39' W	C. Colorado
ARZM01025	Hunter	Rojas	50	34°15' S	60°32' W	C. Colorado – C. Amarillo
ARZM01026	Ferré	General Arenales	88	34°08' S	61°08' W	Cristalino Colorado
ARZM01027	Carabelas	Rojas	83	34°03' S	60°52' W	Cristalino Colorado
ARZM01028	Colón	Colón	90	33°59' S	61°06' W	Cristalino Colorado
ARZM01030	Conesa	San Nicolás	58	33°36' S	60°22' W	Cristalino Colorado
ARZM01033	El Paraíso	Ramallo	33	33°34' S	59°59' W	C. Colorado – C. Amarillo
ARZM01036	Conesa	San Nicolás	58	33°36' S	60°22' W	Cristalino Colorado
ARZM01039	Rancagua	Pergamino	69	34°02' S	60°30' W	Cristalino Colorado
ARZM01044	Chivilcoy	Chivilcoy	55	34°54' S	60°01' W	Cristalino Colorado
ARZM01048	La Violeta	Pergamino	55	33°44' S	60°11' W	Cristalino Colorado
ARZM01058	Chivilcoy	Chivilcoy	55	34°54' S	60°01' W	C. Colorado – C. Amarillo
ARZM01062	Chacabuco	Chacabuco	69	34°38' S	60°29' W	C. Colorado – Amarillo Ocho Hileras
ARZM01066	Rojas	Rojas	69	34°12' S	60°44' W	Cristalino Colorado
ARZM01082	Nueva Roma	Torquinst	285	38°06' S	62°14' W	Cristalino Colorado
ARZM01086	Nueva Roma	Torquinst	285	38°06' S	62°14' W	Cristalino Colorado
ARZM01087	Nueva Roma	Torquinst	285	38°06' S	62°14' W	Cristalino Colorado
ARZM01092	Pigüé	Saavedra	298	37°41' S	62°24' W	Cristalino Colorado
ARZM01096	Coronel Suárez	Coronel Suárez	234	37°28' S	61°56' W	Cristalino Colorado
ARZM01102	Carhue	Adolfo Alsina	112	37°11' S	62°45' W	Cristalino Colorado
ARZM01124	Trenque Lauquen	Trenque Lauquen	96	35°58' S	62°44' W	Cristalino Colorado
ARZM01151	Mones Cazón	Pehuajó	88	35°48' S	61°53' W	Cristalino Colorado
ARZM01152	Carlos Tejedor	Carlos Tejedor	96	35°23' S	62°25' W	Cristalino Colorado

detected by silver nitrate staining (Benbouza *et al*, 2006). The relative allelic frequencies were calculated using the direct counting method from the individual genotypes found in each landrace.

The Prevosti distance (Prevosti, 1974) was calculated from the relative allelic frequencies of the molecular markers for each landrace to infer the relationship between landraces using a principal coordinate analysis (PCoA). The allele number was determined for each group.
Subsequently, expected heterozygosity (He) was calculated for each group identified in the PCoA, which was calculated using the following equation:

$$He = 1 - \sum_{i=1}^{k} p_i^2$$

where, p_i is the frequency of the $i^{\text{-th}}$ allele, and k is the number of alleles.

Joint analysis

For joint analysis, generalized procrustes analysis (GPA) (Gower, 1975) was performed. This method gets a better adjustment for the information provided by both PCA and PCoA. A consensus configuration was performed and represented in a two-dimensional space. A minimum spanning tree (MST) from the Euclidean matrix obtained from the first

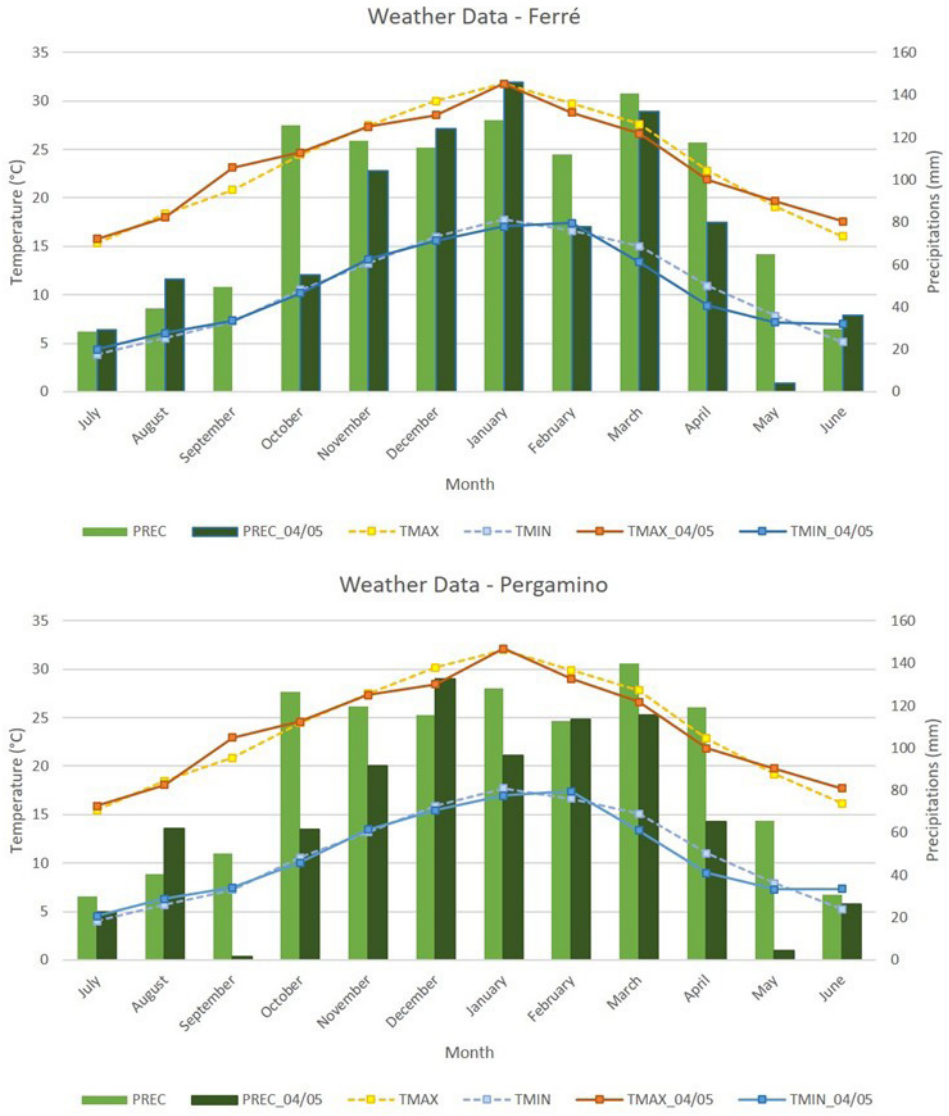

Figure 1. Meteorological data recorded as long-term averages and during the 2004/2005 growing season in Ferré (a) and Pergamino (b), Buenos Aires province, Argentina. PREC, long-term precipitations; PREC_04/05, precipitations in 2004/2005 growing season; TMAX, long-term maximum temperatures; TMAX_04/05, maximum temperatures in 2004/2005 growing season; TMIN, long-term minimum temperatures; TMIN_04/05, minimum temperatures in 2004/2005 growing season.

two GPA coordinates was added in the principal plane.

A Mantel test was performed to quantify the relationship between the molecular, agro-morphological and consensus distance matrix.

To determine concordance of molecular and agro-morphological characterizations at the landrace level, we calculated the Euclidean distance between two analogous points, i.e. points corresponding to a single landrace in the agro-morphological and molecular configurations.

All statistical analyses were carried out using the NTSYS programme (Numerical Taxonomic System ver. 2.11) (Rohlf, 2002).

Results and discussion

Agro-morphological characterization

Table 2 presents the mean values, standard errors, and ranges for each trait, calculated across landraces in each environment under evaluation.

In the Ferré 2004/2005 growing season, plants exhibited shorter height, a shorter anthesis-silking interval, and higher grain yields. These differences may be attributed to the higher precipitation levels recorded in Ferré during January, which match with the critical period for determining grain yield (30 days centred around flowering (Fischer and Palmer, 1984)). Maize is particularly sensitive during this stage, and any stress can increase the anthesis-silking interval, potentially leading to pollination failure and grain yield loss (Tao *et al*, 2023). The other traits did not show differences between the two environments.

The correlation matrix (Table 3) revealed strong and significant ($p < 0.01$) correlations between several traits: days to silking and days to anthesis ($r = 0.88$), plant height and ear height ($r = 0.87$), yield and ear diameter ($r = 0.79$), ear height and plant height/ear height ($r = -0.78$), yield and ear length ($r = 0.74$), and kernel length and ear diameter ($r = 0.73$). Similar correlations among these traits have been reported by other authors during the evaluation of maize landraces (Defacio, 2009; Javed *et al*, 2021; de Faria *et al*, 2022).

Table 2. Mean values, standard errors (S.E.), and ranges for each trait evaluated in two environments.

Environment	**Pergamino 2004/2005**			**Ferré 2004/2005**		
Trait	**Mean**	**S.E.**	**Range**	**Mean**	**S.E.**	**Range**
Ear length (cm)	15.99	0.14	13.10–19.50	16.56	0.11	14.00–18.70
Ear diameter (mm)	41.46	0.31	33.40–48.70	42.04	0.30	34.50–48.10
Number of kernel rows	13.16	0.17	10 .00–16.60	13.09	0.15	9.80–16.40
Kernel width (mm)	8.48	0.09	6.00–9.80	8.77	0.07	7.20–10.20
Kernel length (mm)	8.19	0.12	5.40–12.00	10.11	0.10	8.00–12.00
Kernels per row	31.25	0.40	21.60–38.40	34.90	0.28	29.90–40.00
Prolificacy (index)	0.94	0.02	0–1.36	1.01	0.01	0.66–1.33
1,000-kernel weight (g)	281.30	3.78	174.00–355.00	285.67	3.31	201.00–346.00
Yield (Kg/ ha)	5576.90	176.80	1341.10–9440.80	6838.30	172.90	2623.80–11561.30
Plant height (cm)	163.98	1.56	122.50–193.00	143.42	1.18	118.50–165.50
Ear height (cm)	96.88	1.22	68.00–117.50	84.20	1.10	65.00–106.50
Plant height/ear height ratio (index)	1.70	0.01	1.52–2.03	1.71	0.01	1.43–2.09
Days to anthesis (GDU)	1032.30	3.86	944.70–1100.15	903.23	3.49	837.60–1017.55
Days to silking (GDU)	1069.75	3.09	991.10–1143.75	974.92	5.10	895.90–1089.80
Anthesis-silking interval (GDU)	37.45	1.79	12.80–108.12	71.69	2.91	15.00–155.30

Table 3. Correlation matrix between evaluated traits. EL, ear length; ED, ear diameter; NKR, number of kernel rows; KW, kernel width; KL, kernel length; KR, kernels per row; HKW, 1,000-kernel weight; PH, plant height; EH, ear height; PH/EH, plant height/ear height ratio; PROL, prolificacy; DA, days to anthesis; DS, days to silking; ASI, anthesis–silking interval; NS, non-significant ($p > 0.05$); *, significant at $p < 0.05$; **, significant at $p < 0.01$.

	EL	**ED**	**NKR**	**KW**	**KL**	**KR**	**HKW**	**Yield**	**PH**	**EH**	**PH/ EH**	**PROL**	**DA**	**DS**	**ASI**
EL	1	**	NS	NS	**	**	**	**	**	NS	*	NS	NS	NS	NS
ED	0.56	1	**	NS	**	NS	**	**	NS	NS	NS	NS	NS	NS	NS
NKR	0.12	0.49	1	**	*	NS	NS	*	*	NS	NS	NS	NS	NS	NS
KW	0.16	-0.08	-0.46	1	NS	NS	*	NS	**	*	NS	*	NS	NS	NS
KL	0.51	0.73	0.36	0.10	1	NS	**	**	NS	NS	NS	NS	NS	NS	NS
KR	0.61	0.10	-0.17	0.18	0.26	1	NS	NS	*	NS	NS	NS	NS	NS	NS
HKW	0.62	0.64	0.09	0.38	0.66	0.11	1	**	*	NS	NS	*	NS	NS	NS
Yield	0.74	0.79	0.34	0.05	0.70	0.27	0.75	1	NS	NS	NS	**	NS	NS	NS
PH	0.46	0.15	-0.32	0.50	0.20	0.40	0.43	0.28	1	**	*	NS	NS	NS	NS
EH	0.23	0.02	-0.28	0.41	0.11	0.27	0.26	0.11	0.87	1	**	NS	*	*	NS
PH/EH	0.14	0.11	0.10	-0.13	0.03	-0.02	0.04	0.11	-0.37	-0.78	1	NS	**	**	NS
PROL	0.27	0.24	-0.16	0.33	0.27	0.10	0.35	0.44	0.22	0.07	0.14	1	NS	NS	NS
DA	-0.04	-0.03	-0.07	0.06	0.18	0.09	-0.11	-0.11	0.25	0.43	-0.50	-0.01	1	**	NS
DS	-0.02	0.04	-0.09	0.11	0.10	-0.02	-0.04	-0.09	0.29	0.43	-0.45	0.10	0.88	1	**
ASI	0.04	0.15	-0.06	0.12	-0.13	-0.21	0.13	0.03	0.14	0.10	-0.01	0.22	-0.03	0.45	1

Table 4. Axis loadings corresponding to PC1 and PC2

Trait	PC1	PC2
Ear lenght	0.8324	0.0588
Ear diameter	0.7851	0.3346
Number of kernel rows	0.2442	0.6234
Kernel width	0.2345	-0.5806
Kernel lenght	0.7884	0.1752
Kernels per row	0.4068	-0.2193
Prolificacy	0.3596	-0.1912
1,000-kernel weight	0.8402	0.0398
Yield	0.8861	0.2641
Plant height	0.5499	-0.6681
Ear height (cm)	0.3948	-0.7835
Plant height/ear height ratio	-0.0741	0.6170
Days to antesis	0.1024	-0.5616
Days to silking	0.1209	-0.6186
Anthesis-silking interval	0.0623	-0.2467

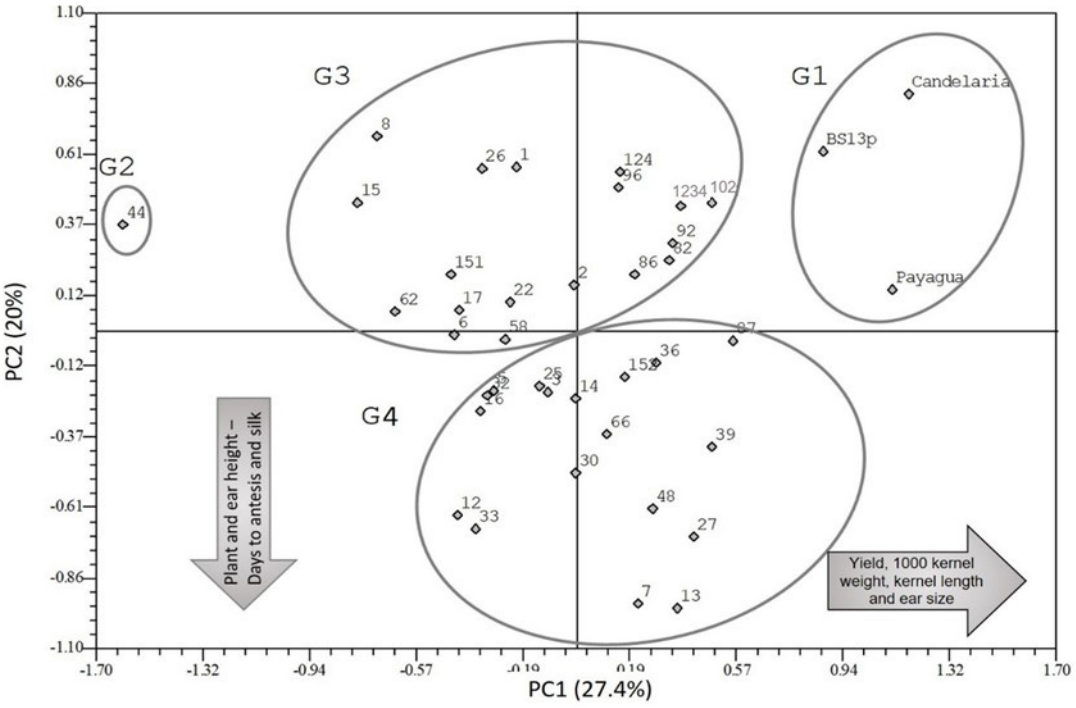

Figure 2. Principal Component Analysis of agro-morphological traits. Landraces are identified with the last numbers in their identifier (e.g. 13 corresponds to ARZM1013).

Results from the PCA (Figure 2) show that the first and second principal components (PC1 and PC2, respectively) accounted for 27.42% and 20.86% of the total variation, respectively. The axis loadings corresponding to PC1 and PC2 are shown in Table 4. PC1 was positively and strongly associated with yield, 1000-kernel weight, kernel length, ear diameter and ear length. PC2 was negatively and moderately associated with plant architecture traits (plant height and ear height), days to anthesis, and days to silking.

Landraces were classified in four groups by PCA, based on the distances observed between individuals in the direction of both established gradients.

G1. This group included BS13p, Candelaria INTA and Payagua INTA. These OP varieties were associated with the highest values of yield and its components, as well as shorter plant and ear height and fewer days to anthesis and silking than the rest of the evaluated landraces. This result agrees with the fact that these genotypes were selected for yield purposes.

G2. Represented by only one accession (ARZM01044) that showed the lowest yield and the smallest ear, grain size and 1,000-kernel weight of all landraces. This accession presented medium to low plant height and intermediate to fewer days to anthesis and silking.

G3. This group included landraces with average yield, 1,000-kernel weight, kernel length, ear diameter, and ear length, displaying shorter plants, lower ear height and fewer days to anthesis and silking than the G4 cluster.

G4. Represented by landraces with average yield, 1,000-kernel weight, kernel length, ear diameter and ear length, and highest values for days to anthesis and silking, high plant and ear height.

Molecular characterization

In this study, a set of five SSR markers was employed for the preliminary molecular characterization of maize landraces. Other authors (Di Pasquale *et al*, 2024; Joshi *et al*, 2020; Ignjatović Micić *et al*. 2013) have also used a low number of SSRs, ranging from 5 to 10, to evaluate maize landraces.

A total of 48 alleles were detected. The overall number of alleles per locus varied from 6 (phi034 and phi072) to 21 (bnlg439), with an average of 9.6 (Table 5). Six alleles were unique to landraces (unique or private alleles) while five other different alleles were present in two landraces (rare alleles).

The average number of alleles per locus obtained from landraces (9.6) was higher than the values reported by Reif *et al* (2003) (5.9), Warburton *et al* (2002) (6.3), Labate *et al* (2003) (6.5) and Di Pasquale *et al* (2024) (7.4), but lower than those reported by Barcaccia *et al* (2003) (20.75), Rivas *et al* (2022) (19.05) and Torres-Morales *et al* (2023) (25.39).

In the OP varieties, the assayed loci scored a mean number of alleles equal to 7.6, lower than landraces (9.6). This result is consistent with that obtained by Barcaccia *et al* (2003) of 10.25 vs. 19.75, showing that even though OP varieties have genetic variability, they originated from a narrow genetic base.

First and second principal axes of PCoA (Figure 3) accounted for 10.79 and 8.96% of the total variation, respectively. Landraces were distributed in four groups based on their relative distances on the principal plane, which differed from those obtained using PCA.

Table 5. Numbers of alleles per locus across landraces

SSR markers	No. of alleles
phi080	8
phi072	6
phi034	6
phi96100	7
bnlg439	21
Average	9.6

G1. Represented by ARZM01082 and ARZM01086 landraces.

G2. This group included ARZM01124, ARZM01102, ARZM01152, BS13p and Candelaria INTA.

G3 and G4. These groups included most of the evaluated landraces and were separated by the dispersion of the second principal coordinate (PCO2).

This clustering was not associated with the presence of rare or private alleles. Some landraces exhibited rare or private alleles (ARZM01003, ARZM01049, ARZM01102 and SP1234) but were clustered with other landraces.

Allele numbers for five SSR markers and expected heterozygosity were calculated for each group identified by the PCoA (Figure 4).

The number of alleles observed varied among groups, increasing from G1 to G4 in parallel with the number of landraces included in each group. This pattern suggests greater genetic variability in G4, in concordance with the broader dispersion of landraces observed for this group in the PCoA (Figure 3). The expected heterozygosity (He) also varied among groups, but no clear relationship was observed with the total number of alleles.

Joint analysis

The correlation between agro-morphological and molecular data matrices was very low and not significant (r = 0.07, p-value = 0.77). The different configurations obtained with both types of variables indicate that individual characterization offers additional information that can be used complementarily to know the genetic variability among landraces. Low correlation values between agronomic and molecular traits were found in 41 varieties of cucumber (*Cucumis sativus L.*) (Bramardi *et al*, 2005), in 37 Patagonian isolates of yeast (*Saccharomyces cerevisiae*) (Lopes *et al*, 2006), in 57 red clover landraces (*Trifolium pratense* L) (Dias *et al*, 2008), and in a set of banana (*Musa sp*.) clones (Ermini *et al*, 2016). For this reason, it is necessary to use a technique that gathers molecular and agro-morphological information.

According to the GPA results (Figure 5), seven groups of landraces were identified.

Some landraces denoted a high correspondence between molecular and agro-morphological characterizations. However, most landraces denoted a great discordance between agro-morphological and molecular data. Distance between both types of data presents a range between 0.02

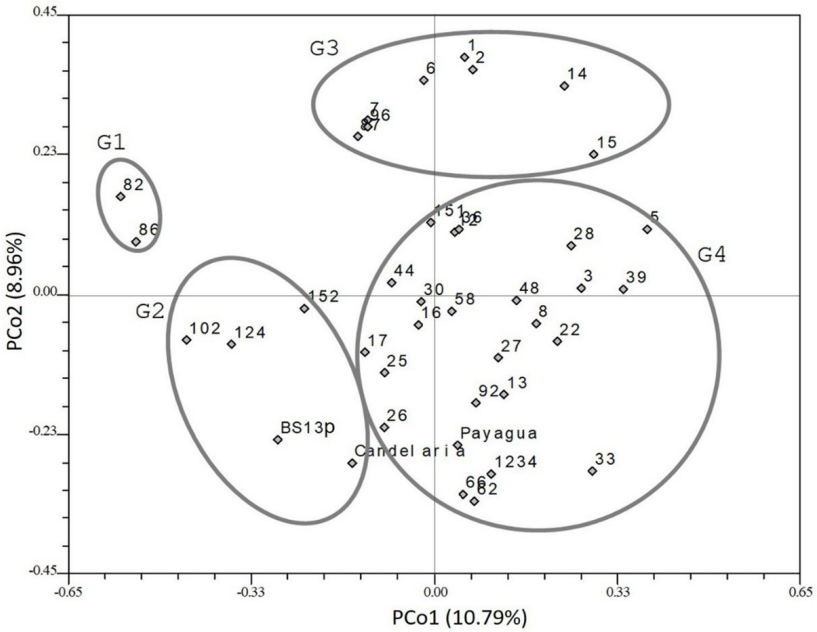

Figure 3. Principal coordinate analysis of molecular traits. Landraces are identified with the last numbers in their identifier (e.g. 13 corresponds to ARZM1013).

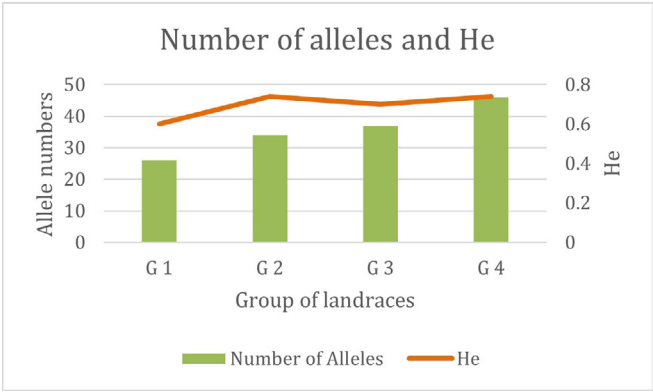

Figure 4. Allele numbers for five SSR markers and expected heterozygosity (He) for each group identified by the PCoA

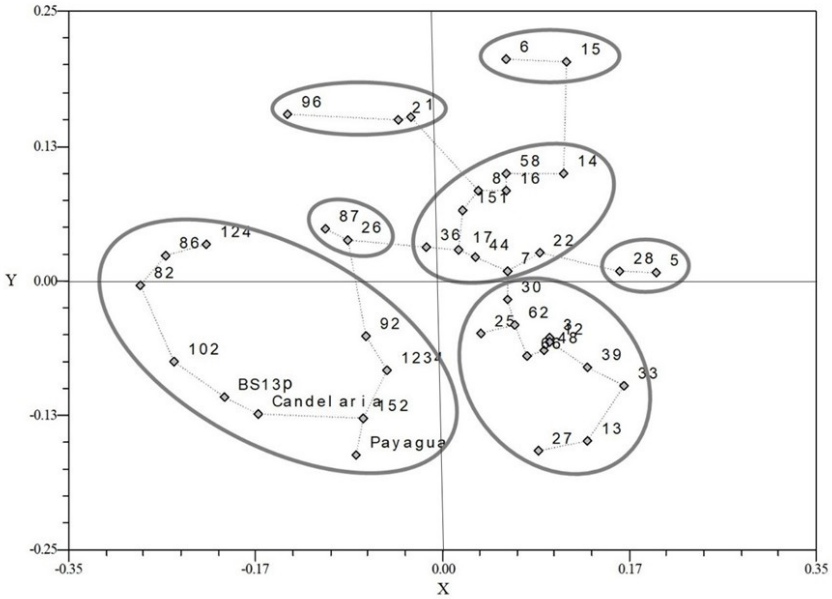

Figure 5. Generalized procrustes analysis of agro-morphological and molecular traits in the first two axis with Minimum Spanning Trees. Landraces are identified with the last numbers in their identifier (e.g. 13 corresponds to ARZM1013).

(ARZM01017) and 0.45 (ARZM01082), with an average of 0.24 (Table 6). The discrepancies observed between agro-morphological and molecular markers may be explained by the fact that the SSR used in this work are neutral and not linked to the agro-morphological traits under evaluation. Expression of these phenotypic traits is strongly influenced by environmental conditions and the selection performed by farmers according to local preferences. This selection contributed to phenotypic differentiation, which may not be reflected in neutral genomic regions, such as those assessed by SSR markers (Javed *et al*, 2021).

Table 6. Distance between two analogous points, i.e. points corresponding to a single landrace in the agro-morphological and molecular configurations

Identifier	Distance between molecular and agro-morphological traits
ARZM01001	0.26
ARZM01002	0.27
ARZM01003	0.21
ARZM01005	0.27
ARZM01006	0.23
ARZM01007	0.37
ARZM01008	0.24
ARZM01012	0.32
ARZM01013	0.42
ARZM01014	0.10
ARZM01015	0.19
ARZM01016	0.06
ARZM01017	0.02
ARZM01022	0.17
ARZM01025	0.14
ARZM01026	0.30
ARZM01027	0.37
ARZM01028	0.23
ARZM01030	0.15
ARZM01033	0.40
ARZM01036	0.06
ARZM01039	0.31
ARZM01044	0.08
ARZM01048	0.24
ARZM01058	0.11
ARZM01062	0.24
ARZM01066	0.24
ARZM01082	0.45
ARZM01086	0.42
ARZM01087	0.25
ARZM01092	0.21
ARZM01096	0.43
ARZM01102	0.26
ARZM01124	0.37
ARZM01151	0.08
ARZM01152	0.23
BS13P	0.16
Candelaria INTA	0.16
Payagua INTA	0.17
SP 1234	0.26

Consensus configuration grouped the four OP varieties and the six landraces. Interestingly, ARZM01044 formed a group by itself when evaluated by agro-morphological traits and was included in a group with a larger number of landraces when evaluated by molecular markers and in consensus analysis. ARZM01082 and ARZM01086 landraces were assigned to a separate group, according to molecular markers. However, according to agro-morphological traits, these landraces were grouped with other landraces. In GPA, these landraces were grouped with OP varieties. A similar situation was observed with checks Candelaria INTA, Payagua INTA and BS13p, which formed a distinct group according to agro-morphological analysis but grouped together with other landraces according to the molecular markers and GPA. The correlations among the three distance matrices (molecular, agro-morphological and consensus) were estimated using a Mantel test. A greater correlation was found between the consensus and the molecular and agro-morphological characterization (0.20 and 0.47, respectively) than between molecular and agro-morphological characterization (0.07). This result indicates that GPA allows the simultaneous characterization of a set of accessions with agro-morphological traits and SSR markers. There is no unique pattern of association among landraces, which emphasizes the importance of studying the different descriptors jointly to obtain the best description and interpretation of genetic diversity.

In conclusion, both agro-morphological and molecular variation were detected among the studied landraces, highlighting the importance of integrating both types of characterization to evaluate genetic diversity. GPA is a powerful statistical technique to align genetic and agro-morphological descriptors. Increasing the knowledge of the available genetic diversity in maize germplasm will facilitate the establishment of core collections. Furthermore, integrating agronomic performance with genetic data is critical to developing and optimizing future breeding strategies. Currently, maize breeding relies on a narrow genetic base. Incorporating landraces into crosses with elite varieties offers a promising approach to introduce novel alleles and broaden the genetic base of maize breeding. Moreover, the local adaptation exhibited by landraces represents a valuable source of germplasm for future needs in sustainable agriculture, particularly in the context of climate change.

To enhance the understanding of the genetic diversity of the conserved landraces, it is recommended to incorporate more molecular markers as well as landraces from other races and origins.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Raquel Defacio, Natalia Paz and Sergio Bramardi. The first draft of the manuscript was written by Raquel Defacio and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability statement

Accession-level data are available from the corresponding author upon reasonable request.

Conflict of interest statement

The authors have no competing interests to declare that are relevant to the content of this article.

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