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Genetic divergence in popcorn lines detected by microsatellite markers

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ABSTRACT - *The purpose of this study was to use microsatellite markers to evaluate the genetic divergence in 10 popcorn lines. The number of alleles per locus ranged from 2 to 5. The proportion of polymorphic loci was highest (50%) in line Curagua, while Zélia II was the most monomorphic (100%). The arithmetic complement of similarity of Roger and Tanimoto for the 10 lines indicated lower genetic similarity between the lines Zélia II and Avati Pichinga I and higher similarity between Zélia I and Zélia II. The mean distance between groups was greatest between the group formed by lines IAC 112 I 112 IAC II, Avati Pichinga I, Avati Pichinga II and the group containing Yellow Pear Popcorn II. Strategies on how to use the heterosis between different lines in partial diallel crosses are indicated.*

Key words: *Zea mays* L, genetic similarity, microsatellites, heterotic groups.

INTRODUCTION

The most recent trend in breeding programs is to integrate traditional techniques and biotechnology. Particularly for the hybrid breeding programs the potential of DNA markers provides the identification of divergent genotypes which exploit heterosis for the yield components in populations and improved hybrids (Benchimol et al. 2000, Warburton et al. 2002, Aguiar et al. 2008, Vilela et al. 2008).

In the case of popcorn, the use of DNA markers has been rather rudimentary in the identification of lines and varieties in different heterotic groups as well as other application possibilities of the technology (Pereira et al. 2008)

More research work has been invested in breeding programs of maize, in which the efficiency of identifying heterotic groups of lines by RFLP procedures (Ajmone-Marsan et al. 1998, Benchimol et al. 2000, Pinto et al. 2003), by AFLP (Oliveira et al. 2004) and, more recently, by SSR markers has become evident (Reif et al. 2003, Barata and Carena 2006). Compared to conventional methods, molecular markers are advantageous since they discriminate little different lines and, consequently, heterotic groups are composed containing genotypes that unequivocally represent the differences in allele frequencies of the populations.

These studies are consistent, especially since the highly efficient biometric information for the

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identification of promising lines in crosses is not discarded, such as estimates of combining ability or knowledge on the germplasm pedigree.

In popcorn, additional attention is required in the formation of the hybrid lines, since expansion volume is a second focus, besides yield. While yield depends in the first place on dominance effects, the expansion volume - main trait of grain quality - is influenced almost exclusively by additive effects Lyerly (1942), Pereira and Amaral Júnior (2001), Scapim et al. (2002), Simon et al. (2004), Freitas Júnior et al. (2006), Rangel et al. (2007), Rangel et al. (2008), Santos et al. (2008).

From the ethnobotanist aspect, popcorn is derived from selections of maize germplasm, with incorporation of dent maize genes, which results in a loss of expansion volume in popcorn, indicating that the genetic basis of popcorn germplasm is narrow (Kantety et al. 1995). In an evaluation of the genetic variability in 90 inbred popcorn lines – derived from the heterotic groups «South American», «Supergold» and «Amber Pearl» - and eight dent lines by ISSR markers, Kantety et al. (1995) noted the formation of different groups among the popcorn and dent lines and that the popcorn lines are, although derived from different groups, genetically very similar.

Studies with SSR markers have received some attention in the scientific community. Li et al. (2004) studied the genetic diversity in 56 popcorn lines that represent the broad genetic basis of the crop, apart from 21 maize lines from different heterotic groups of breeding programs in China. The authors investigated the clustering of differentiated popcorn and maize lines. Seven heterotic groups were identified with high concordance between the intragroup lines and previous breeding studies. They also emphasized the importance of the use of SSR markers in studies of genetic divergence with popcorn and maize lines.

Santacruz-Varela et al. (2004) used 29 morphological markers, 18 isoenzyme loci and 31 SSR loci to evaluate the relationship between popcorn germplasm representative of the Americas. The study included 56 populations from the US and 9 from Latin American countries, which were separated into groups, based on analyses of groups and main components. Three groups were proposed by genotype clustering, namely: i) " Yellow Pearl Popcorn," which represents the largest US trade group, ii) "North American Pointed Rice Popcorns", which is probably originated from a complex of traditional popcorn races from Latin America and iii) "North American and North American Early Popcorns" which are closely related with the flint lines from northern USA.

In Brazil the use of SSR markers in the identification of genetic variability of popcorn germplasm has not been reported so far. The rare studies, restricted to classical breeding methods for popcorn, are limiting for the development of superior hybrids for a great part of the popcorn-producing areas of the country. This is also true in the case of South America, Africa and Oceania.

In view of the high earning potential of the crop, this situation is paradox. In the opinion of Scapim et al. (2002) the possibility of mechanization and the total absence of a government price control boost the commercial value of popcorn to at least three times as high as common maize.

The purpose of this study was to evaluate the genetic similarity of the lines Yellow Pear Popcorn I, Yellow Pear Popcorn II, Zélia I, Zélia II, Curagua, IAC 112 I, IAC 112 II, Avati Pichinga I, Avati Pichinga II and Pisankalla, using microsatellite markers.

MATERIAL AND METHODS

Plant material

Ten popcorn lines were used. The lines Yellow Pearl Popcorn I and II were derived from an American compound. Zélia I and II were obtained from a triple hybrid of Pioneer. The line Curagua was originated from the Curagua population, from Chile. Lines IAC112 I and IAC 112 II were obtained from the modified triple hybrid IAC 112, Brazil. Lines Avati Pichinga I and II were derived from the Pichinga population from Paraguay. The line Pisankalla, originated from Bolivia, was provided by CIMMYT.

Seeds were sown in small earth-containing baskets and maintained at ideal temperature for their development.

DNA extraction

The genomic DNA of seven plants of each line was extracted from the leaf tissue, using the methodology described by Hoisington et al. (1994), with minor modifications.

Thereafter, the DNA concentration was estimated. Bands of total genomic DNA, separated by 0.8% agarose gel electrophoresis were to check the integrity and purity of the extracted DNA, by comparing the samples with a DNA of known phage λ concentration. After the quantification, DNA samples of good quality were diluted to a concentration of 10 ng μL^{-1}.

DNA amplification

The SSR amplification reactions were performed in a total of 20 µL, with 25 ng genomic DNA, 2.0 µL of the 10x reaction buffer (10 mM Tris-HCl, pH 8.8), 2.0 mM MgCl2 , 0.1 mM of each dNTP, 1 unit of Taq-DNA polymerase (Invitrogen), 0.2 mM of specific primers F and R and milli-Q water to a volume of 20 µL. The amplifications were performed in a thermal cycler, using Touchdown PCR (Don et al. 1991).

Fifty-one primers from the site <http://www.maizegdb.org> were tested. The amplified samples were fractionated in 4% agarose gel (50% agarose and 50% agarose MS-8) in TBE buffer X 0.5 (44.5 mM Tris, 44.5 mM boric acid and 1 mM EDTA). To estimate the amplification fragment size, we used a 100 bp molecular weight marker (Invitrogen). The gels were exposed to an electric field of 60 volts for about 5 h and then stained with 0.5 µg mL^{-1} ethidium bromide solution and photographed under UV light.

Statistical analysis

Seven plants from each S_7 line were analyzed. After amplification, a dissimilarity matrix was constructed expressed by the arithmetic complement of similarity of Roger and Tanimoto (Cruz 2006). Based on the dissimilarity matrix, the UPGMA (Unweighted Pair-Group Method Using Arithmetic Averages) was used for all pairs of lines as well as the Tocher optimization procedure, where the number of genotypes is partitioned in non-empty and not mutually exclusive subgroups. The clustering techniques were complemented by the graphic dispersion, based on a projection of spatial distances in three dimensions. All tests were performed using software GENES (Cruz 2006).

RESULTS AND DISCUSSION

Of the 51 primer pairs tested 27.4% were polymorphic, using the most common allele as criterion, with a frequency of less than 95%. The genetic variability among the 10 popcorn lines is expressed by the primers displayed in Table 1.

Table 1. Primer sequences of the microsatellite used to estimate genetic diversity of the 10 popcorn lines, number of alleles detected by each primer and their location in maize chromosomes

Locus	Nucleotide Sequence	Number of Alleles	Chromosome
umc2350	CGAATCGAGGATGGTTTGTTTTT (Reverse)	2	10
umc2350	AGTAGCGACTCCTCTGCGTGAG (Forward)		
umc 1636	GTACTGGTACAGGTCGTCGCTCTT (Reverse)	4	9
umc 1636	CATATCAGTCGTTCGTCCAGCTAA (Forward)		
umc 2262	CGTTCCCTGGTACCCTGTCTATAA (Reverse)	4	3
umc 2262	TCTGTTCGGGATTCTTCTTCAGTC (Forward)		
umc2227	AGCTGAGCCTTCTCTTCTTGGCT (Reverse)	4	1
umc2227	ACCTTGAGCGTGGAGTCGGT (Forward)		
umc 1422	CTCATCGCGATCTCCCAGTC (Reverse)	3	2
umc 1422	GAGATAAGCTTCGCCCTGTACCTC (Forward)		
umc 2343	GACTGACAACTCAGATTTCACCCA (Reverse)	3	9
umc 2343	TCATCTTCCCCACAAATTTTCATT (Forward)		
umc 2292	ACTTCCGGCATGTCTTGTGTTT (Reverse)	3	5
umc 2292	AGCAGAAGAGGACAAACCAGATTC (Forward)		
umc 1336	CTCTGTTTTGGAAGAAGCTTTTGG (Reverse)	2	10
umc 1336	GTACAAATGATAAGCAAGGGGCAG (Forward)		
umc 2245	CGTCGTCTTCGACATGTACTTCAC (Reverse)	3	2
umc 2245	GCCCTGTTATTGGAACAGTTTACG (Forward)		
umc1071	GTGGTTGTCGAGTTCGTCGTATT (Reverse)	3	1
umc1071	GTGGTTGTCGAGTTCGTCGTATT (Forward)		
umc2280	AAAAGAAGACGCTTTGTTTGTTGC (Reverse)	3	4
umc2280	TTTTCGTCAACTTGATGTTTATGAGAGT (Forward)		
umc2281	ATGATGATCTGCAGAGCCTAGTCC (Reverse)	5	4
umc2281	CAATGATTGGAGCCTAACCCCT (Forward)		
umc2293	ATGTTCCGTTTATTATTTGCCCG (Reverse)	3	5
umc2293	AAAGAACAGACGCGATCCAATC (Forward)		
umc1653	GCCGCCCACGTACATCTATC (Reverse)	5	6
umc1653	GAGACATGGCAGACTCACTGACA (Forward)		

The number of alleles per locus for the lines ranged from two to five, totaling 47 alleles. The largest number of alleles was observed for loci umc1653 and umc2281, while four alleles were found for umc2227, umc1636 and umc2262 (different alleles for the loci identified shown in Figure 1).

The occurrence of a null allele for locus umc2343 was clear for the lines Yellow Pear Popcorn II and IAC 112 I. For locus umc2227, the null allele was found for the lines Yellow Pear Popcorn II, IAC 112 I, Pisankalla, and Avati Pichinga II. According to Alvarez et al. (2001) null alleles may occur in microsatellite loci because not all primer regions determine an effective amplification in all samples analyzed, due to possible mutations in annealing regions, complementary to the primers used.

The proportion of polymorphic loci was highest (50%) in line Curagua, while Zélia II was the most monomorphic, with monomorphism in all loci. Based on the genetic distances, the lowest genetic similarity (0.8495) was detected for the lines and Zélia I and Avati Pichinga I and the greatest (0.2092) for Zélia I and Zélia II (Table 2).

At a vertical cut-off at 50% in the dendrogram, we find the formation of four groups, as follows: Group I, formed by lines 2 (Zélia I), 4 (Zélia II), 3 (Curagua) and 1 (Yellow Pearl Popcorn I); Group II, with only line 5 (Yellow Pearl Popcorn II), group III, comprising the inbred lines 6 (IAC112 I), 7 (IAC112 II), 8 (Avati Pichinga I) and 10 (Avati Pichinga II) and group IV, with only line 9 (Pisankalla) (Figure 2). A reliable relationship between the group and the genetic background of lines can be established, since genotypes derived from Zélia are contained in group I and genotypes derived from IAC and Avati Pichinga in Group III. Furthermore, the reliability of the group is reinforced by lines from germplasm of different regions distributed in separate groups as in the case of the genotypes from Chile and Paraguay, assigned to groups I and II, respectively.

To date there is no consensus on the establishment of an appropriate cut-off value in a dendrogram. Assuming that the cut-off point would be set at the level of greatest change - at around 60% - there would be two major groups. The line Yellow Pearl Popcorn II appears to be closer to the group of Zélias and Pisankalla closest to the group of IACs and Avatis. Since there is no conclusive method regarding the cut-off point of a dendrogram, the level was established at 50% for comparison purposes with other techniques that will be discussed below.

The Tocher grouping distributed the lines into four groups (Table 3). Group I comprised the lines Yellow Pear Popcorn I, Zélia I, Curagua and Zélia II; Group II, IAC 112 I, IAC 112 II, Avati Pichinga I and Avati Pichinga II; and finally, groups III and IV contained the lines Pisankalla and Yellow Pearl Popcorn II, respectively.

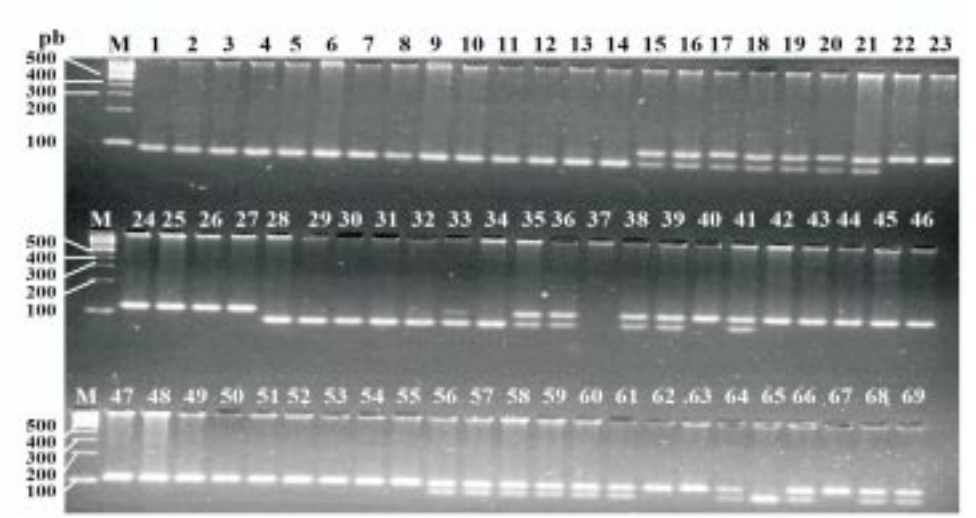

Figure 1. Amplification of the genomic DNA of the popcorn lines: Yellow Popcorn I (1 to 7); Zélia I (1 to 14), Curagua (15 to 21), Zélia II (22 to 28); Yellow Popcorn II (29 to 35); IAC 112 I (36 to 42): IAC 112 II (43 to 49), Avati Pichinga I (50 to 56); Pisankalla (57 to 63) and Avati Pichinga II (63 to 69), using Primer UMC 1071. M corresponds to the molecular weight marker (100 bp ladder) of Invitrogen

Table 2. Dissimilarity matrix constructed based on the arithmetic complement of genetic similarity of Roger and Tanimoto of 10 popcorn lines

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	0.2677	0.3289	0.3618	0.3402	0.6100	0.6387	0.6443	0.6501	0.6575
2	-	0.3056	0.2092	0.4046	0.6403	0.6228	0.7681	0.6945	0.6609
3		-	0.3117	0.4581	0.5374	0.6006	0.6436	0.6498	0.5657
4			-	0.3985	0.7424	0.6966	0.8495	0.7377	0.7559
5				-	0.7698	0.8359	0.7879	0.7194	0.7255
6					-	0.2194	0.3028	0.4212	0.2668
7						-	0.2861	0.3791	0.3221
8							-	0.4358	0.2649
9								-	0.3469

1 - Yellow Pearl Popcorn I; 2 - Zélia I; 3 - Curagua; 4 - Zélia II; 5 - Yellow Pearl Popcorn II; 6 - IAC112 I; 7- IAC112 II; 8 - Avati Pichinga I; 9 - Pisankalla; e 10 - Avati Pichinga II

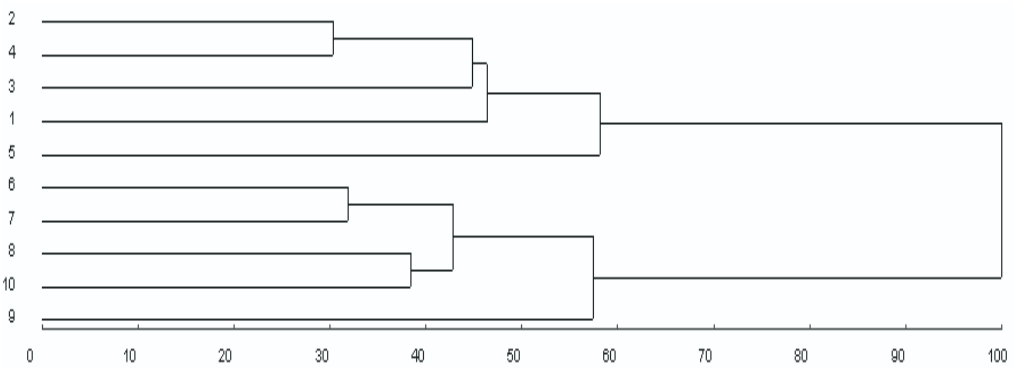

Figure 2. Grouping by the UPGMA method based on dissimilarity measures between 10 popcorn lines, expressed by Rogers genetic distances. The inbred lines are**:** 1 - Yellow Pearl Popcorn I; 2 - Zélia I; 3 – Curagua; 4 - Zélia II; 5 - Yellow Pearl Popcorn II; 6 - IAC112 I; 7 - IAC112 II; 8 - Avati Pichinga I; 9 – Pisankalla; and 10 - Avati Pichinga II

Table 3. Grouping of 10 popcorn lines, according to the Tocher procedure, applied to the matrix of genetic distances

Limit of the distance between groups	**Group formation**		**Mean distance between groups**	
	Group	**Lines**	**Group**	**Distance**
(1) 0.2677	1	2, 4, 3, 1	1	0.2975
(2) 0.2092	2	6, 7, 8, 10	1 x 2	0.6646
(3) 0.3056	3	9	1 x 3	0.6830
(4) 0.2092	4	5	1 x 4	0.4004
(5) 0.3402			2	0.2770
(6) 0.2194			2 x 3	0.3958
(7) 0.2194			2 x 4	0.7798
(8) 0.2649			3	.
(9) 0.3469			3 x 4	0.7194
(10) 0.2649			4	.

1 - Yellow Pearl Popcorn I; 2 - Zélia I; 3 - Curagua; 4 - Zélia II; 5 - Yellow Pearl Popcorn II; 6 - IAC112 I; 7 - IAC112 II; 8 - Avati Pichinga I; 9 - Pisankalla; and 10 - Avati Pichinga II

Although the algorithms underlying clustering were different, the groups by UPGMA and Tocher are closely associated, since the same genotypes were grouped together by both techniques. The groups are therefore highly consistent, which makes the accuracy of SSR markers high in the discrimination of popcorn genotypes, as reported by Li et al. (2004) and Santacruz-Varela et al. (2004).

Estimates of mean distance between groups (Table 3) show that heterotic levels are highest in hybridizations between lines of Group II and IV. At least four single hybrids are promising between crosses of Yellow Pearl Popcorn II with: IAC 112 I, IAC 112 II, Avati Pichinga I and Avati Pichinga II. This does however not invalidate the perspective of crosses between lines of other groups to explore a possible heterosis of hybrids therefrom. It is believed that better use should be made of the allelic complementation of the lines in partial diallel crosses, focusing on crosses between genetically more distant lines.

The relative position of the lines arranged in three-dimensional projection (Figure 3), revealed the formation of the same groups as by UPGMA and Tocher. The stress was estimated at 0.20, a value considered moderate, according to the scale of Kruskall (1964), which corroborates the reliability of the interpretation of the projection. The relative position of the lines arranged in three dimensions suggests that within the group formed by the lines Yellow Pear Popcorn I, Zélia I, Curagua and Zélia II, there is a clearer approximation between Yellow Pear Popcorn and I Curagua, similarly to the lines IAC 112 I and Avati Pichinga II, in Group II.

In the three-dimensional projection, some caution is needed when drawing conclusions based on the closeness or distance indicated by this technique, since not all multivariate vectors include the area where the lines should appear. However, it was clearly verified that the three-dimensional projection revealed the same results as observed for the UPGMA and Tocher groupings, indicating an interesting complementary research technique.

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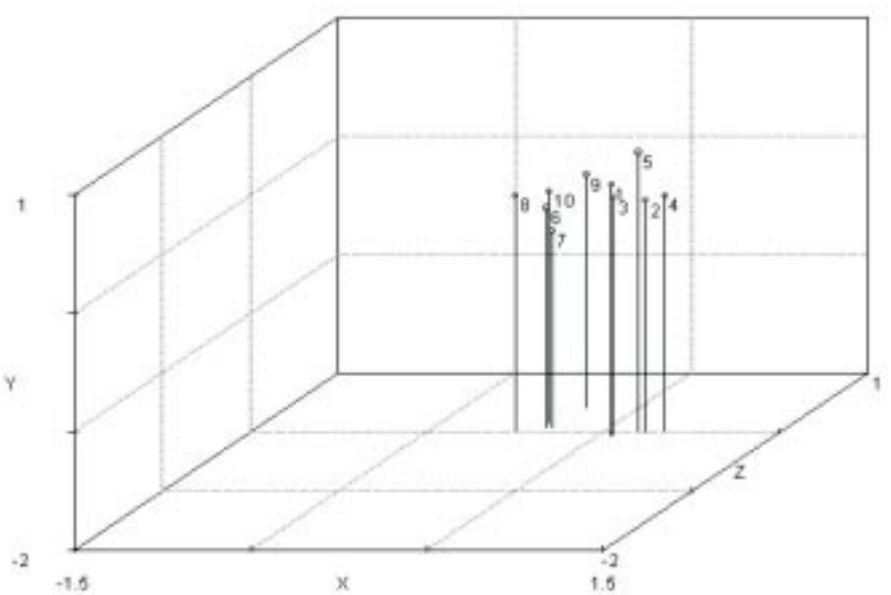

Figure 3. Three-dimensional projection of genetic distances among all pairs of the 10 popcorn lines without processing (root) and no data rotation. 1 - Yellow Pearl Popcorn I; 2 - Zélia I; 3 – Curagua; 4 - Zélia II; 5 - Yellow Pearl Popcorn II; 6 - IAC112 I; 7 - IAC112 II; 8 - Avati Pichinga I; 9 – Pisankalla; and 10 - Avati Pichinga II

Divergência genética entre linhagens de milho pipoca por marcadores SSR

RESUMO - *Objetivou-se utilizar marcadores SSR para avaliar a divergência genética entre dez linhagens de milho pipoca. O número de alelos por loco variou de dois a cinco. A linhagem Curagua foi a que apresentou a maior proporção de locos polimórficos (50%), enquanto a Zélia II foi a mais monomórfica (100%). Os complementos aritméticos do coeficiente de similaridade de Roger e Tanimoto entre as dez linhagens revelaram menor similaridade genética entre as linhagens Zélia II e Avati Pichinga I e maior similaridade entre Zélia I e Zélia II. A distância média entre os grupos revelou maior divergência entre o grupo formados pelas linhagens IAC 112 I, IAC 112 II, Avati Pichinga I, Avati Pichinga II em relação ao grupo formado pela linhagem Yellow Pear Popcorn II. Indicam-se estratégias de cruzamentos dialélicos parciais para aproveitamento da heterose entre linhagens divergentes.*

Palavras-chave: *Zea mays* L, similaridade genética, microssatélites, grupos heteróticos.

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