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Genetic diversity and diallel analysis of elite popcorn lines[1]

Diversidade genética e análise dialélica de linhagens elite de milho pipoca

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ABSTRACT – Popcorn is an important agricultural crop that is consumed as a snack food worldwide. In Brazil, the lack of cultivars with high potential grain yield and popping expansion volume is a vital problem. Therefore, the present study aimed to evaluate the genetic divergence among elite popcorn lines using amplified fragment length polymorphism (AFLP) markers to estimate the general and specific combining ability (GCA and SCA, respectively), and correlate the genetic distance with the SCA and hybrid means. For the genetic divergence study, 45 popcorn inbred lines of different hybrids and varieties were evaluated. Subsequently, 10 inbred lines were selected for diallel analysis; grain yield (GY) and popping expansion (PE) were evaluated in three environments. A wide variability of popcorn inbred lines was observed based on the AFLP markers. After evaluating the hybrids, a significant effect was observed for GY and PE, with some experimental hybrids with higher potential GY and PE than the commercial cultivars. From the diallel analysis, a significant effect was observed for GCA for GY and PE; therefore, the lines differed in the frequency of favorable alleles, with promising inbred lines for forming hybrids or synthetic varieties. However, for SCA, no significant effect was observed for these traits. Genetic distance did not correlate with GY and $\hat{S}_{ij}$, demonstrating an absence of consistency in the prediction of heterotic groups for popcorn using AFLP markers.

Key words: *Zea mays* everta Sturt.. Molecular marker. AFLP. Combining ability.

RESUMO – O milho pipoca é uma importante cultura agrícola, consumida como petisco pela população mundial. No Brasil, a falta de cultivares com alto potencial de rendimento de grãos e elevado volume de expansão é considerada uma das principais dificuldades. Nesse contexto, o presente trabalho teve como objetivo avaliar a divergência genética entre linhagens elite de milho pipoca por meio de marcadores AFLP (Amplified Fragment Length Polymorphism), estimar a capacidade geral e específica de combinação (GCA e SCA, respectivamente) e correlacionar a distância genética com a SCA e a média dos híbridos. Para o estudo de divergência genética, foram avaliadas 45 linhagens de milho pipoca provenientes de diferentes híbridos e variedades. Posteriormente, dez linhagens foram selecionadas para análise dialélica; o rendimento de grãos (GY) e a capacidade de expansão (PE) foram avaliados em três ambientes. Com base nos marcadores AFLP, foi observada uma ampla variabilidade entre as linhagens avaliadas. Na avaliação dos híbridos, foi observado efeito significativo para GY e PE, sendo que alguns híbridos apresentaram alto potencial de GY e PE quando comparados às testemunhas comerciais. Por meio da análise dialélica, foi observado efeito significativo da GCA para GY e PE, indicando que as linhagens diferem na frequência de alelos favoráveis, com linhagens promissoras para a formação de híbridos ou variedades sintéticas. Por outro lado, para SCA, nenhum efeito significativo foi observado para essas características. A distância genética não se correlacionou com GY e $\hat{S}_{ij}$, demonstrando ausência de consistência na predição de grupos heteróticos para milho pipoca via marcadores AFLP.

Palavras-chave: *Zea mays everta* Sturt.. Marcadores moleculares. AFLP. Capacidade combinatória.

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INTRODUCTION

Popcorn (*Zea mays* everta Sturt.) is a special type of corn that is consumed worldwide and has small, hard kernels that burst when heated. Based on archeological and molecular evidence, popcorn is one of the oldest types of corn (MANGELSDORF, 1949). However, the genetic basis of the main cultivars used today is narrow; therefore, knowledge of heterotic patterns is extremely important (de CARVALHO *et al*., 2013).

For hybrid development, knowledge of the heterotic patterns among genetically different groups is crucial for maximum heterosis exploration (HALLAUER; CARENA; MIRANDA, 2010; BADU-APRAKU *et al*., 2016). The term heterotic groups refers to groups of related or non-related genotypes of similar or different populations that present a combining ability and heterotic response when crossed with genotypes from other genetically distinct groups (GUPTA *et al*., 2020).

For popcorn, heterotic groups are not well defined. Santacruz-Varela *et al*. (2004) evaluated different popcorn populations from the USA and Latin America based on morphological and molecular descriptors and observed the formation of three main groups: i) Yellow Pearl, which is an important source for commercial production in the USA, ii) Pointed Rice, which probably gave rise to the complex of traditional popcorn races in Latin America, and iii) North American Early, which revealed genetic traces to Flint corn. In contrast, Blanco *et al*. (2005) evaluated the Iowa State University popcorn germplasm and defined three heterotic groups: i) Amber Pearl, ii) South American, and iii) Supergold.

Genotypes can be classified into heterotic groups based on their genealogy, genetic-quantitative analysis, heterosis, and molecular data (HALLAUER; CARENA; MIRANDA, 2010; BERNARDO, 2020). Among genetic-quantitative analyses, diallel analysis is an efficient strategy for defining genotypes in heterotic groups (BADU-APRAKU *et al*., 2013; LAUDE; CARENA, 2014; COELHO *et al*., 2020). This analysis estimates genetic components and their interactions with the environment, facilitating the determination of heterotic groups. However, this procedure is restrictive because of the limited number of genotypes that can be crossed and evaluated. In this context, the application of molecular markers can be used as an additional tool for the definition of heterotic groups because they provide reliable measures of genetic diversity and, therefore, can be used to determine genealogy (AKINWALE *et al*., 2014; MUNDIN *et al*., 2015; KULKA *et al*., 2018; SILVA *et al*., 2019).

The aims of the present study were to evaluate the genetic divergence among elite popcorn inbred lines from the genetic breeding program at the Universidade Estadual de Maringá (UEM) using amplified fragment length polymorphism (AFLP) markers, estimate the combining ability among inbred lines using diallel analysis, and correlate the genetic distance estimated by the molecular marker with the specific combining ability and the average of the obtained hybrids.

MATERIAL AND METHODS

Plant material

A total of 45 elite popcorn lines from the UEM breeding program were evaluated from different hybrids and varieties grown in Brazil (Table 1). For molecular evaluation, these lines were sown in plastic pots in a greenhouse at the UEM. Approximately 15 days after sowing, young leaves were collected from 20 individuals from each line for genomic DNA extraction.

Table 1 – Identification, name, and origin of the 45 popcorn inbred lines from the Universidade Estadual de Maringá germplasm collection

Identification	Inbred lines	Origin	Plant material / Institution
L1	GP1	Zélia	Triple–cross hybrid / Pioneer
L2	P1.2	Zélia	Triple–cross hybrid / Pioneer
L3	P1.3	Zélia	Triple–cross hybrid / Pioneer
L4	P1.17	Zélia	Triple–cross hybrid / Pioneer
L5	P1.19	Zélia	Triple–cross hybrid / Pioneer
L6	GP3	CMS–42	Composite variety of Embrapa
L7	P3.1.2	CMS–42	Composite variety of Embrapa
L8	GP4	CMS–43	Composite variety of Embrapa
L9	P4.4	CMS–43	Composite variety of Embrapa
L10	GP5	UEM–J1	Open pollinated variety of UEM

Continuation Table 1

L11	P5.1	UEM–J1	Open–pollinated variety of UEM
L12	P6.1	Catedral	Open–pollinated variety of UEM
L13	P7.2.4	UEM–M2	Open–pollinated variety of UEM
L14	P7.4.11	UEM–M2	Open–pollinated variety of UEM
L15	P7.17.1	UEM–M2	Open–pollinated variety of UEM
L16	P8.1.1	Zaeli	Unknown genealogy
L17	P8.1.5.5	Zaeli	Unknown genealogy
L18	P8.1.5.9	Zaeli	Unknown genealogy
L19	P8.1.5.10	Zaeli	Unknown genealogy
L20	P8.2 M	Zaeli	Unknown genealogy
L21	P8.2.2.4	Zaeli	Unknown genealogy
L22	P8.2	Zaeli	Unknown genealogy
L23	P9.1	IAC–112	Modified single–cross hybrid / IAC
L24	P9.1.1	IAC–112	Modified single–cross hybrid / IAC
L25	P9.1.5	IAC–112	Modified single–cross hybrid / IAC
L26	P9.1.6	IAC–112	Modified single–cross hybrid / IAC
L27	P9.2.3	IAC–112	Modified single–cross hybrid / IAC
L28	P9.4.1	IAC–112	Modified single–cross hybrid / IAC
L29	P9.4.6	IAC–112	Modified single–cross hybrid / IAC
L30	P9.5.1	IAC–112	Modified single–cross hybrid / IAC
L31	P9.5.2	IAC–112	Modified single–cross hybrid / IAC
L32	P9.6.3	IAC–112	Modified single–cross hybrid / IAC
L33	P9.6.1	IAC–112	Modified single–cross hybrid / IAC
L34	P9.7.3	IAC–112	Modified single–cross hybrid / IAC
L35	P9.10.1	IAC–112	Modified single–cross hybrid / IAC
L36	P9.11.1	IAC–112	Modified single–cross hybrid / IAC
L37	P9.12	IAC–112	Modified single–cross hybrid / IAC
L38	GP10	Angela	Open–pollinated variety of Embrapa
L39	GP11	IAC–125	Triple–cross hybrid / IAC
L40	P11.1	IAC–125	Triple–cross hybrid / IAC
L41	P12.2	IAC–125	Triple–cross hybrid / IAC
L42	GP12	IAC–125	Triple–cross hybrid / IAC
L43	GP13	Jade	Triple–cross hybrid / Pioneer
L44	GP 14	Maradona	Open–pollinated variety of UEM
L45	GP 15	Colombiana	Open–pollinated variety of UEM

Molecular marker – AFLP

The genomic DNA of the popcorn lines was extracted based on the modified protocol of Ferreira and Grattapaglia (1998), using CTAB buffer associated with isopropanol precipitation. All samples were treated with RNAse (110 ng mL^{-1}). DNA integrity was confirmed by electrophoresis in a 1% agarose gel, whereas the concentration and purity were determined by spectrophotometry using a NanoDrop® 2000 / 2000c (Thermo Fisher Scientific). Samples with A260/280 nm ratios between 1.8 and 2.0 were used.

The AFLP technique was performed following the protocol described by Vos *et al.* (1995), with some modifications. Approximately 700 ng of DNA from each

popcorn inbred line was doubly digested by *Eco*RI and *Mse*I (5 U each) enzymes in the presence of 2 µL of 10X *Mse*I assay buffer, with a final volume of 20 µL, and incubated for 18 h at 37 °C. The fragments generated were ligated to the adapters for *Eco*RI (0.5 µM) and *Mse*I (5 µM), using the T4 DNA ligase enzyme (1 U), 1X T4 DNA ligase buffer, NaCl (0.05 M), BSA (50 µg/µL), and DTT (0.25 mM) in a final volume of 10 µL. The reaction was incubated in a thermocycler at 37 °C for 3 h, 17 °C for 30 min, and 70 °C for 10 min. After confirming the digestion and binding process of PCR amplification by electrophoresis in 1% agarose gel, the final amplified binding product was diluted 1:4 times in ultrapure water.

Subsequently, the fragments were amplified using a pair of pre-selective primers containing a selective base. Pre-selective amplification was performed in a final volume of 10 µL, using 3.5 µL of the GoTaq® Green Master Mix kit (Promega), 0.58 µL of the pre-selective primer (4.75 µM), and 3.0 µL of the restriction/binding reaction dilution. The thermocycler program consisted of 2 min at 72 °C, followed by 20 cycles of 1 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C, and finally, 30 min at 60 °C. Confirmation of pre-selective polymerase chain reaction was performed on a 2% agarose gel, and the amplified product was diluted 1:16 times in ultrapure water.

For the selective amplification, 2.5 µL of the diluted pre-selective product was used, composed of 0.54 µL of each selective primer of *Mse*I (5 µM) and *Eco*RI (1 µM), and 3.5 µL GoTaq® Green Master Mix (Promega), in a final volume of 10 µL. The selective reactions were performed in the thermocycler, as follows: initial cycle of 2 min at 94 °C; 30 s at 65 °C, and 2 min at 72 °C; 8 cycles of 1 s at 94 °C, 30 s at 64 °C and 2 min at 72 °C, decreasing 1 °C for each cycle; 23 cycles of 1 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C; and finally, 30 min at 60 °C. Three combinations of primers *Eco*RI and *Mse*I (E-ACG/M-CAG, E-AGC/M-CAG, and E-AGG/M-CTG) were tested.

The products of the three selective amplifications were subjected to capillary electrophoresis in an automated system (Applied Biosystems, 3500xL). For this, the same combinations of primer sets described above were used, each labeled with one of the fluorophores FAM, NED, and VIC, in blue, yellow, and green, respectively. The amplified samples with the labeled primers were combined based on the following proportions: 1 µL of FAM: 2 µL of NED: 2 µL of VIC, and 3.0 µL of ultrapure water. For the sequencer run, a final volume equal to 10 µL was used, consisting of 1.0 µL of the primer mixture, 0.2 µL of GeneScan™ 600 LIZ. ® Size Standard v 2.0, and 8.8 µL of Hi-Di formamide (Applied Biosystems). The reaction proceeded through a denaturation process at 95 °C for 3 min before performing capillary electrophoresis.

The electrophoresis results of DNA fragments were combined in a binary matrix using the GenMapMap® v. 4.1 software (Applied Biosystems). All amplifications were performed using GeneAmp PCR System 9700 (Applied Biosystems).

Diallel analysis

Among the 45 popcorn lines characterized by the AFLP marker, 10 were selected based on the genetic divergence group and prior agronomic performance knowledge. These lines were L4, L14, L16, L22, L26, L38, L39, L42, L44, and L45. The selected lines were crossed in a balanced diallel scheme, according to method 4 described by Griffing (1956), to obtain 45 simple popcorn hybrids. Due to the genetic divergence of the lines, they were sown in two seasons to guarantee the coincidence of flowering and complete pollination.

The 45 simple hybrids resulting from the diallel and the three commercial cultivars used as checks (IAC 125, POPTOP, and POPTEN) were evaluated in three environments (Sabáudia, Maringá, and Londrina) in Paraná State, Brazil. The experimental design consisted of a randomized block with three replications, with each plot consisting of two 4.0 m long rows with 0.90 m spacing between the rows and 0.20 m between the plants, with a usable area of 7.2 m^2.

For the basic fertilization, 280 kg ha^{-1} of the 8–20–20 formulation was applied. Nitrogen topdressing was performed for 30 days with 250 kg ha^{-1} of urea. Pest control was undertaken as needed, following agronomic recommendations with neonicotinoids and organophosphates. Weed control was performed using atrazine and tembotrione herbicides.

The agronomic traits of grain yield (GY, in kg ha^{-1}) and popping expansion (PE, in mL g^{-1}) were evaluated. For GY, the correction for an ideal humidity of 13.5% and the stand was performed according to the methodology of Schmildt *et al*. (2001). The PE was determined in the laboratory using 30 g of kernels in a hot air electric popper at 280 °C with a popping time of 2 min and 10 s. The PE was determined from the ratio between the total popped volume (mL) and the kernel weight, measured in a 2.000 mL graduated cylinder.

Data analysis

The Jaccard distance matrix was calculated based on the AFLP data and Ward cluster analysis. The GY and PE data were subjected to joint variance analysis anddiallel analysis using Griffing's model 4 (GRIFFING, 1956) considering all effects as fixed, except for the experimental error. The statistical model adopted was as follows: $Y_{ij} = \mu + g_i + g_j + s_{ij} + a_k + ga_{ik} + ga_{jk} + sa_{ijk} + \bar{e}_{ijk}$, where Y_{ij} is the observation of the hybrid combination involving the parents *i* and *j*; µ is the general average; g_i and g_j are the general combining

ability of the *i*-th and *j*-th parent, respectively; s_{ij} is the specific combining ability for crosses between the parents of order *i* and *j*; a_k is the effect of the environment *k*; ga_{ik} and ga_{jk} are the effect of the interaction between the general combining ability (GCA) associated with the *i* and *j*-th parent with *k* environments, respectively; sa_{ijk} is the effect of the interaction between the specific combining ability (SCA) associated with *i* and *j* parents with k environments; and $\bar{e}_{ijk}$ is the average experimental error.

For the correlation studies, Pearso''s correlation coefficient (*r*) was estimated between the genetic distances of the popcorn inbred lines obtained by the AFLP marker, with SCA effects and the average of the hybrids obtained by the diallel crosses for GY and PE. All analyses were performed using R (http://www.r-project.org) and Genes (CRUZ, 2016) software.

RESULTS AND DISCUSSION

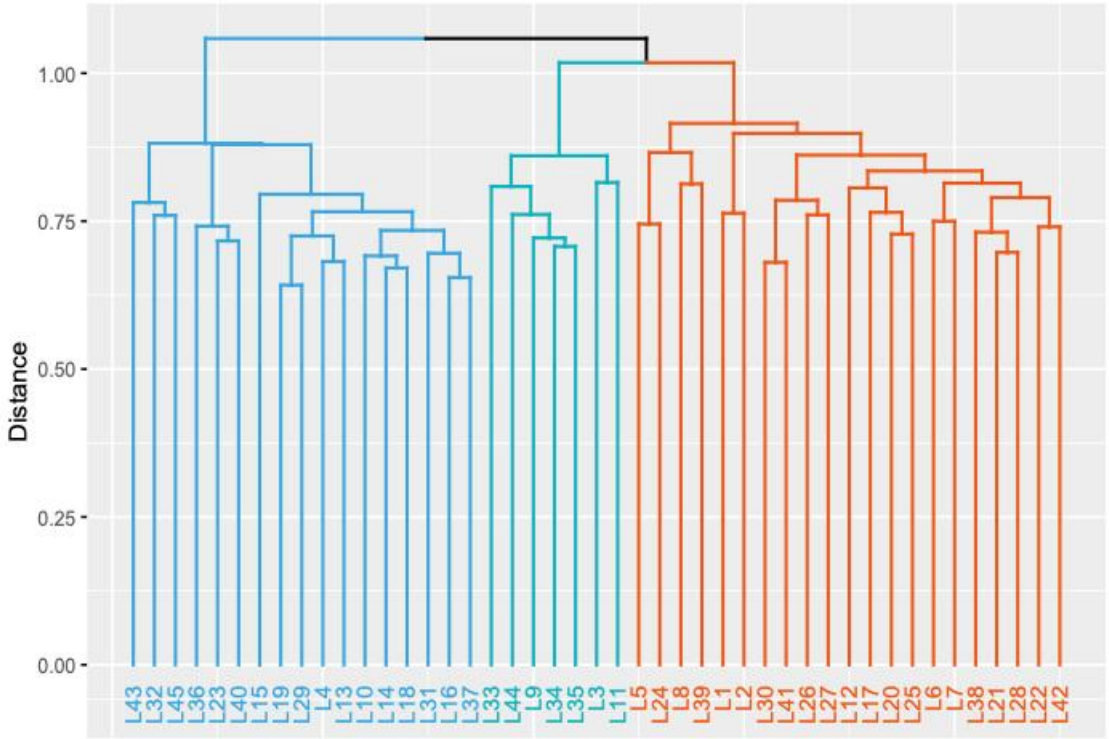
Genetic diversity based on AFLP marker

The AFLP markers were efficient in detecting genetic variability among the popcorn inbred lines evaluated in the present study. The three combinations of the AFLP primers produced 684 polymorphic bands, which were distributed between 60 and 500 base pairs. The E-ACA/M-CAC, E-ACC/M-CAA, and M-CAA/E-ACG combinations produced 59, 231, and 394 polymorphic bands, respectively. The AFLP marker associated with capillary electrophoresis in an automated system is an important tool for exploring genetic divergence in several agricultural species (BABA *et al*., 2016; CARDOSO *et al*., 2018; CONSTANTINO *et al*., 2020; MASSUCATO *et al*., 2020). In a genetic diversity study of 145 corn inbred lines, Giordani *et al*. (2019) obtained 1008 polymorphic bands, of which 97% were polymorphic.

The analysis of the frequency distribution of the pairwise dissimilarity distances ranged from 0.64 to 0.88, with an average distance of 0.78 (± 0.04). The 0.75⊣0.80 and 0.80⊣0.85 classes presented the highest frequencies (45% and 34%, respectively). The greatest distance was observed between the inbred lines L1 × L33, whereas L19 × L29 were closest to each other. This divergence was in agreement with that observed by Dos Santos *et al.* (2017), who evaluated 18 popcorn lines using microsatellite markers.

Based on Ward's hierarchical cluster analysis, three groups were identified (Figure 1). Groups I (dark blue), II (light blue), and III (orange) contained 17, 7, and 21 inbred lines, respectively. We did not observe a relationship between the origin of the inbred lines and the groups formed, mainly of the inbred lines from the IAC 112, IAC 125, Zaeli, and Jade hybrids. Embrapa's open-pollinated

Figure 1 – Ward's hierarchical clustering analysis of 45 popcorn inbred lines based on Jaccard distances generated by the polymorphism of 684 AFLP markers



varieties were allocated to groups II and III, whereas the inbred lines from UEM were allocated to groups I and II. The Catedral, Maradona, and Colombiana landraces were clustered in groups III, II, and I, respectively. Considering the genetic diversity, genealogy, and agronomic traits, 10 inbred lines were selected for diallel analysis, five from group III (L22, L26, L38, L39, and L42), four from group I (L4, L14, L16, and L45), and one from group II (L44).

Analysis of variance

The analysis of joint variance showed a significant effect of the hybrid × environment interaction for all traits (Table 2). The existence of this interaction is associated with two factors: simple and complex. Based on the proposal by Cruz and Castoldi (1991), a predominance of the complex part was observed for GY, whereas for PE, the simple type interaction predominated. Significant effects were also observed for both traits as sources of variation in hybrids and environments. Several studies have indicated that PE has a predominance of additive effects and is influenced by a few genes (LU; BERNARDO; OHM, 2003; COAN *et al*., 2019). Studying the inheritance of PE in a popcorn × Flint maize crossing, Coan *et al.* (2019) verified that this trait was controlled by a main additive gene and polygenic modifiers that act both additive and dominant.

The GY varied from 2322 to 3861 kg ha^{-1} in Sabáudia, from 841 to 2576 kg ha^{-1} in Maringá, and from 2105 to 3425 kg ha^{-1} in Londrina. For PE, the experimental hybrids ranged from 28 to 38 mL g^{-1} in Sabáudia and Maringá, and from 19 to 30 mL g^{-1} in Londrina. Based on the Scott-Knott test, the experimental hybrids that obtained the highest GY values were UEM–3 (L4 × L22), UEM–5 (L4 × L38), and UEM–20 (L16 × L38) (Table 3). For PE, the highest values were observed for the POPTOP and POPTEN checks, which did not differ from the six and 18 experimental hybrids in Sabáudia and Londrina, respectively.

Therefore, some experimental hybrids with high productive potential and good PE were observed, especially for UEM–3 hybrid, which presented, in Sabáudia and Londrina, high GY (3265 and 3295 kg ha^{-1}, respectively) and PE values (29 and 36 mL g^{-1}, respectively). In Maringá, even though GY did not exceed 3000 kg ha^{-1}, this hybrid showed excellent PE (36 mL g^{-1}).

Diallel analysis

Diallel analysis showed a significant effect on the GCA for GY and PE, indicating that the inbred lines differed in the frequency of favorable alleles, with more promising lines for the formation of hybrids (Table 4). However, for SCA, no significant effect was observed, indicating that only the presence of additive gene action was significant between the loci related to GY and PE. For PE, these results were expected because of the predominance of additive gene effects (PEREIRA *et al.*, 2001). For GY, the predominance of dominant gene action was expected. However, Scapim *et al*. (2002) also found a predominance of additive gene action in the control of GY.

In the GCA × environment interaction, significant effects were observed for GY and PE, whereas for SCA × environment, significant effects were observed only for GY. Therefore, there was a tendency of crossings to maintain the PE constant in different environments. However, this did not avoid a differentiated response in the productive performance of hybrid combinations in response to environmental variations.

Table 2 – Analysis of joint variance with mean squares and significance of the *F* test, in Sabáudia, Maringá, and Londrina for grain yield and popping expansion with general average and average of checks cultivars

Source of variation	DF	Mean square	
		Grain yield (kg ha^{-1})	Popping expansion (mL g^{-1})
Blocks/Environment	6	911639	6.0347
Hybrids (H)	47	603267*	50.7560*
Environment (E)	2	9925829*	3163.0800*
H × E	94	251436*	6.6544*
Residual	282	134980	4.8361
Mean			
IAC–125		2772	34
POPTOP		1978	37
POPTEN		1963	38
General		2514	31
CV (%)		14.61	7.10

* Significant at $p < 0.05$, as determined by the *F* test. DF: degrees of freedom; CV: coefficient of variation

Table 3 – Grain yield (GY, kg ha^{-1}) and popping expansion (PE, mL g^{-1}) of 45 simple hybrids popcorn resulting from the diallel and three checks cultivars

Inbred lines[1]	Hybrids	Sabáudia		Maringá		Londrina	
		GY	PE	GY	PE	GY	PE
L4 × L14	UEM–1	2968 b	32 c	1318 c	35 b	2641 b	27 a
L4 × L16	UEM–2	3062 b	31 d	2576 a	34 c	3169 a	23 b
L4 × L22	UEM–3	3265 a	36 a	2214 a	36 b	3295 a	29 a
L4 × L26	UEM–4	2898 b	31 d	1550 c	32d	2739 a	22 b
L4 × L38	UEM–5	3741 a	32 c	2284 a	33 c	2786 a	24 b
L4 × L39	UEM–6	3598 a	33 c	1902 b	34 c	3134 a	26 a
L4 × L42	UEM–7	3861 a	36 a	1338 c	38 b	3323 a	27 a
L4 × L44	UEM–8	3342 a	34 b	1872 b	36 b	2300 b	25 b
L4 × L45	UEM–9	3034 b	32 c	1601 c	32 d	2895 a	24 b
L14 × L16	UEM–10	3404 a	33 c	1400 c	34 c	3120 a	22 b
L14 × L22	UEM–11	3773 a	35 b	1312 c	36 b	2621 b	30 a
L14 × L26	UEM–12	2322 b	35 b	1314 c	34 c	2493 b	23 b
L14 × L38	UEM–13	3370 a	33 c	1360 c	37 b	2995 a	26 b
L14 × L39	UEM–14	3168 b	36 a	1429 c	37 b	2750 a	27 a
L14 × L42	UEM–15	3107 b	37 a	940 c	34 c	2358 b	27 a
L14 × L44	UEM–16	3051 b	34 b	1321 c	35 b	2604 b	27 a
L14 × L45	UEM–17	3103 b	33 c	841 c	31 d	2701 a	25 b
L16 × L22	UEM–18	3304 a	31 d	2193 a	31 d	2605 b	25 b
L16 × L26	UEM–19	3546 a	28 d	1764 b	29 d	2818 a	19 b
L16 × L38	UEM–20	3822 a	31 d	2135 a	34 c	2827 a	20 b
L16 × L39	UEM–21	3268 a	33 c	1870 b	30 d	2909 a	23 b
L16 × L42	UEM–22	3431 a	31 d	1637 b	35 c	2769 a	26 a
L16 × L44	UEM–23	3806 a	30 d	1452 c	29 d	2708 a	25 b
L16 × L45	UEM–24	3040 b	29 d	1666 b	28 d	3256 a	21 b
L22 × L26	UEM–25	3693 a	31 d	1555 c	31 d	2796 a	24 b
L22 × L38	UEM–26	3279 a	34 b	1730 b	35 c	2989 a	27 a
L22 × L39	UEM–27	3758 a	34 b	1898 b	32d	3425 a	27 a
L22 × L42	UEM–28	3188 b	36 a	1262 c	35 b	2761 a	28 a
L22 × L44	UEM–29	3106 b	35 b	1570 c	33 c	2256 b	26 a
L22 × L45	UEM–30	3564 a	33 c	1208 c	31d	2847 a	23 b
L26 × L38	UEM–31	3031 b	33 c	1334 c	34 c	2615 b	25 b
L26 × L39	UEM–32	3000 b	34 b	1292 c	33 c	2826 a	26 b
L26 × L42	UEM–33	2974 b	35 b	1132 c	34 c	2889 a	29 a
L26 × L44	UEM–34	3018 b	31 d	1575 c	35 c	2225 b	25 b
L26 × L45	UEM–35	2972 b	30 d	1175 c	34 c	2754 a	22 b
L38 × L39	UEM–36	3122 b	29 d	1854 b	34 c	2532 b	28 a
L38 × L42	UEM–37	2958 b	35 b	2168 a	32 c	2728 a	26 b
L38 × L44	UEM–38	2958 b	35 b	1796 b	36 b	2483 b	29 a
L38 × L45	UEM–39	3385 a	32 c	1840 b	34 c	2422 b	23 b
L39 × L42	UEM–40	3035 b	34 b	1244 c	33 c	2326 b	28 a

Continuation Table 3

L39 × L44	UEM–41	3280 a	34 b	2090 a	33 c	2565 b	25 b
L39 × L45	UEM–42	3139 b	32 c	1668 b	32 c	2420 b	24 b
L42 × L44	UEM–43	3133 b	38 a	1511 c	34 c	2679 a	26 a
L42 × L45	UEM–44	3421 a	32 c	1683 b	33 c	2779 a	25 b
L44 × L45	UEM–45	3010 b	33 c	1450 c	32d	2105 b	24 b
–	IAC–125	3822 a	35 b	1711 b	37 b	2782 a	28 a
–	POPTOP	2454 b	39 a	1192 c	41 a	2288 b	32 a
–	POPTEN	2193 b	38 a	1639 b	44 a	2058 b	32 a
General mean		3224	33	1601	34	2716	26
CV (%)		13.08	5.85	19.88	6.67	13.05	9.24

[1]Means followed by the same letter belong to the same group according to the Scott-Knott test at 5% probability. CV: coefficient of variation

Table 4 – Analysis of joint variance, effect of general (GCA) and specific combining ability (SCA) for grain yield and popping expansion of diallel elite popcorn

Source of variation	DF	Mean square	
		Grain yield (kg ha^{-1})	Popping expansion (mL g^{-1})
Treatments (T)	(44)	506934*	33.4222*
GCA	9	1628653*	126.200*
SCA	35	218492^{ns}	9.5650^{ns}
Environment (E)	2	95534361*	2917.4800*
T × E	(88)	239425*	6.7616*
GCA × E	18	368482 *	8.1416*
SCA × E	70	206239 *	6.4067^{ns}
Residual	264	139227	4.9133
General mean		2532.00	31.00

*: significant at $p < 0.05$, F test; *ns*, non-significant; DF, degrees of freedom

The estimates of $\hat{g}_l$ (GCA) for GY, L4, L16, L22, and L39 lines were the ones that presented the highest positive values for all environments, indicating a higher frequency of favorable alleles for this trait (Table 5). Regarding PE, the L14, L42, and L44 lines had the highest positive estimates of $\hat{g}_l$. Inbred lines L4, L22, and L39 showed positive estimates of $\hat{g}_l$ for PE in at least two environments evaluated. Therefore, the merits of GCA should not be disregarded because the combination of high productive potential and PE, in the same genotype, constitutes a challenge for popcorn breeding (MARINO *et al.*, 2019).

Table 6 shows the estimates of $\hat{s}_{ij}$ (SCA) among the lines for GY by environment and PE based on the average of the environments. However, as the isolated effect of SCA was not significant for both traits, it is not possible to infer which combinations are more favorable for exploring heterosis according to the estimates of $\hat{s}_{ij}$.

Correlation between genetic divergence and estimates of genetic parameters

For correlation analysis, only the GY trait was used because PE did not have a significant effect on SCA, indicating a strong concentration of additive alleles. For GY, no correlation was observed between the genetic distance and average GY, demonstrating an absence of consistency in the prediction of heterotic groups for popcorn using the AFLP marker. Among the different factors related to this lack of correlation was the non-significant dominance effect for GY, allelic frequency of the parent lines not being negatively correlated, and absence of the association of marks with QTLs influencing GY (BERNARDO, 1992). These results observed in the present study were in agreement with Fernandes *et al.* (2015). For GY and $\hat{s}_{ij}$, the correlations for the Sabáudia, Maringá, and Londrina counties were 0.74, 0.62, and 0.71, respectively.

Table 5 – Estimates of general combining ability ($\hat{g}_l$) effects of the diallel of 10 popcorn lines for grain yield (GY, kg ha^{-1}) and popping expansion (PE, mL g^{-1})

Inbreed lines	$\hat{g}_l$ (GY)				$\hat{g}_l$ (PE)			
	Sabáudia	Maringá	Londrina	Mean	Sabáudia	Maringá	Londrina	Mean
L4	63.4	273.7	204.3	180.5	–0.02	1.15	0.05	0.39
L14	–124.4	–403.7	–45.5	–191.2	1.35	1.52	0.92	1.26
L16	177.6	278.5	191.6	215.9	–2.52	–2.10	–2.82	–2.48
L22	208.5	59.6	118.4	128.8	0.97	–0.10	1.55	0.80
L26	–225.9	–221.7	–61.5	–169.7	–1.15	–0.60	–1.45	–1.06
L38	50.5	254.5	–33.8	90.4	-0.40	1.02	0.17	0.26
L39	13.3	97.7	29.9	47.0	0.22	–0.35	0.92	0.26
L42	–19.2	–193.7	–4.4	–72.4	2.10	0.90	1.92	1.64
L44	–69.7	21.5	–340.3	–129.5	0.85	0.27	0.67	0.60
L45	–74.2	–166.6	–58.5	–99.7	–1.40	–1.72	–1.95	–1.69
SD[1] ($\hat{g}_l$)	83	63	68	–	0.38	0.44	0.45	–

[1]SD: standard deviation

Table 6 – Estimates of the specific combining ability ($\hat{s}_{ij}$) of 45 simple popcorn hybrids of the diallel for grain yield (GY, kg ha^{-1}) and popping expansion (PE, mL g^{-1})

Inbred lines	Hybrids	$\hat{s}_{ij}$ (GY)			$\hat{s}_{ij}$ (PE)
		Sabáudia	Maringá	Londrina	Mean
L4 × L14	UEM–1	–222.264	–159.250	–256.347	–0.866
L4 × L16	UEM–2	–430.389	416.500	34.403	0.884
L4 × L22	UEM–3	–258.264	273.375	233.653	1.926
L4 × L26	UEM–4	–190.764	–109.250	–142.347	–1.532
L4 × L38	UEM–5	375.736	148.500	–123.097	–1.532
L4 × L39	UEM–6	269.986	–76.750	161.153	–0.199
L4 × L42	UEM–7	565.486	–349.250	384.528	1.093
L4 × L44	UEM–8	96.986	–30.500	–302.597	0.134
L4 × L45	UEM–9	–206.514	–113.375	10.653	0.093
L14 × L16	UEM–10	99.486	–82.000	235.278	0.343
L14 × L22	UEM–11	437.611	48.875	–190.472	1.051
L14 × L26	UEM–12	–578.889	332.250	–138.472	–0.074
L14 × L38	UEM–13	192.611	–98.000	335.778	–0.074
L14 × L39	UEM–14	27.861	127.750	27.028	1.259
L14 × L42	UEM–15	–0.639	–69.750	–330.597	–0.782
L14 × L44	UEM–16	–6.139	96.000	251.278	–0.407
L14 × L45	UEM–17	50.361	–195.875	66.528	–0.449
L16 × L22	UEM–18	–333.514	247.625	–443.722	0.134
L16 × L26	UEM–19	342.986	100.000	–50.722	–1.657
L16 × L38	UEM–20	342.486	–5.250	–69.472	0.009
L16 × L39	UEM–21	–174.264	–113.500	–51.222	0.343

Continuation Table 6

L16 × L42	UEM–22	21.236	–55.000	–156.847	0.968
L16 × L44	UEM–23	446.736	–455.250	118.028	–0.657
L16 × L45	UEM–24	–314.764	–53.125	384.278	–0.366
L22 × L26	UEM–25	459.111	109.875	0.528	–1.616
L22 × L38	UEM–26	–231.389	–191.375	165.778	0.384
L22 × L39	UEM–27	284.861	133.375	538.028	–0.616
L22 × L42	UEM–28	–252.639	–211.125	–91.597	0.009
L22 × L44	UEM–29	–284.139	–118.375	–260.722	–0.616
L22 × L45	UEM–30	178.361	–292.250	48.528	–0.657
L26 × L38	UEM–31	–44.889	–306.000	–28.222	0.926
L26 × L39	UEM–32	–38.639	–191.250	119.028	1.259
L26 × L42	UEM–33	–32.139	–59.750	216.403	1.551
L26 × L44	UEM–34	62.361	168.000	–111.722	0.259
L26 × L45	UEM–35	20.861	–43.875	135.528	0.884
L38 × L39	UEM–36	–193.139	–105.500	–202.722	–0.741
L38 × L42	UEM–37	–324.639	500.000	27.653	–1.449
L38 × L44	UEM–38	–274.139	–87.250	118.528	1.926
L38 × L45	UEM–39	157.361	144.875	–224.222	0.551
L39 × L42	UEM–40	–210.389	–267.250	–438.097	–0.782
L39 × L44	UEM–41	85.111	363.500	136.778	–0.741
L39 × L45	UEM–42	–51.389	129.625	–289.972	0.218
L42 × L44	UEM–43	–29.389	76.000	285.153	–0.116
L42 × L45	UEM–44	263.111	436.125	103.403	–0.491
L44 × L45	UEM–45	–97.389	–12.125	–234.722	0.218
SD[1] ($\hat{s}_{ij}$)		218	165	179	0.650

1SD: standard deviation

CONCLUSION

1. A wide genetic variability was observed among popcorn inbred lines;
2. Molecular markers were not efficient for predicting heterotic groups;
3. Based on the combining ability, promising hybrids were observed for the development of new popcorn cultivars.

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