LEONARDO ALVES RISSO

HETEROSIS AND GENETIC DIVERSITY ANALYSES INVOLVING TROPICAL AND TEMPERATE POPCORN POPULATIONS

Dissertation presented to the Universidade Federal de Viçosa as part of the requirements of the Genetic and Breeding Graduate Program for the obtention of the degree of *Magister Scientiae.*

VIÇOSA
MINAS GERAIS - BRAZIL
2019

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T
R595h
2019

Risso, Leonardo Alves, 1992-
Heterosis and genetic diversity analyses involving tropical and temperate popcorn populations / Leonardo Alves Risso. – Viçosa, MG, 2019.
vi, 26f. : il. ; 29 cm.

Texto em inglês.
Orientador: José Marcelo Soriano Viana.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Referências bibliográficas: f.11-16.

1. Milho pipoca. 2. Heterose. 3. Diversidade Genética. I. Universidade Federal de Viçosa. Departamento de Biologia Geral. Programa de Pós-Graduação em Genética e Melhoramento. II. Título.

CDD 22 ed. 635.677

LEONARDO ALVES RISSO

HETEROSIS AND GENETIC DIVERSITY ANALYSES INVOLVING TROPICAL AND TEMPERATE POPCORN POPULATIONS

Dissertation presented to the Universidade Federal de Viçosa as part of the requirements of the Genetic and Plant Breeding Graduate Program for the obtention of the degree of *Magister Scientiae.*

APPROVED: April 4th, 2019

Rodrigo Oliveira de Lima

Eveline Teixeira Caixeta

Plínio César Soares

José Marcelo Soriano Viana
(Advisor)

To God,

My Mom Dalcina,

My Father Donisete,

My Sisters Aline and Larissa,

I DEDICATE

ACKNOWLEDGMENTS

To God, through faith guiding my steps.

To my deceased and dear mother Dalcina, for passing me her knowledge and example.

To my father Donisete, for the support and believing in me.

To my sisters Aline and Larissa, for supporting me and their friendships.

To my brother-in-laws Michel Todd Sherrill e Daniel Uliano for their friendships.

To my girlfriend Deborah, for her love, and for supporting me in the bad moments. Her parents José de Lima Lucas and Iracema Fantuzzi Lucas, for their friendships.

To my friends from "República Zagaia" who have become my second family.

To the Federal University of Viçosa and Genetic and Plant Breeding Graduate Program, for the opportunity to develop this work.

To the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES), and the "Fundação de Apoio à Pesquisa do Estado de Minas Gerais" (FAPEMIG), for financial support.

To Prof. José Marcelo Soriano Viana, for his advice, instruction, trust, and support whenever I needed it.

To Professors and Researchers Rodrigo Oliveira de Lima, Eveline Teixeira Caixeta, and Plínio César Soares for their availability and valuable contributions.

To my friends from the "Popcorn Breeding program" Vinícius, Hélcio, Aloísio, and Victor for every lesson, and for every piece of advice and friendship.

To all my friends of Biotechnology and vegetal Breeding Laboratory. Thank you for always helping me.

To Paulo Fernando (Dinho), for providing his farm to do the experiment.

To Márcio and Antônio field technicians, for supporting me and sharing their knowledge.

Finally, I am sincerely grateful to all those who contributed to make this work possible.

THANK YOU!

SUMMARY

ABSTRACT

RISSO, Leonardo Alves, M.Sc., Universidade Federal de Viçosa, April, 2019. **Heterosis and genetic diversity analyses involving tropical and temperate popcorn populations.** Adviser: José Marcelo Soriano Viana.

Understanding the heterosis and genetic diversity is crucial to choose potential popcorn (*Zea mays* L. ssp. mays) populations to be used in future hybrid programs. The objective of this study was to analyze heterosis and genetic diversity between tropical and temperate popcorn populations. Nine popcorn populations from different origins were crossed by diallel design and genotyped using 23 microsatelite markers. The parental populations, selfed populations, the hybrids and reciprocals were evaluated in 2017/18 growing season for expansion volume and grain yield at two locations in Minas Gerais, Brazil. The heterosis analysis were performed according to the analysis II of Gardner and Eberhart and fitted to the unrestricted model. The genetic diversity analysis was assessed by the complement of Jaccard similarity. There were genetic variability and dominance for expansion volume and grain yield. The cluster analysis based on microsatelite markers grouped nine popcorn populations into two groups. The grouping with hierarchical and Tocher's procedures were in accordance with the origins. The genetic distance was weakly correlated with heterosis for grain yield. Regarding expansion volume, there was no significant correlation. The midparent heterosis was used to assign the nine populations into heterotic groups, since that correlation was irrelevant between genetic distance and heterosis. The Tocher's optimization procedure established four heterotic groups, and a promising combination (Sintético-UFV × UFV-MP5) was identified.

RESUMO

RISSO, Leonardo Alves, M.Sc., Universidade Federal de Viçosa, abril de 2019. **Análise de Heterose e de diversidade genética envolvendo populações tropicais e temperadas de milho-pipoca.** Orientador: José Marcelo Soriano Viana.

Compreender a heterose e a diversidade genética é crucial para escolher populações potenciais de milho-pipoca (*Zea mays* L. ssp. Mays) para serem utilizadas em programas de melhoramento de híbridos. O objetivo deste estudo foi analisar a heterose e a diversidade genética entre populações tropicais e temperadas de milho-pipoca. Nove populações de milho pipoca de diferentes origens foram cruzadas em esquema dialélico e genotipadas usando 23 marcadores microssatélites. As populações parentais, as autofecundadas, os híbridos e os recíprocos foram avaliados na safra 2017/18 para capacidade de expansão e produtividade de grãos em dois locais em Minas Gerais, Brasil. A análise de heterose foi realizada de acordo com a análise II de Gardner e Eberhart, ajustada para o modelo irrestrito. A matriz de diversidade genética foi obtida pelo complemento da similaridade de Jaccard. Houve variabilidade genética e dominância para a capacidade de expansão e produtividade de grãos. A análise de agrupamento baseada em marcadores microssatélites agrupou as nove populações de milho-pipoca em dois grupos. O agrupamento com os procedimentos hierárquicos e pelo método Tocher foram condizentes com as origens das populações. A distância genética foi fracamente correlacionada com a heterose para a produtividade de grãos e não houve correlação para capacidade de expansão. A heterose foi utilizada para atribuir as nove populações em grupos heteróticos, uma vez que essa correlação foi irrelevante entre a distância genética e a heterose para ambos caracteres. O procedimento de otimização de Tocher estabeleceu quatro grupos heteróticos, e uma combinação mais promissora (Sintético-UFV × UFV-MP5) foi identificada.

1. INTRODUCTION

The maize breeding programs are fundamentally based on utilization of heterotic patterns and groups (Melani and Carena 2005). The establishment of maize germplasm into a known heterotic group allows the maximum exploitation of heterosis, which provides increase yield potential (Hochholdinger and Baldauf 2018). According to Lee (1995), a heterotic group is comprised of a set of germplasm that tends to display higher degree of heterosis when crossed with an external group than when crossed with a member of its own group. Whereas, heterotic pattern refers to specific pair of genotypes of two distinct heterotic groups (Melchinger and Gumber 1998). Based on phenotypic data, the heterotic groups have been classified by two main approaches, the diallel analysis (Soengas et al. 2003, Cherchali et al. 2018) and testcrosses (Barata and Carena 2006, Fan et al. 2018). However, due to environmental influence in the morphological traits, molecular markers has also been employed to classify heterotic groupings (Reid et al. 2011, Richard et al. 2016).

Revilla et al. (2006) used diallel mating design to identify heterotic patterns among twelve Spanish and French maize populations across four environments. Based on the high midparent heterosis for grain yield, they established one heterotic pattern for each location, and a most promising heterotic pattern across sites. Laude and Carena (2015) employed diallel analysis and genetic diversity among 16 tropical and temperate adapted maize populations to classify them into heterotic groups. They reported high correlation between specific heterosis and genetic diversity for morphological traits, and defined the specific heterosis for grain yield as the best strategy to establish heterotic groups. Melani and Carena (2005) investigated the combining ability analysis and midparent heterosis among 10 maize populations from opposite heterotic groups, and revealed promising heterotic patterns, finding an average midparent heterosis for grain yield of 19.5%. Soengas et al. (2003) attempted to identify heterotic patterns within flint germplasm, crossing 10 flint maize population by the diallel design, and based on midparent heterosis for grain yield they found that Flint × Flint crosses could be used to develop hybrids.

Studies involving molecular markers have been used to predict heterosis, and to establish heterotic groups. Reif et al. (2003a) reported high relationship between genetic distance from 85 SSR (Single Sequence Repeat) markers and midparent heterosis of a diallel among 7 maize populations (r=0.63), and concluded that SSR markers are a valuable tool in the identification

of heterotic groups. Mundim et al. (2015) found correlation greater than 0.6 between genetic distance and heterosis for expansion volume and grain yield in inbred line popcorn (*Zea mays* L. ssp. *mays*), using 90 SSR markers. On the other hand, weak correlation or the absence of correlation between molecular markers and heterosis has been observed (Devi and Singh 2011, Makumbi *et al.* 2018). Vancetovic et al. (2015) used diallel analysis and 16 SSR markers among six drough tolerant maize populations to determine potential heterotic patterns. They reported low correlation for genetic distance based on SSR and midparent heterosis (r=0.179) for grain yield, and defined possible heterotic patterns using the midparent heterosis.

Previous works have attempted to assess the heterotic relationship among maize germplasms using diversity analyses only. In popcorn, Santacruz-Varela et al. (2004) evaluated the genetic relationship among 56 populations from North America and nine from Latin American based on 31 SSR markers, 29 morphological traits, and 18 isozyme loci. They identified three different groups for the North American popcorn populations, namely, Yellow Pearl, Pointed Rice, and Early popcorns, and two groups for Latin American populations. Oliveira et al. (2004) using AFLP (Amplified Fragment Length Polymorphism) markers, investigated the relationship among 96 maize inbred lines. Using Tocher's optimization procedure from Jaccard's complement, they classified the lines into 17 heterotic groups. Li et al. (2004) using 113 SSR markers by UPGMA clustering, identified seven heterotic groups among 56 popcorn inbred lines and 21 common maize lines from well-known heterotic groups from China, highlighting that the SSR markers are important to assign popcorn and maize lines into heterotic groups.

Genetic diversity based on DNA markers has given different results for predicting of heterosis in maize, thus, field evaluation has been recommended simultaneously in classification of maize germplasm into heterotic groups (Barata and Carena 2006). Little is known about heterosis and diversity analysis among tropical and temperate popcorn populations. Furthermore, understanding heterosis is crucial to choose the potential popcorn populations to be used in future hybrid programs. This study aimed to analyze heterosis and genetic diversity between tropical and temperate popcorn populations through diallel analysis and molecular markers, to establish them into heterotic groups.

2. MATERIALS AND METHODS

2.1. Populations

Nine popcorn populations of different origins were used in this study. Three tropical populations were used, Viçosa C4 and Beija-Flor C4, obtained from Viçosa and Beija-Flor populations after four cycles of half-sib selection, and Sintético-UFV, derived by random crossing 20 inbred lines from Viçosa and 20 inbred lines from Beija-Flor populations, and selected based on expansion volume. The five temperate populations, UFV-MP1, UFV-MP2, UFV-MP3, UFV-MP4 and UFV-MP5, are derived from the North American hybrids P622, P625, P802, AP2501, and AP4502, respectively, developed by Agricultural Alumni Seed Improvement Association, Romney, IN, USA. The population ARZM 07-49, was collected in Argentina and developed by International Maize and Wheat Improvement Center-CIMMYT. In 2016/17, each popcorn population was increased, selfed and intercrossed in a diallel mating design.

2.2. Field trials

In the 2017/18 growing season, the nine parental populations, 36 hybrids, 36 reciprocals, and nine selfed populations were evaluated in two experiments arranged in a 9 x 10 alpha lattice design with three replications. One experiment was conducted at an experimental station of Universidade Federal de Viçosa (20°50′ S, 42°48′ W; alt. 640 m asl) and the other in a farm in Madre de Deus de Minas (21°28′ S, 44°19′ W; alt. 990 m asl), both are located in Minas Gerais State, Brazil. The experimental plot consisted of two rows of 4 m, spaced 0.8 m, with a plant density of approximately 62.500 plants ha^{-1}. At maturity, all the ears from each plot were harvest and shelled, and the grain weight and grain moisture were recorded. The grain yield (kg ha^{-1}) was calculated at 145 g/kg moisture. The expansion volume (mL g^{-1}) was assessed in the Cretor's Metric Weight Volume Tester (MWVT), using 250 g of grains of each plot.

2.3. Genotyping

To perform the genetic diversity study, DNA samples of 20 plants from each parental population were genotyped with SSR markers, using the ‘population bulk DNA strategy’, described by Dubreuil et al. (1999). Each population was represented by two DNA bulks, composed by equal volume of the genomic DNA of 10 individuals. The DNA of individuals was isolated using the CTAB procedure (Diniz et al. 2005), and DNA concentration was quantified to 25 ng/µL using Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA).

Twenty three polymorphic SSR markers were chosen from the MaizeGDB (http://www.maizegdb.org), based on bin location, to provide uniform coverage (Table 1). The polymerase chain reactions (PCR) were carried out using the bulks of DNA in Touchdown PCR program according to CIMMYT Applied Biotechnology Center Manual of Laboratory Protocols (CIMMYT 2005). Amplification products were separated on a 6.0% (v/v) denaturing polyacrylamide gels, using the electrophoretic system Sequi-Gen GT (Bio-Rad, Hercules, CA, USA), and the bands were visualized with silver nitrate staining, according to Brito et al. (2010).

2.4. Diallel analysis

The analysis II of Gardner and Eberhart (1966) was performed but fitting the unrestricted model described by Viana (2000), including inbred populations and reciprocals F1's according to Viana and Matta (2003) and Griffing (1956), respectively. The unrestricted model allows the estimation of the population mean, heterosis, average heterosis, population heterosis, specific heterosis, change in the population mean due to inbreeding, and reciprocal effect. The phenotypic means of the populations, hybrids, reciprocals hybrids, and selfed populations were defined as, respectively:

$$y_j = m + v_j + \bar{e}_j$$

$$y_{jj'} = m + \frac{1}{2}v_j + \frac{1}{2}v_{j'} + H_{jj'} + r_{jj'} + \bar{e}_{jj'} = m + \frac{1}{2}v_j + \frac{1}{2}v_{j'} + H + H_j + H_{j'} + S_{jj'} + r_{jj'} + \bar{e}_{jj'}$$

$$y_{j'j} = m + \frac{1}{2}v_{j'} + \frac{1}{2}v_j + H_{j'j} - r_{jj'} + \bar{e}_{j'j} = m + \frac{1}{2}v_{j'} + \frac{1}{2}v_j + H + H_j + H_{j'} + S_{j'j} - r_{jj'} + \bar{e}_{j'j}$$

$$y_{js} = m + v_j + d_j + \bar{e}_{js}$$

where m is a constant, v_j is the population effect, $H_{jj'}$ is the heterosis, $r_{jj'}$ is the reciprocal effect, H is the average heterosis, H_j is the population heterosis, $S_{jj'}$ is the specific heterosis, d_j is the change in the genotypic mean due to one generation of selfing, and $\bar{e}$ is a mean error. The population means were submitted to the grouping test of Scott and Knott (1974), assuming a significance level of 5%. Moreover, we calculated the midparent heterosis dividing the heterosis estimated by average of j and j' population means.

2.5. Genetic diversity analysis

As the populations were analyzed using the bulk of DNA, each amplified fragment was scored as presence (1) versus absence (0). If the presence of an SRR allele was observed in at least one bulk, it was classified as present in the population. The binary matrix was assembled to compute the Jaccard similarity coefficient (GS) (Jaccard, 1908), and a cluster analysis was

performed through Unweighted Pair Group Method with the Arithmetic average (UPGMA) algorithm. The genetic distance was determined using the complement of the Jaccard's similarity coefficient (GD= 1-GS). The diversity analysis and the UPGMA clustering were performed using NTSYS-pc 2.2 software (Numerical Taxonomy and Multivariate Analysis System) (Rohlf 2009). The cophenetic correlation coefficient between the similarity matrix and the clustering was also calculated. A complementary cluster analysis was also carried out to obtain the groups using Tocher's optimization procedure (Rao, 1952). This approach was performed through the use of the 'Biotools' package of R Software. Additionally, we computed Pearson correlation between pairwise genetic distances and heterosis for each trait, and tested using the Student's test.

3. RESULTS AND DISCUSSION

3.1. Analysis of variance

The combined analysis of variance showed significant differences ($P<0.01$) for population means, for expansion volume and grain yield (Table 2). The population means × environment interaction was also significant ($P<0.01$) for the two traits. The significance of population means test indicated that there are differences in the gene frequencies between the populations for both traits. The coefficients of variation for expansion volume and grain yield were 11% and 14%, respectively. These coefficients are agreement with the previous works by Scapim et al. (2006a), Miranda et al. (2008), and Munhoz et al. (2009).

The heterosis differed significantly for expansion volume and grain yield ($P<0.01$). As there are differences in the gene frequencies between populations, the significance of heterosis test showed that there is dominance (Viana 2000). The average heterosis, variety heterosis, and specific heterosis were also significantly different for the two traits. The significance of average heterosis test indicated unidirectional dominance for both traits. Whereas, the test for the population and specific heterosis showed that there is a degree of divergence of each population in relation to the other diallel's parents and that the magnitude of the divergence between the pairs of populations is not a constant, respectively. The corresponding interaction between the parameters of heterosis and environment were all statistically significant, showing differences in the locations.

The change in the mean of the populations due to inbreeding was significant ($P<0.01$) for both traits, as well as their interaction with environment. The interpretation of test to inbreeding is redundant to the heterosis test (Viana and Matta 2003), confirming the contribution due to dominance to the genotypic values. The reciprocals were statistically significant ($P<0.05$) for both traits, and also for the interaction reciprocals × environment. The knowledge about the reciprocals might be useful in indicating when to use the parents as male or female in crosses. Furthermore, the significance of reciprocal effects in the populations may to suggest the existence of maternally determined traits (Jumbo and Carena 2008), such as the cytoplasmic genetic factors (Mukanga et al. 2010). Although there are significant differences of reciprocals effect for both traits, in accordance with Andrade et al. (2002) and Cabral et al. (2015), our study will not considered it in subsequent analyses.

3.2. Population mean analysis

Based on unrestricted model, the population effects cannot be estimated. However, it is possible to estimate the population means, which can be considered an indicator of superiority relative to other populations in terms of the frequency of the favorable genes (Viana 2000). Therefore, a given population with high a population mean possesses great frequencies of genes for increasing trait expression. Thus, regarding the expansion volume, two tropical and three temperate populations showed superiority in frequency of favorable genes (Table 3), highlighting the temperate population UFV-MP4 (33.65 mL. g^{-1}). The temperate and tropical populations showed on average similar values of population means for expansion volume. This result is in disagreement with Santacruz-Varela et al. (2004) and Miranda et al. (2008) who found higher perfomances for expansion volume in the temperate compared to tropical populations.

For grain yield, the result showed that the population from Argentina (ARZM 07-49) is superior to the other populations in terms of frequency of favorable genes. This population gave the higher value for population mean (4480.1 Kg. ha^{-1}). In general, the temperate populations had low values for the populations mean, showing an average yield of 2270 Kg. ha^{-1}, whereas tropical populations yielded on average 3130 Kg. ha^{-1}. The most likely explanation for this seems to be the superior adaptation of them to the tested environments.

3.3. Heterosis analysis

The sign of average heterosis for expansion volume indicates the predominance of dominant genes towards the increase this trait, however, possessing low magnitude (1.60 mL.

g^{-1}). Solalinde et al. (2014) also reported similar values of average heterosis for expansion volume (1.9 mL. mL^{-1}) among seven popcorn population from the Avatí Pichingá (Paraguay). Similarly, Silva et al. (2010) detected positive unidirectional dominance for expansion volume, evaluating 10 popcorn inbred lines based on Griffing's (1956) method. As expected, for grain yield, average heterosis was positive and high magnitude (486.8 Kg. ha^{-1}) (Table 3), indicating positive unidirectional dominance.

The varietal heterosis, in absolute value, indicates divergence of the population compared to the other genitors (Viana 2000). Thus, the most divergent population from the set of populations are the populations UFV-MP2 (4.90 mL. g^{-1}) and ARZM 07-49 (762.3 Kg. ha^{-1}) for expansion volume and grain yield, respectively (Table 3). Moreover, the UFV-MP5 population was the most divergent from the other populations for expansion volume (2.90 mL. g^{-1}) and grain yield (311.6 Kg. ha^{-1}) when associated.

The heterosis estimates is an indicator of the populations with the greatest gene frequency differences (Viana 2000). We observed for expansion volume that the populations with the greatest gene frequency differences were Sintético-UFV × UFV-MP2 (9.55 mL. g^{-1}) and UFV MP5 × UFV-MP2 (5.97 mL. g^{-1}). While for grain yield, the highest differences of gene frequencies were observed between the populations Sintético-UFV and UFV-MP1 (1092 Kg. ha^{-1}), and Sintético-UFV and ARZM 07-49 (1077 Kg. ha^{-1}) (Table 5). Notably, the UFV-MP2 and Sintético-UFV populations were between the best combinations for expansion volume and grain yield, respectively.

According to Viana (2000), the estimates of specific heterosis by unrestricted model is redundant to heterosis analysis and, in like manner, indicates a degree of divergence between themselves and in relation to the parental group. Additionally, when there is positive unidirectional dominance (like the result mentioned), the greatest specific heterosis values indicate populations with the greatest differences of gene frequencies between themselves and in relation to the parental group. For expansion volume and grain yield, the combinations which most diverge between themselves and in relation to parental group were Sintético-UFV × UFV-MP2 (2.782 mL. g^{-1}) and Sintético-UFV × UFV-MP1 (-505.43 Kg. ha^{-1}), respectively.

3.4. Inbreeding analysis

The alteration of mean due to inbreeding can also be used to indicate the direction of dominance (Viana and Matta 2003). Additionally, inferences about gene frequency can be done. The greatest values (in the absolute value) of inbreeding, the closer to ½ should be the gene frequency in the populations. The results of inbreeding estimates suggest bidirectional dominance (positive and negative dominance deviations) for expansion volume, outstanding to the UFV-MP4 population as source of desirable dominant genes to an increase of expansion volume. Viana and Matta (2003) and Munhoz et al. (2009) also found bidirectional dominance for expansion volume. While for grain yield, the temperate and tropical populations showed opposite unidirectional dominance. The temperate populations have recessive genes, whereas tropical populations have dominant genes contributing to an increase of grain yield. Furthermore, the populations UFV-MP4 and ARZM 07-49 showed the gene frequency closer to 1/2 for expansion volume and grain yield, respectively. In general, regarding to grain yield, tropical popcorn populations and Argentinean population showed highest estimates of inbreeding. Pacheco et al. (2002) evaluated yield for 28 maize populations also found greater inbreeding for population with broad genetic base. Vianna et al. (1982) concluded that populations with a broad genetic base, and which had never been exposed to inbreeding, tend to show higher inbreeding depression compared to narrow genetic base populations.

Regarding the expansion volume, the inbreeding estimates varied from -7.62 (UFV-MP4) to 4.32 (UFV-MP2) mL. g^{-1}, and in percentage was -24.0 to 23.7 %, respectively. While for grain yield, it varied from -36.0 (ARZM 07-49) to 28.8 % (UFV-MP2). Arnhold et al. (2007) found similar variation of inbreeding depression (-33.7 to 24.8%) between six tropical popcorn population for expansion volume. In contrast, Scapim et al. (2006b) reported positive inbreeding depression, ranging from 7.2 to 14.3% for expansion volume, and 10.5 to 45.2% for grain yield.

3.5. Genetic diversity and relationship with heterosis

Twenty-three primers SSR were used, and produced 111 alleles with an average of 4.8 alleles per locus. Dubreuil et al. (2006) using SSR bulk analysis in maize populations found average alleles per locus of 7.8. Similarly, Oppong et al. (2014) detected 145 alleles and 7.3 alleles per SSR locus using bulk DNA strategy for maize populations. The number of alleles per locus found here seems to be low if compared to the studies above. The genetic distance values ranged from 0.111 to 0.721, with an average GD of 0.51 (Table 7). The most closely

related population were UFV-MP1 and UFV-MP2 (GD= 0.111), while the most genetically distance populations were ARZM 07-49 and UFV-MP4 (GD=0.721). Two groups and one singleton were found based on Tocher's optimization procedure (Table 8). The first group included the tropical populations. The second group included all temperate populations. The singleton included the ARZM 07-49 population. The UPGMA dendrogram was correlated with similarity matrix, and a high cophenetic correlation (0.94) was obtained. da Silva et al. (2009) found a cophenetic correlation of 0.8 between UPGMA and genetic distance from bulk DNA of SSR markers. These authors also grouped 25 popcorn populations similarly between the UPGMA cluster and Tocher's method. Notably, if the cut-off point was defined at 0.49 (average of similarity), the same three groups would be defined by UPGMA (Figure 1), in agreement with Tocher's procedure.

The correlation between genetic distance based on SSR and heterosis was positive and low (r= 0.376, P<0.05) for grain yield, and was in accordance with Makumbi et al. (2018) that reported positive and low correlation (ranged from 0.14 to 0.40) between heterosis and genetic diversity from SSR markers in maize populations. On the other hand, Reif et al. (2003b) reported high correlation (r=0.63) between genetic distance and heterosis for grain yield in tropical maize populations. While for expansion volume, the correlation was non-significant (r= 0.064). This result is similar to findings in other studies that demonstrated absence of correlation between genetic distance and heterosis for expansion volume (Rinaldi et al. 2007; Munhoz et al. 2009). Some theoretical considerations has showed that the low correlation between heterosis and genetic diversity could be attributed to low genome coverage (Bernardo 1992). Therefore, the results suggest that the groups formed by genetic distance cannot be validated by the heterosis for grain yield and expansion volume

3.6. Evaluation of heterotic group

Since the correlation between genetic distance and heterosis was irrelevant, another UPGMA clustering (Figure 2) and Tocher's optimization procedure (Table 8) were performed to assess the heterotic relationship among nine popcorn populations. The midparent heterosis for grain yield was used as dissimilarity matrix (data not shown). Recent studies had used the specific heterosis and midparent heterosis as dissimilarity matrix to obtain heterotic groups (Laude and Carena 2015, Vancetovic et al. 2015).

The UPGMA cluster method from midparent heterosis did not have a good agreement with genetic background of the populations. The Sintético-UFV population, derived from Viçosa and Beija-Flor population, should be allocated in the same group of tropical populations. The cophenetic correlation coefficient between the UPGMA method and midparent heterosis dissimilarity was 0.604, indicating a limited reliability to represent heterotic relationship among the nine populations. Thus, Tocher's procedure was chosen to classify the heterotic groups. The optimization procedure established four heterotic groups. Heterotic group 1 contained Viçosa C4, Beija-Flor C4, Sintético-UFV, UFV-MP2, UFV-MP3 and UFV-MP4 populations. The heterotic group 2 contained UFV-MP1. Heterotic group 3 contained UFV-MP5, and the last group, contained the Argentinean population ARZM 07-49.

The Tocher's classification by midparent heterosis showed agreement with hierarchical and optimization procedure from SSR markers in the establishment of the tropical populations in same group. However, three temperate populations were grouped with the tropical populations, showing disagreement with origin. The Argentinean population was allocated separately in both methods of grouping. Vittorazzi et al. (2018) using Bayesian model from 15 SSR markers allocated the popcorn populations from South America (Argentina, Bolivia, Chile, Paraguay and Brazil) in the same group.

The midparent heterosis for grain yield was 17 %, and ranged from -1.13% to 42%. The results were intermediate to the results of some authors that reported midparent heterosis from well-known heterotic groups for grain yield. Rasmussen and Hallauer (2006) obtained heterosis values ranging from 18 to 72% (the average 34.6 %) for crosses between Iowa Stiff Stalk Synthetic (BSSS) and Non-BSSS populations. Keeratinijakal and Lamkey (1993) assessing cycles of reciprocal recurrent selection in the Iowa Stiff Stalk Synthetic (BSSS) and Iowa Corn Borer Synthetic (BSCB) heterotic groups, and reported an average heterosis of 25.44% when C0 populations were initially crossed.

The most outstanding midparent heterosis were for Sintético-UFV × UFV-MP1 (HG1 × HG2) and Sintético-UFV × UFV-MP5 (HG1 × HG4) crosses that showed for a grain yield 42 and 34%, respectively. Moreover, this last cross showed superior population means for expansion volume, which makes them potential populations to be used in hybrid development. Melchinger and Gumber (1998) recommended as criteria for identification of heterotic group in hybrid populations the superiority of mean performance. The Argentinean popcorn

population, ARZM 07-49, has a highlighted average midparent heterosis for grain yield, however it should not be used, since, for expansion volume, it had the worst performance.

Some temperate populations from different heterotic groups, but same origin, such as UFV-MP2 × UFV-MP5, had a great midparent heterosis for grain yield (28%), however, cannot be considered good combination, because has very poor *per se* performance for grain yield.

4. CONCLUSIONS

Based on the results from heterosis analysis, there were genetic variability and dominance for expansion volume and grain yield. The heterosis and inbreeding analysis showed different dominance direction for expansion volume. Weak correlation was found between genetic distance SSR markers and heterosis from phenotypic data. The Tocher's optimization procedure from midparent heterosis showed four heterotic groups, and was agreement with origin and SSR clustering for tropical populations grouping. A promising heterotic group was created from population cross, Sintético-UFV and UFV-MP5, high heterosis for grain yield and superiority for expansion volume was observed.

5. REFERENCES

Andrade RA de, Cruz CD, Scapim CA, Silvério L, Pinto RJB and Tonet A (2002) Análise dialélica da capacidade combinatória de variedades de milho-pipoca. **Acta Scientiarum - Agronomy** 1197–1204.

Arnhold E, Silva DJH da, Filho OL de M and Viana JMS (2007) Inbreeding depression simulation in popcorn cultivars to estimate the effective population size for germplasm. **Crop Breeding and Applied Biotechnology 7**: 87–93.

Barata C and Carena MJ (2006) Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. **Euphytica 151**: 339–349.

Bernardo R (1992) Relationship between single-cross performance and molecular marker heterozygosity. **Theoretical and Applied Genetics 83**: 628–634.

Brito GG de, Caixeta ET, Gallina AP, Zambolim EM, Zambolim L, Diola V and Loureiro ME (2010) Inheritance of coffee leaf rust resistance and identification of AFLP markers linked to the resistance gene. **Euphytica** 255–264.

Cabral PDS, Júnior AT do A, Viana AP, Viera HD, Freitas IL de J, Vittorazzi C and Vivas M

(2015) Combining ability between tropical and temperate popcorn lines for seed quality and agronomic traits. **Australian Journal of Crop Science 9**: 256–263.

Cherchali FZ, Ordás B, Revilla P, Pedrol N and Djemel A (2018) Heterotic Patterns among Algerian , US Corn Belt , and European Flint Maize Populations under the Mediterranean Conditions of North Africa. **Crop Science 58**: 1–11.

CIMMYT (2005) **Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory**, Third Edit. Mexico, D.F.

da Silva TA, Pinto RJB, Scapim CA, Mangolin CA, Machado M de FP da S and Carvalho MSN (2009) Genetic divergence in popcorn genotypes using microsatellites in bulk genomic DNA. **Crop Breeding and Applied Biotechnology** 31–36.

Devi P and Singh NK (2011) Heterosis, molecular diversity, combining ability and their interrelationships in short duration maize (Zea mays L.) across the environments. **Euphytica 178**: 71–81.

Diniz LEC, Sakiyama NS, Lashermes P, Caixeta ET, Oliveira ACB, Zambolim EM, Loureiro ME, Pereira AA and Zambolim L (2005) Analysis of AFLP markers associated to the Mex -1 resistance locus in Icatu progenies. **Crop Breeding and Applied Biotechnology** 387–393.

Dubreuil P, Rebourg C, Merlino M and Charcosset A (1999) Evaluation of a DNA pooled-sampling strategy for estimating the RFLP diversity of maize populations. **Plant molecular biology reporter 17**: 123–138.

Dubreuil P, Warburton M, Chastanet M, Hoisington D and Charcosset A (2006) More on the introduction of temperate maize into Europe: Large-scale bulk SSR genotyping and new historical elements. **Maydica 51**: 281–291.

Fan X, Bi Y, Zhang Y, Jeffers D, Yin X and Kang M (2018) Improving Breeding Efficiency of a Hybrid Maize Breeding Program Using a Three Heterotic-Group Classification. **Agronomy Journal 110**: 1209–1216.

Gardner C and Eberhart S (1966) Analysis and Interpretation of the Variety Cross Diallel and Related Populations Author (s): C . O . Gardner and S . A . Eberhart Published by : International Biometric Society Stable. **Biometrics 22**: 439–452.

Griffing B (1956) Concept of General and Specific Combining Ability in Relation to Diallel Crossing Systems. **Australian Journal of Biological Sciences 9**: 463.

Hochholdinger F and Baldauf JA (2018) Heterosis in plants. **Current Biology 28**: R1089–

R1092.

Jaccard P (1908) Nouvelles recherche's sur la distribution florale. Bull Soc Vaud Sci Nat 44:223-270.

Jumbo MB and Carena MJ (2008) Combining ability , maternal , and reciprocal effects of elite early-maturing maize population hybrids. **Euphytica** 325–333.

Keeratinijakal V and Lamkey KR (1993) Responses to Reciprocal Recurrent Selection in BSSS and BSCB1 Maize Populations. **Crop Science** 73–77.

Laude TP and M.J.Carena (2015) Genetic diversity and heterotic grouping of tropical and temperate maize populations adapted to the northern. **Euphytica** 661–677.

Lee M (1995) Dna markers and plant programs. **advances in agronomy 55**: 265–344.

Li YL, Lv DB, Wang YZ, Chen SJ and Tang JH (2004) Study on the genetic diversity of popcorn inbreds and their germplasm relationship with normal corn inbreds using SSR markers. **Maydica 49**: 3–9.

Makumbi D, Assanga S, Diallo A, Magorokosho C, Asea G, Worku M and Bänziger M (2018) Genetic Analysis of Tropical Midaltitude- Adapted Maize Populations under Stress and Nonstress Conditions. **Crop Science** 1492–1507.

Melani MD and Carena MJ (2005) Alternative maize heterotic patterns for the northern Corn Belt. **Crop Science 45**: 2186–2194.

Melchinger AE and Gumber RK (1998) Overview of Heterosis and Heterotic Groups in Agronomic Crops. **In : K.R. Lamkey, J.E. Staub (Eds.), Concepts and Breeding of Hetero- sis in Crop Plants. CSSA, Madison, WI.** 29–44.

Miranda GV, De Souza LV, Galvão JCC, Guimarães LJM, De Melo AV and Dos Santos IC (2008) Genetic variability and heterotic groups of Brazilian popcorn populations. **Euphytica 162**: 431–440.

Mukanga M, Derera J and Tongoona P (2010) Gene action and reciprocal effects for ear rot resistance in crosses derived from five tropical maize populations. **Euphytica 174**: 293–301.

Mundim GB, Viana JMS, Maia C, Paes GP, DeLima RO and Valente MSF (2015) Inferring tropical popcorn gene pools based on molecular and phenotypic data. **Euphytica 202**: 55–68.

Munhoz REF, Prioli AJ, Amaral AT, Scapim CA and Simon GA (2009) Genetic distances between popcorn populations based on molecular markers and correlations with heterosis

estimates made by diallel analysis of hybrids. **Genetics and Molecular Research 8**: 951–962.

Oliveira KM, Laborda PR, Garcia AAF, Paterniani MEAGZ and Souza APDE (2004) Evaluating genetic relationships between tropical maize inbred lines by means of AFLP profiling. **Hereditas 33**: 24–33.

Oppong A, Bedoya CA, Ewool MB, Asante MD, Thompson RN, Adu-Dapaah H, Lamptey JNL, Ofori K, Offei SK and Warburton ML (2014) Bulk genetic characterization of Ghanaian maize landraces using microsatellite markers. **Maydica 59**: 1–8.

Pacheco CAP, Santos MX dos, Cruz CD, Parentoni SN, Guimarães PE de O, Gama EEG e, Silva ÁE da, Carvalho HWL de and Júnior PAV (2002) Inbreeding depression of 28 maize elite open pollinated varieties. **Genetics and Molecular Biology 25**: 441–448.

Rao CR (1952) Advanced statistical methods in biometric research. John Wiley & Sons, New York, 390p.

Rasmussen, C.C., A. R. Hallauer. 2006. Evaluation of heterotic patterns of Iowa Stiff Stalk Synthetic maiz populations. **Maydica** 51:177-186.

Reid LM, Xiang K, Zhu X, Baum BR and Molnar SJ (2011) Genetic diversity analysis of 119 Canadian maize inbred lines based on pedigree and simple sequence repeat markers. **Canadian Journal of Plant Science** 651–661.

Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA, Vasal SK, Beck D, Bohn M and Frisch M (2003) Use of SSRs for establishing heterotic groups in subtropical maize. **Theoretical and Applied Genetics** 947–957.

Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA, Vasal SK, Srinivasan G, Bohn M and Frisch M (2003) Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. **Crop Science 43**: 1275–1282.

Revilla P, Boyat A, Álvarez Á, Gouesnard B, Soengas P, Ordás A and Malvar RA (2006) Heterotic patterns among french and spanish maize populations. **Maydica 51**: 525–535.

Richard C, Osiru DS, Mwala MS and Lubberstedt T (2016) Genetic diversity and heterotic grouping of the core set of southern African and temperate maize (Zea mays L) Inbred lines using SNP markers. **Maydica** 1–9.

Rinaldi DA, Pípolo VC, Gerage AC, Ruas C de F, Júnior N da SF, Souza A de, Souza SGH de and Garbuglio DD (2007) Correlation between heterosis and genetic divergence estimated of diallel crosses and RAPD molecular markers in populations of popcorn. **Bragantia 66**:

183–192.

Rohlf FH (2009) **NTSYSpc: numerical taxonomy and multi- variate analysis system**.

Santacruz-Varela A, Widrlechner MP, Ziegler KE, Salvador RJ, Millard MJ and Bretting PK (2004) Phylogenetic relationships among North American popcorns and their evolutionary links to Mexican and South American popcorns. **Crop Science 44**: 1456–1467.

Scapim CA, Braccini A de L e, Pinto RJB, do Amaral AT, Rodovalho MA, Silva RM da and Moterle LM (2006) Average genetic components and inbreeding depression in popcorn populations. **Ciencia Rural 36**: 36–41.

Scapim CA, Pinto RJB, Amaral AT Do, Mora F and Dandolini TS (2006) Combining ability of white grain popcorn populations. **Crop Breeding and Applied Biotechnology 6**: 136–143.

Scott AJ and Knott M (1974) A Cluster Analysis Method for Grouping Means in the Analysis of Variance. **Biometrics 30**: 507–512.

Silva VQR da, Júnior AT do A, Gonçalves LSA, Júnior SPF, Candido LS, Vittorazzi C, Moterle LM, Vieira RA and Scapim CA (2010) Combining ability of tropical and temperate inbred lines of popcorn. **Genetics and Molecular Research 9**: 1742–1750.

Soengas P, Ordás B, Malvar RA, Revilla P and Ordás A (2003) Heterotic patterns among flint maize populations. **Crop Science 43**: 844–849.

Solalinde JMQ, Scapim CA, Viera RA, Junior AT do A, Vivas M, Pinto RJB, Mora F and Viana AP (2014) Performance of popcorn maize populations in South American Avatí Pichingá using diallel analysis. **Australian Journal of Crop Science 8**: 1632–1638.

Vancetovic J, Bozinovic S, Ignjatovic-Micic D, Delic N, Kravic N and Nikolic A (2015) A diallel cross among drought tolerant maize populations. **Euphytica 205**: 1–16.

Viana JMS (2000) The parametric restrictions of the Gardner and Eberhart diallel analysis model: Heterosis analysis. **Genetics and Molecular Biology 23**: 869–875.

Viana JMS and Matta F de P (2003) Analysis of general and specific combining abilities of popcorn populations, including selfed parents. **Genetics and Molecular Biology 26**: 465–471.

Vianna RT, Gama EEG, Naspolini Filho V, Morô JR and Vencovsky R (1982) Inbreeding depression of several introduced populations of maize (Zea mays L.). **Maydica** 27:151-157.

Vittorazzi C, Junior ATA, Guimarães AG, Silva FHL, Pena GF, Daher RF, Gerhardt IFS,

Oliveira GHF, Santos PHAD, Souza YP, Kamphorst SH and Lima VJ (2018) Evaluation of genetic variability to form heterotic groups in popcorn. **Genetics and Molecular Research 17**: 1–17.

Table 1 SSR primers, Bin, motif, and number of alleles found.

Primer	Bin	Motif	Alleles
umc1676	1.05	(GCC)7	4
umc2223	1.10	(GCG)4	2
umc1605	1.12	(GGC)4	5
bnlg1297	2.02	(AG)32	6
umc2214	2.10	(CTT)4	7
umc1167	3.05	(AG)12	2
umc1594	3.09	(TA)10	7
umc1288	4.02	(TCCA)4	4
phi096	4.04	(AGGTG)	6
phi079	4.05	(AGATG)	6
umc1447	5.03	(CTT)4	2
bnlg161	5.05	-	12
umc1006	6.02	(GA)9	7
umc2165	6.07	(TTC)12	5
umc2057	7.02	(GCT)8	6
bnlg1070	7.03	(AG)15	3
phi115	8.03	(AT/ATAC)	3
bnlg240	8.06	-	5
phi233376	8.09	(CCG)	4
umc1078	9.05	(GT)13	6
umc1505	9.08	(AAAAC)4	2
umc1336	10.03	(ACCAG)4	2
bnlg1518	10.04	(AG)15	5

Table 2 Combined analyses of variance of nine populations, their hybrids and reciprocals, and the selfed populations, and coefficients of variation concerning expansion volume and grain yield

Source of variation	Expansion vol.		Grain yield	
	df	MS	df	MS
Environments	1	60.8822**	1	2819143.7**
Replications/Env.	4	3.0107	4	76844.3
Blocks/Rep./Env.	54	4.2446**	54	48762.4
Entries	89	16.5936**	89	396379.8**
Population	8	208.5680**	8	3330319.3**
Heterosis	36	4.5307**	36	125621.6**
Average heterosis	1	16.2853**	1	2094203.9**
Population heterosis	8	7.1881**	8	82162.8*
Specific heterosis	27	3.3080**	27	65587.8*
Inbreeding	9	7.7620**	9	249903.9**
Reciprocals	36	2.8140*	36	67136.9**
Entries x Env.	84	2.4050*	84	96487.8**
Var. x Env.	8	228.0094**	8	4630462**
Het. x Env.	36	8.8308**	36	278713.4**
Av. het. x Env.	1	23.9933**	1	2148178.0**
Pop. het. x Env.	8	8.6699**	8	174848.5**
Spec. het. x Env.	27	8.3169**	27	240248.7**
Inbr. x Env.	9	14.6828**	9	346959.8**
Rec. x Env.	36	8.2609**	36	147097.5**
Pooled error	251	1.7078	250	36531.5
CV%		10.8		14.3

*Significant at 1% by the F test.

Table 3 Estimates of the population mean ($\hat{m} + \hat{v}_j$), average heterosis ($\hat{H}$), population heterosis ($\hat{H}_j$), and change in the population mean due to inbreeding ($\hat{d}_j$), relative to expansion volume and grain yield

Population	Expansion volume			Grain yield		
	$\hat{m} + \hat{v}_j$	$\hat{H}_j$	$\hat{d}_j$	$\hat{m} + \hat{v}_j$	$\hat{H}_j$	$\hat{d}_j$
Viçosa C4	27.65[b]	0.74	1.24	3052.83[b]	431.91	-543.42
Beija-Flor C4	31.32[a]	0.47	-4.35	3288.65[b]	408.56	-622.40
Sintético-UFV	31.25[a]	0.25	-4.55	3054.46[b]	611.82	-623.57
ARZM 07-49	13.52[c]	0.90	3.21	4480.18[a]	762.30	-1654.22
UFV-MP1	28.92[b]	2.42	0.67	2166.71[c]	499.28	357.91
UFV-MP2	28.77[b]	4.90	4.32	2188.32[c]	369.14	631.88
UFV-MP3	33.65[a]	1.44	-0.05	2545.17[c]	399.98	64.13
UFV-MP4	31.61[a]	1.20	-7.62	2378.09[c]	360.42	248.35
UFV-MP5	30.51[a]	2.09	3.30	2070.74[c]	538.52	311.65
$\hat{H}$		1.60			486.8	

Means followed by the same letter belongs to the same group.

Table 4 Estimates of the heterosis (below the diagonal) and specific heterosis (above the diagonal) for expansion volume.

Population	Viçosa C4	Beija-Flor C4	Sintético-UFV	ARZM 07-49	UFV-MP1	UFV-MP2	UFV-MP3	UFV-MP4	UFV-MP5
Viçosa C4		-2.64	-5.70	-4.61	-3.49	-2.70	-0.60	-1.84	-4.96
Beija-Flor C4	0.18		-4.93	-4.80	-2.41	-2.93	-2.19	-3.73	-3.19
Sintético-UFV	-3.09	-2.59		-5.09	-4.01	2.78	-3.829	-3.71	-2.54
ARZM 07-49	-1.35	-1.81	-2.32		-2.04	-3.35	-3.06	-2.49	-0.94
UFV-MP1	1.28	2.09	0.27	2.89		-3.79	-3.36	-3.63	-2.13
UFV-MP2	4.55	4.05	9.55	4.07	5.14		-5.91	-3.86	-2.62
UFV-MP3	3.19	1.33	-0.52	0.90	2.11	2.03		-2.47	-4.43
UFV-MP4	1.72	-0.44	-0.65	1.22	1.60	3.85	1.78		-4.34
UFV-MP5	-0.53	0.98	1.41	3.65	3.98	5.97	0.70	0.55	

Table 5 Estimates of the heterosis (below the diagonal) and specific heterosis (above the diagonal) for grain yield.

Population	Viçosa C4	Beija-Flor C4	Sintético-UFV	ARZM 07-49	UFV-MP1	UFV-MP2	UFV-MP3	UFV-MP4	UFV-MP5
içosa C4		-1132.80	-1029.58	-825.59	-1070.39	-852.44	-1225.42	-962.06	-746.80
Beija-Flor C4	194.55		-1179.64	-927.13	-1069.99	-883.25	-686.46	-873.81	-1115.35
Sintético-UFV	501.03	327.62		-783.83	-505.44	-1497.45	-980.60	-919.41	-769.20
ARZM 07-49	855.49	730.61	1077.17		-900.33	-1063.15	-976.63	-1003.70	-1034.33
UFV-MP1	347.68	324.73	1092.54	848.13		-871.00	-1243.76	-1031.75	-1085.05
UFV-MP2	435.49	381.33	-29.61	555.17	484.30		-731.54	-1209.79	-799.22
UFV-MP3	93.35	608.97	518.08	672.53	142.38	524.47		-880.07	-1152.52
UFV-MP4	317.16	382.06	539.71	605.90	314.83	6.66	367.22		-1035.97
UFV-MP5	710.51	318.62	868.02	753.37	439.62	595.32	272.86	349.86	

Table 6 Estimates of the reciprocals effects for expansion volume (above the diagonal) and grain yield (below the diagonal).

Population	Viçosa C4	Beija-Flor C4	Sintético-UFV	ARZM 07-49	UFV-MP1	UFV-MP2	UFV-MP3	UFV-MP4	UFV-MP5
Viçosa C4		-0.43	-2.59	-1.43	-1.37	-0.35	-0.28	2.38	-0.01
Beija-Flor C4	-87.06		-0.12	-1.09	-0.15	-1.28	1.66	0.08	-0.01
Sintético-UFV	-290.58	-65.36		2.61	-0.98	-0.08	-1.10	0.69	0.26
ARZM 07-49	-125.49	216.62	-151.04		-2.08	-1.19	-1.54	0.58	1.04
UFV-MP1	-85.27	1.80	51.24	207.5		-0.51	-0.15	0.29	-0.22
UFV-MP2	-27.72	31.17	206.17	262.60	-51.44		1.26	-1.21	0.43
UFV-MP3	-22.24	13.14	295.77	288.26	7.70	235.88		1.35	1.91
UFV-MP4	-321.44	-66.76	81.12	-574.17	12.13	-35.97	224.49		0.28
UFV-MP5	-4.55	-52.19	24.01	323.99	-135.77	12.84	-5.33	-90.24	

Table 7 Genetic distances between the populations.

Population	Viçosa C4	Beija-Flor C4	Sintético-UFV	ARZM 07-49	UFV-MP1	UFV-MP2	UFV-MP3	UFV-MP4
Beija-Flor C4	0.260							
Sintético-UFV	0.264	0.254						
ARZM 07-49	0.567	0.529	0.615					
UFV-MP1	0.568	0.542	0.507	0.679				
UFV-MP2	0.592	0.568	0.535	0.667	0.111			
UFV-MP3	0.600	0.595	0.543	0.691	0.306	0.353		
UFV-MP4	0.603	0.560	0.568	0.721	0.327	0.340	0.491	
UFV-MP5	0.590	0.584	0.554	0.646	0.483	0.517	0.389	0.578

Table 8 Different groups of popcorn populations using Tocher's optimization procedure.

Method	Groups	Populations
SSR	1	Viçosa C4, Beija-Flor C4, Sintético-UFV
	2	UFV-MP1,UFV-MP2,UFV-MP3,UFV-MP4, UFV-MP5
	3	ARZM 07-49
Midparent Heterosis	1	Viçosa C4, Beija-Flor C4, Sintético-UFV, UFV-MP2, UFV-MP3, UFV-MP4
	2	UFV-MP1
	3	UFV-MP5
	4	ARZM 07-49

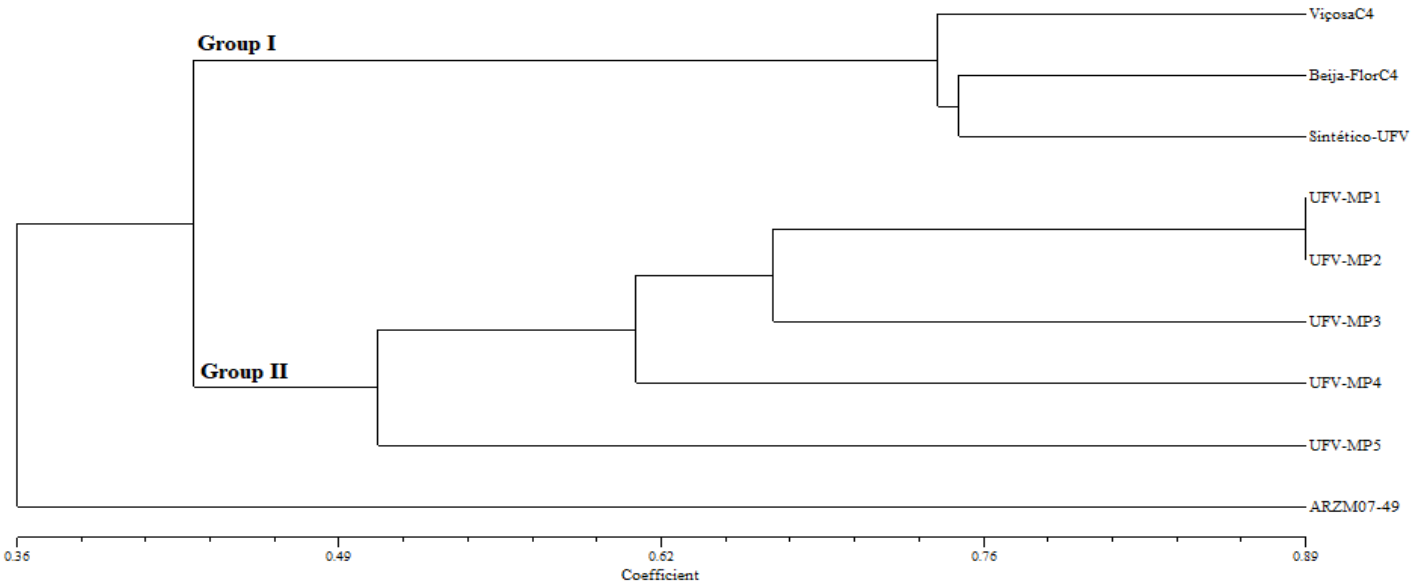

Figure 1 Dendrogram of the nine popcorn populations constructed based on Jaccard similarity matrix from 23 SSR markers and revealed by UPGMA.

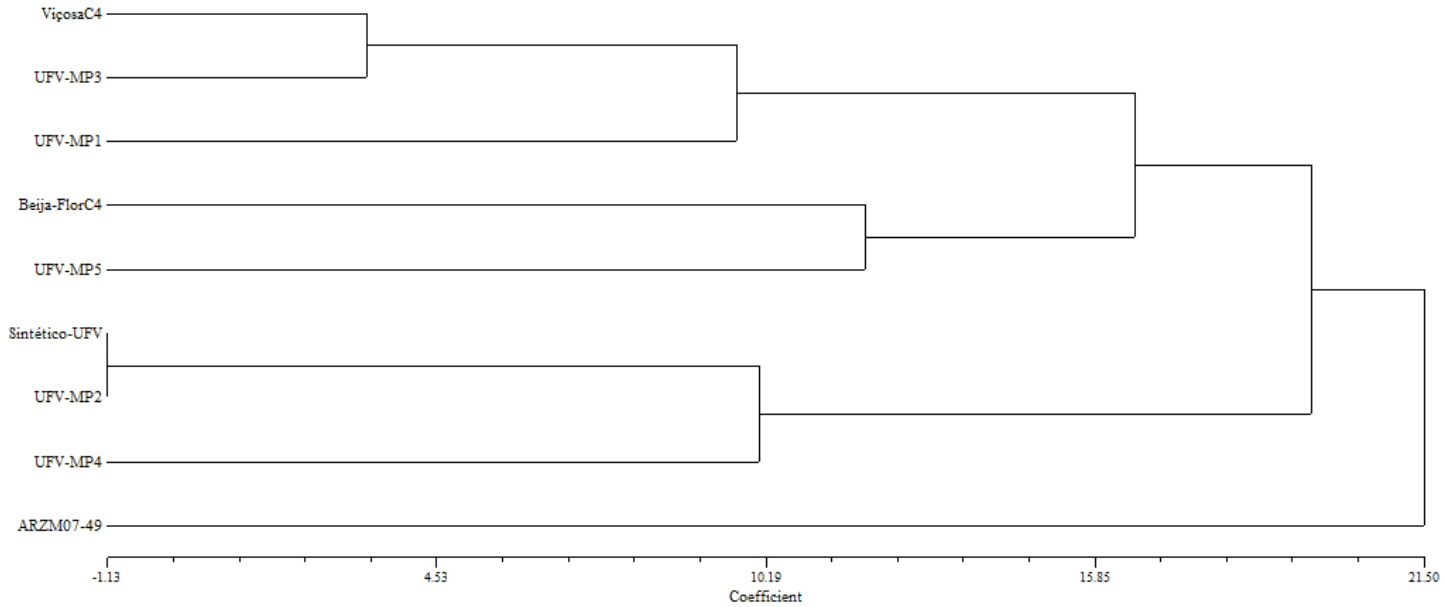

Figure 2 Dendrogram of heterotic group among nine popcorn population based on percentage of heterosis for grain yield and revealed by UPGMA.