Popcorn parental selection based on genetic divergence

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ABSTRACT

Nine popcorn genotypes were assessed for eight traits and their genetic divergence was estimated by multivariated techniques to identify parents for inclusion in breeding programs involving hybridization. The nine genotypes were divided into two groups by the Tocher method using the generalized Mahalanobis distances. The popping expansion, popcorn size and grain mass per plant were considered the most important agronomic traits for selecting parents for crossing. Using the divergence and these trait scores as criteria for crossing, the crosses between genotype 9 (group II) and genotypes 8, 3, 7, and 5 (group I) were recommended. Therefore, only four (11%) out of 36 possible (diallel) crosses among the nine assessed genotypes would be made.

KEY WORDS: Grouping, multivariate analysis, popping expansion, popcorn.

INTRODUCTION

Popcorn culture in Brazil has not received the same attention as the common corn culture, and little progress has been reported by improvement programs and researches. In addition, the recommended cultivars have shown problems of susceptibility to diseases and low quality compared to their corresponding American hybrids.

Popcorn quality is conditioned to popping expansion (PE), which must be above 25, i.e., a grain volume equivalent to 25 volumes of expanded popcorn. The greater the popping expansion of a specific variety the greater its tenderness and commercial value. This trait has a negative correlation with the majority of the agronomic traits, thus, in general, popcorn cultivars are more fragile and susceptible to diseases, layering and breakage (Gama et al., 1990; Pípolo et al., 2002).

The main PE genetic variation component is given by the additive genetic effects, and, in some cases, by the dominance effects. In crosses between low PE lines and high PE lines, hybrids with the PE close to the average, and with clear additive effect dominance, are obtained (Lyerly, 1942; Alexander and Creech, 1977; Dofing et al., 1991; Sawazaki, 1996).

One of the most important steps in genetic improvement programs involving hybridization is the selection of parentals with an effective performance and a wide genetic basis. One alternative for choosing a parental is to analyze its behavior in isolation or in diallel crosses. However, the needed to carry out a large number of manual crosses and experiments involving a great number of hybrids has limited the use of diallel crosses (Miranda Filho, 1974; Naspolini Filho et al., 1981; Môro, 1987).

Therefore, genetic divergence measurements obtained prior to any cross can help improvers focus on the most promising combinations only. The heterosis manifested in the crosses is related directly to the genetic divergence found among the parentals (Falconer, 1981).

Smith (1984) studied the genetic variability in corn hybrids in the USA, based on the allelic frequency of 21 loci obtained through the use of isoenzymes. By analyzing the dispersion in relation to the first and second components, which involved 27 and 15 % of the total variation, as well as the dispersion to the third and fourth component, which involved 14,4 and 10% of the variation, the same author concluded that 10 out of the 111 hybrids were identical or very close, and that around 90% of them showed different allelic frequencies. However, in many cases, these differences were due to the small allelic frequency variations found in a few loci.

Although the importance of genetic divergence in selecting parental for improvement programs by hybridization has been highly emphasized, the relationship between parental divergence and the production potential of its hybrids is still being discussed. In experiments with barley carried out by Chaudhary and Singh (1975), the relationship between genetic divergence, measured by the intragroup distance and heterosis in yield, was not evidenced. These authors credit these results to the complexity of the grain yield resulted from the large number of inter and intragenic interactions, when only a few characters are used during the divergence estimation. Nonsignificant correlations between parental divergence and grain yield heterosis were also found in vicia faba L. by Ghaderi et al. (1984). Melo et al. (2001) found genetic divergence in ten corn hybrids. However, they concluded that this high parental divergence estimation does not guarantee high heterosis in crosses. Thus, it was not possible to infer on the heterosis expressed in one cross from the genetic divergence found among the parentals under study.

Cruz (1990) concluded that multivaried procedures such as the analysis of conglomerations based on Euclidian or Mahalanobis distances and the analysis of main components or canonical variables are efficient in predicting the behavior of corn hybrids via information from parentals. This finding can be evidenced by the Spearman correlation between the average behavior of hybrids, or their heterosis for grain production per plant, and the divergence among parentals, quantified by the generalized Mahalanobis distances, which ranged from 0,77 to 0,73, respectively. In alogamus crosses, the genetic divergence is a necessary but insufficient condition for heterosis to take place, since it depends on the allelic frequency differences and on the dominance (Cress, 1966).

The objective of this work was to estimate the genetic divergence in nine popcorn genotypes regarding eight agronomic traits, using multivariate techniques, in order to identify adequate parentals for genetic improvement programs.

MATERIAL AND METHODS

Nine popcorn populations 1- UEL-PAMP, 2- UEL-PAPYY, 3- UEL-PASHA, 4- UEL-PAMPGA, 5- UEL-PAMPCB, 6- UEL-PAPA, 7- UEL-PAAPC, 8- UEL-ZP and 9- UEL-PP from three generations selected from commercial popcorn genotypes were evaluated in an experiment carried out on the Universidade Estadual de Londrina, Londrina, Paraná, Brazil, during the agricultural years of 1997/98.

A randomized block experimental design with nine

treatments and eight replications was used. Parcels were constituted of two rows 5.0 m long with a density of 50.000 plants/ha. The soil in the experimental area was classified as Dark Red Dystrophic Latossolo, with an average texture and fertility level. Fertilization during planting was composed of 500 kg/ha of concentrate 8-28-16. Cover crop fertilization was composed of 100 kg/ha of urea, and was carried out 30 days after emergence (Instituto Agronômico de Campinas, 1996). The other culture treatments were those usually recommended for corn culture (EMBRAPA, 1996).

The following traits were evaluated: ear average length in cm (EL) from a randomized sample of 10 ears per parcel; ear total mass, without the husk, in grams (TEMP), estimated mean from the ear total mass, without the husk, per plant; cob weight in grams (CW); grain mass per plant in grams (GMP), determined by the grain total mass, per plant; grain yield in kg/ha (GY), estimated by the GMP, number of plants per parcel and parcel area; number of grain in 40 cm³ (NGV), determined by a grain sample of 40 cm³, taken from the central basal part of 10 ears per parcel; popping expansion (PE) measured by the relationship between popped popcorn volume and the number of grain used. Popping expansion was obtained from a sample containing 40 cm³ grain taken from the central basal part of 10 ears per parcel. After humidity became uniform (11%), grain expansion was obtained using a conventional popcorn maker, and the volume of the popcorn measured in one test tube of 1000 cm³. Popping standard time was set at 48 seconds, after the temperature in the popcorn maker reached 180 degrees C. They were popped in 2,5 mL of vegetal oil per 40 cm³ of grain. The temperature in the popcorn maker was measured by a Termopar equipment (Radionave TDR 1000 - Indústrias Eletrônicas, Londrina-Pr). This methodology was based on the studies of Sawazaki et al. (1986) and Nascimento and Boiteux (1994); on the % of unpopped grain (NUG); popcorn size in cm³ (PS), obtained by the relationship between popped grain volume and the number of popped grain.

In addition to the multivariate analyses of variance and the comparison of means by the criterion proposed by Duncan (5%), this study also: a) determined the genotypic coefficient, using computational resources from the Genes Program (Cruz, 1997); b) estimated the generalized Mahalanobis (1936) distances (D²); c) established similar groups through the optimization method proposed by Tocher and cited by Rao (1952). A detailed example of this method is presented by Singh and Chaudhary (1977); d) carried out the graphic analysis of the divergence using the canonical variables. Information on the genetic distance by each genotype dispersion scores in the Cartesian system, whose axis is established by the first canonical pair (Rao, 1952), were also determined.

Phenotypic, genotypic and environmental correlations were also estimated for the traits studied. The phenotypic (r_p) , genotypic (r_G) and environmental (r_E) correlation coefficients among all traits were calculated by the following formulas (Cruz and Regazzi, 1997):

$$r_{p} = COV_{P(x,y)} / (s_{Px}^{2} \cdot s_{Py}^{2})^{0.5}$$

$$r_{G} = COV_{G(x,y)} / (s_{Gx}^{2} \cdot s_{Gy}^{2})^{0.5}$$

$$r_{E} = COV_{E(x,y)} / (s_{Ex}^{2} \cdot s_{Ey}^{2})^{0.5}$$

where: $\text{COV}_{P(x,y)}$, $\text{COV}_{G(x,y)}$ and $\text{COV}_{E(x,y)}$ corresponds to the phenotypic, genotypic and environmental covariances, respectively, among traits x and y; s^2_{Px} , s^2_{Gx} and s^2_{Ex} correspond to the phenotypic, genotypic and environmental variances, respectively, for trait x; s^2_{Py} , s^2_{Gy} and s^2_{Ey} correspond to the phenotypic, genotypic and environmental variances, respectively, for trait y.

The phenotypic, genotypic and environmental correlation coefficient were estimated and tested to evaluate their significance using the t-test. The degree of freedom used for the phenotypic and genotypic correlation was the number of genotypes minus two (d.f.=9-2=7). The values found were 0,666 and 0,798

at 5% and 1% of significance, respectively. The degree of freedom of the residue (56) was adopted during the environmental correlation test. The values found were 0,259 and 0,337 at 5% and 1% of significance, respectively (Cruz and Regazzi, 1997).

RESULTS AND DISCUSSION

Except for popcorn size (PS) and number of grain per volume (NGV), the analysis of variance showed significant differences for all traits studied. The coefficient of variation (CV%) was inferior to 25% for the evaluated traits, except for number of unpopped grain (NUG, CV = 84,22%), which revealed good levels of experimental precision in the field trials. The genotypic determination coefficients (b%) for traits PE and GMP were 72,89% and 88,85%, respectively, suggesting the possibility of efficiently with efficiency superior parental genotypes in the nine lines evaluated (Table 1).

Genotypes UEL-ZP and UEL-PASHA showed a popping expansion (PE) of over 27,00 cm³ and popcorn size (PS) larger than 4,50 cm³. Next, the genotype UEL-PAAPC presented a PE equal to 24,40 cm³ and PS equal to 4,04 cm³, matching the commercial demands, according to Gama et al. (1990). These genotypes showed higher values for NGV, i.e., 244,75, 248,50 and 248,75, respectively (Table 2). Such results can be confirmed by the work of Souza Júnior et al. (1985), where popcorn popping expansion had a negative relationship with popcorn size and high yield. To provide a more thorough analysis, Table 2 includes genotype grain yield (kg/ ha), comparatively. It can be observed that although UEL-PP presented higher values for GY, UEL-ZP

Table 1. Analysis of variance of eight traits in nine popcorn genotypes, with their respective means, coefficients of variation (CV%) and genotypic determination (b%). Londrina, PR, UEL. 1997/98.

		Mean Square									
F.V.	d.f.	PE	PS	NGV	NUG	EL	TEMP	CW	GMP		
Blocks	7	15.3868	0.9575	1064.3174	8.2108	5.0510	228.8877	21.1059	130.8671		
Genotypes	8	83.64401/	0.9308	2045.0625	21.6644 ^{1/}	12.0815 ^{1/}	2170.9464 ^{1/}	90.6851 ^{1/}	1433.4988 ^{1/}		
Residues	56	22.6748	0.8195	1340.6656	8.1086	3.0042	256.5459	18.1256	159.7304		
Means		22.56	4.10	228.83	3.38	16.62	90.41	17.41	73.04		
C.V. (%)		21.11	22.07	16.00	84.22	10.43	17.72	24.45	17.30		
b (%)		72.89	11.95	34.44	62.57	75.13	88.18	80.01	88.85		

^{1/}: significant at 5% of probability by the test F; d.f.: degree of freedon; PE: popping expansion (cm³); PS: popcorn size (cm³); NGV: number of grain in 40 cm³; NUG: number of unpopped grain (%); EL: ear length (cm); TEMP: total ear mass per plant (g); CW: cob weight (g); GMP: grain mass per plant (g).

showed higher selective potential, with an PE value of 27,50 cm³ and a GY over 3300 kg/ha (Table 2).

The clustering method proposed by Tocher (cited by Rao, 1952), using the generalized Mahalanobis distances (D²) divided the nine genotypes into two groups. Group I contained eight genotypes, corresponding to 88,89% of the genotypes studied; group II contained one genotype, corresponding to 11,11% of the genotypes studied. Genotypes four and five were the most genetically related, the distance between them being of a lesser magnitude (D²= 1,2343). The most genetically divergent genotypes were two and nine, presenting greater distances (D²= 35,2639) (Table 3).

Some authors have recommended the use of parentals with the highest divergence level to maximize the heterosis manifested by the hybrids, to increase the probability of superior segregants to occur in advanced generations and to widen its genetic basis (Cruz et al., 1994; Fuzatto et al., 2002). Thus, data in Table 3 should be used for cross recommendations. Taking into consideration the multivariate statistics, a high degree of similarity among genotypes of the same group is expected. Therefore, within group crosses must be avoided (Destro, 1991). Pípolo et al. (1995) evaluated the behavior of 25 genotypes in relation to eight traits to identify adequate parentals for pigeon pea genetic improvement program. The genotypes were divided into five groups according to plant height and protein and oil percentages. Twenty-four crosses were recommended and 8% of all possible crosses involved 25 of the evaluated genotypes.

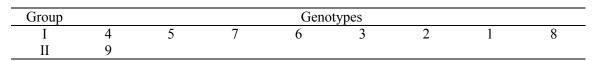
The clustering technique based on the generalized Mahalanobis distances, together with the analysis of the canonical variables, were used to quantify genetic divergence among parentals. In the latter case, the genetic divergence is evaluated by the graphic dispersion of the scores related to the genotypes studied in Cartesian axes (Figure 1). With this great concentration of total variance in the first variables, in general over 80%, the study of the genetic divergence through geometric distances among parentals in dispersion graphics becomes viable (Cruz and Regazzi, 1997). In this study, the dispersion shown in Figure 1 was found in relation to the two first canonical variables, which retained 83% of the total variation, considered satisfactory by this study.

Table 2. Comparison of means obtained from nine popcorn genotypes in relation to the nine traits evaluated.Londrina, PR, UEL. 1997/98.

	Genotype	PE	PS	NGV	NUG	EL	TEMP	CW	GMP	GY
8	UEL-ZP	27.50 a	4.61	244.75	2.29 b	19.43 a	104.39 b	21.84 ab	82.55 b	3302.00 b
3	UEL-PASHA	27.15 a	4.55	248.50	3.21 ab	16.81 b	83.59 cd	17.67 bc	65.92 cd	2636.80 cd
7	UEL-PAAPC	24.40 ab	4.04	248.75	1.73 b	15.68 b	81.81 cd	14.98 c	66.83 cd	2673.20 cd
5	UEL-PAMPCB	22.71 abc	4.20	225.62	2.18 b	16.00 b	81.05 cd	15.70 c	65.35 cd	2614.00 cd
6	UEL-PAPA	21.87 bc	4.09	230.25	6.08 a	15.87 b	85.40 cd	16.81 c	68.58 c	2743.20 c
4	UEL-PAMPGA	21.06 bc	4.16	215.62	4.00 ab	16.93 b	93.35 bc	17.51 bc	75.84 bc	3033.60 bc
2	UEL-PAPYY	20.68 bc	3.90	218.87	1.79 b	15.18 b	67.60 d	13.14 c	54.49 d	2179.60 d
9	UEL-PP	19.06 bc	3.54	224.87	5.85 a	17.00 b	125.24 a	23.68 a	101.55 a	4062.00 a
1	UEL-PAMP	18.53 c	3.77	202.25	3.26 ab	16.62 b	91.23 bc	15.37 c	76.21 bc	3048.40 bc

PE: popping expansion (cm³); PS: popcorn size (cm³); NGV: number of grain in 40 cm³; NUG: number of unpopped grain (%); EL: ear length (cm); TEMP: total ear mass per plant (g); CW: cob weight (g); GMP: grain mass per plant (g); GY: grain yield (kg/ha); Means followed by the same letter, in the column, do not differ among themselves by the Duncan test at 5% of probability.

Table 3. Group of popcorn parental genotypes established by the clustering method proposed by Tocher and based the generalized Mahalanobis distances.



Maximum Distance: 35,2639 among genotypes 2 and 9; Minimum Distance: 1,2343 among genotypes 4 and 5.

Whenever the dispersion graphic does not provide adequate information on the degree of similarity among the individuals studied, some authors have used the scores from the first components to estimate the Euclidian distance, considering, for that matter, the independence property among these components. Such procedure is, many times, used to complement the graphic dispersion information, providing a less subjective formation of groups than that obtained by visual examinations (Cruz, 1990).

Important agronomic traits such as popping expansion (PE), popcorn size (PS) and grain mass per plant (GMP) were considered for cross recommendation. Based on the genetic divergence and on key agronomic traits, the following crosses were recommended: genotype 9 (group II) with genotypes 8, 3, 7 and 5 (group I) (Figure 1). This way, four crosses could be carried out, representing only 11% of the total to be realized by the partial diallel cross among the nine lines studied, which allowed up to 36 combinations. Multivariate techniques have been used to estimate genetic divergence in order to obtain hybrids effortlessly and concentrate in more promising combinations. In corn studies carried out by Fuzatto et al. (2002), information on the genetic divergence estimates obtained by the multivariate techniques can substitute information on the combining capacity obtained by diallel crosses. The authors concluded that it is possible for improvers to select the most divergent parentals and carry out a smaller number of crosses with the highest chance of success.

The identification of genotypes based only on genetic divergence, disregarding their individual behavior,

cannot be a good improvement program strategy. Thus, crosses among divergent genotypes with superior performance on the main agronomic traits seem to be the most promising for improvement programs.

Correlation estimates are useful whenever one determined character of interest is difficult to evaluate. If this character shows significant phenotypic and genotypic correlation with other of easier access, an indirect selection based on this character of easy access can be done. Difficulties may arise whenever two characters show a positive and significant correlation and one is of no interest, and whenever two characters are of interest but the correlation is negative and significant. Whenever the correlation between two characters is positive and significant, and one of them is of no interest, the solution is to carry out a selection aiming at partially breaking the correlation. In other words, only plants, which show acceptable levels for the two characters, should be selected, e.g., plants with high grain yield and small grain.

Phenotypic, genotypic and environmental correlation coefficient estimates among the eight traits studied in popcorn are shown in Table 4. An important positive phenotypic correlation occurred between popping expansion and number of grain per volume (0,879), which attests the results shown on Table 2 where the genotypes with greater popping expansion show greater number of grain as well. Thus, in popcorn improvement programs, the improver can adopt the indirect selection for the number of grain per volume, i.e., select genotypes with smaller grain, for popping expansion. Similarly the improver could carry out the indirect selection for popping expansion

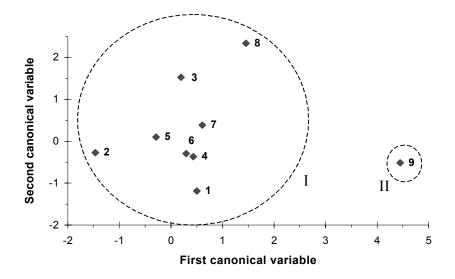


Figure 1. Dispersion scores of nine popcorn genotypes in relation to the first two canonical variables.

using the direct selection for popcorn size (PS), once the correlation among these traits was positive and significant (0,909) (Table 4).

As a matter of fact, investigations have had some difficulty confirming high yield with good popping expansion. These difficulties are due to the negative correlation between the popping expansion and other traits of agronomic importance (Lyerly, 1942; Dofing et al., 1991; Sawasaki, 1996). Based on the genetic parameters from the second cycle of a halfsiblings family selection, Pacheco et al. (1998) reported on the possibility of selecting genotypes by combining high yield and good popping expansion. However, these authors find the selection for greater popping expansion easier than the selection for higher yield, which agrees with the results obtained by Lira (1983).

Correlation studies among plant, ear and grain with popping expansion (PE) traits have been carried out with several popcorn populations. In general, traits with the most agronomic importance such as grain yield, 100 grain weight, plant height, ear height and grain size relate negatively with popping expansion (PE); however, number of rows on the ear and prolificacy shows a positive correlation (Willier and Brunson, 1967; Lyerly, 1942; Lira, 1983; Dofing et al., 1991; Sawazaki, 1996). In Table 4, grain mass per plant, cob weight and total ear mass per plant showed positive phenotypic and genotypic correlation among themselves, allowing the selection for grain mass per plant through the selection of the trait with the highest genetic gain and easy to evaluate.

The environmental correlation coefficients (Table 4) showed that the environmental conditions which favor the number of unpopped grain, total ear mass per plant, cob weight and grain mass per plant, influenced popping expansion negatively, since they presented negative environmental correlation coefficient. In addition, environmental conditions, which favored number of 40 cm³ grain, influenced negatively popcorn size as well.

Although the correlation values found between popping expansion (PE) and ear length (EL) were positive, they were non-significant. Sawazaki (1996) found a positive and significant correlation between popping expansion (PE) and ear length (EL), in six popcorn populations.

Weaver and Thompson (1957) report that the selection for increasing popping expansion resulted in modifications in the correlated traits with grain yield. The long mass selection used to increase popping expansion resulted in the reduction of the unhusked ear diameter and weight.

	PE	PS	NGV	NUG	EL	TEMP	CW	GMP
PE		0.909 ^{1/}	$0.879^{1/}$	-0.409	0.451	-0.148	0.149	-0.223
		$(0.880)^{1/}$	(0.449)	(-0.418)	(0.600)	(-0.164)	(0.243)	(-0.265)
PS	$0.720^{1/}$		0.629	-0.371	0.476	-0.254	0.038	-0.325
			(4.429)	(-1.137)	(1.126)	(-0.204)	(-0.292)	(-0.417)
NGV	0.3621/	-0.354 ^{1/}		-0.214	0.251	-0.015	0.238	-0.084
				(-0.147)	$(0.758)^{2/2}$	(0.302)	(0.853)	(0.155)
NUG	-0.397 ^{1/}	-0.105	-0.293 ^{2/}		0.035	0.561	0.499	0.565
					(-0.090)	(0.656)	(0.559)	$(0.672)^{2/2}$
EL	0.027	$0.295^{2/}$	$-0.334^{2/}$	0.318 ^{2/}		0.633	$0.750^{2/}$	0.590
						(0.638)	$(0.801)^{1/2}$	(0.590)
TEMP	-0.090	0.4251/	-0.651 ^{1/}	0.3491/	$0.662^{1/2}$		0.935 ^{1/}	0.995 ^{1/}
							$(0.959)^{1/2}$	$(0.997)^{1/2}$
CW	-0.164	$0.306^{2/}$	-0.579 ^{1/}	$0.378^{1/}$	$0.578^{1/}$	$0.838^{1/}$		0.896 ^{1/}
								$(0.935)^{1/2}$
GMP	-0.055	$0.437^{1/}$	-0.627 ^{1/}	$0.310^{2/}$	0.641 ^{1/}	0.9831/	$0.722^{1/}$	

Table 4 - Phenotypic (above the diagonal), genotypic (above the diagonal, within parenthesis) and environmental (below the diagonal) correlation coefficients among eight popcorn traits. Londrina, PR, UEL. 1997/98.

PE: popping expansion (cm³); PS: popcorn size (cm³); NGV: number of grain in 40 cm³; NUG: number of unpopped grain (%); EL: ear length (cm); TEMP: total ear mass per plant (g); CW: cob weight (g); GMP: grain mass per plant (g); ^{1/, 2/}: significant by the t-test at 1% and 5% of probability, respectively. Values of 0.666 and 0.798, at 1% and 5% of probability, for the phenotypic and genotypic correlation, respectively. Values of 0.259 and 0.337, at 5% and 1% of probability, for the environmental correlation, respectively.

CONCLUSIONS

1. The genotypes UEL-PAMPGA and UEL-PAMPCB were the most genetically related, showing a distance between them of the smallest magnitude ($D^2= 1,2343$). The most genetically divergent genotypes UEL-PAPYY and UEL-PP showed the longest distance ($D^2= 35,2639$).

2. Based on the genetic divergence and on the key agronomic traits, the following crosses were recommended: genotype UEL-PP (group II), with genotypes UEL-ZP, UEL-PASHA, UEL-PAAPC and UEL-PAMPCB (group I).

3. The popping expansion trait showed a significant and positive correlation with number of grain per volume (0,879) and popcorn size (0,909).

4. Grain mass per plant, cob weight and total ear mass per plant showed positive and significant phenotypic and genotypic correlation among themselves. Therefore, selection for grain mass per plant can be done through the selection of the trait with either the highest genetic gain or with the easiest evaluation process.

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RESUMO

Seleção de parentais de milho pipoca baseado na divergência genética

Nove genótipos de milho pipoca foram avaliados em relação a oito caracteres. Foram estimadas suas divergências genéticas por meio de técnicas multivariadas visando identificar parentais a serem incluídos em programas de melhoramento genético envolvendo hibridações. O agrupamento pelo método de Tocher, a partir das distâncias generalizadas de Mahalanobis, possibilitou a divisão dos nove genótipos em dois grupos. Para recomendação dos cruzamentos foram considerados os caracteres agronômicos mais importantes, sendo esses a capacidade de expansão, o tamanho da pipoca e a massa de grãos por planta. Com base na divergência genética e nos caracteres agronômicos chaves foram recomendados os seguintes cruzamentos: genótipo 9 (grupo II), com os genótipos 8, 3, 7 e 5 (grupo I). Dessa maneira, seriam feitos apenas quatro cruzamentos, representando apenas 11% do total que poderia ser realizado no dialelo parcial entre os nove genótipos avaliados, os quais permitiriam a obtenção de até 36 combinações.

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