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Viability of the UENF popcorn improvement program based on divergence in S_1 families

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ABSTRACT - *With the objective of evaluating the viability of the program of recurrent selection with popcorn of the Universidade Estadual do Norte Fluminense Darcy Ribeiro carried out in Campos dos Goytacazes and Itaocara, state of Rio de Janeiro, 40 families that originated the second cycle were evalutated for 14 morphoagronomical characteristics and RAPD markers, using multivariate analysis. The analyses of variance revealed the existence of variability for most evaluated morphoagronomic traits. Clustering by Tocher's optimization method for the morphoagronomic traits of Campos dos Goytacazes formed eight groups and 16 for those of Itaocara. For the RAPD markers 18 groups of S_1 families were formed by Ward's clustering method. The conclusion was drawn that there is genetic divergence in the selected families, which allows the inference that there is sufficient variability for the continuity of the recurrent selection process with the formation of new cycles.*

Key words: popcorn, genetic variability, recurrent selection, interaction genotype by environment.

INTRODUCTION

Genetic variability is a fundamental condition for the genetic gain in improvement programs (Ferreira et al. 1995). Regarding recurrent selection, it is expected that in the sequence of the consecutive cycles the mean values of the traits of interest increase in function of the concentration of favorable alleles, although without reduction in the genetic variability (Hallauer 1971).

The high genetic variability in the base population associated with methods of genetic improvement that reduce the inbreeding effects is essential for the longevity of an improvement program (Miranda et al. 2003). Many maize breeders have however derived their commercial lines from restricted elite lines; a practice that increases the risk of reducing the genetic diversity on the production fields (Senior et al. 1988).

Examples of reduced variability in cycles of recurrent selection in maize have been described by several authors (Hallauer 1971, Reeder Jr. et al. 1987, Holthaus and Lamkey 1995, Guimarães and Lamkey 2003), generally associated with reduced population sizes.

Despite the studies of genetic variability in recurrent selection cycles of maize, investigations dealing with these

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issues in popcorn are still rare (Granate et al. 2001), especially with the use of molecular markers.

Although popcorn is highly appreciated in Brazil, only seven cultivars are currently listed in the SNPC (Serviço Nacional de Proteção de Cultivares) (Sawazaki 2001). Six of these are restricted to the use of producers who are associate partners of seed supply companies. This setting calls for the implementation of genetic improvement programs for a crop that accounts for an annual turnover of 1.2 billion dollars in the USA (Pacheco et al. 1998, Galvão et al. 2000).

The Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) has developed a recurrent selection program with popcorn, aiming at the establishment of a superior variety of interest for producers and consumers of the northern and northwestern regions of the state of Rio de Janeiro (Pereira and Amaral Júnior 2001, Daros et al. 2002). The improvement of the crop by means of recurrent selection should yet not be exempted from monitoring the genetic variability of the segregating families to guarantee the durability of the program and the establishment of superior varieties in the successive selection cycles.

Our study aimed at an evaluation of the morphoagronomic and molecular diversity in the S_1 families that constitute the population of the second recurrent selection cycle of popcorn by the RAPD technique, with the objective of shedding light on questions about the genetic divergence present in the segregating families and to assess the viability of the popcorn improvement program of the UENF.

MATERIAL AND METHODS

The morphoagronomic evaluations involved the 40 best families selected by Daros et al. (2004) from among 222 S_1 families in experiments conducted at the Colégio Estadual Agrícola Antonio Sarlo, in Campos dos Goytacazes and at the Estação Experimental da PESAGRO-RIO of Itaocara, state of Rio de Janeiro (RJ). The experimental design of complete randomized blocks was used with two replications within sets, each of which consisted of 37 families and five controls. The 40 best inbred families were grown on an area of the Colégio Estadual Agrícola Antônio Sarlo, in Campos dos Goytacazes, RJ, in May 2003, in a design of complete random blocks with two replications in 6.0m long rows with 15 plants each. The rows were spaced 1.0m apart and the plants 0.4m.

The following traits were evaluated: a) plant height (PH): obtained by the mean height of six competitive plants in the plot; b) ear height (EH): mean of the ear heights of the highest ears of the same plants used for the previous trait; c) number of plants in a row (STAND); d) broken plants (BPL): expressed by the number of plants with kinked culm below the highest ear at harvest; e) lodged plant (LPL): expressed by the number of plants inclined 45 degrees or more in relation to the vertical at harvest; e) poorly hulled ears (PHE): number of ears that were not completely covered with straw; f) number of days to flowering (NDF): quantification of the period from planting to the release of the female flowers of at least 50% of the plants in the row, beginning evaluations every other day as soon as the first progeny presented emission of the styles; g) number of healthy ears (NHE): count of the ears harvested on each plot; h) number of diseased ears (NDE): count of the diseased ears harvested on each plot; i) ear weight (EW): obtained by weighing the shelled ears after harvest, expressed in kg $plot^{-1}$; j) weight of diseased ears (WDE): obtained by weighing the diseased shelled ears after harvest, expressed in kg $plot^{-1}$; l) Grain yield (GY): expressed by the weight of the grains after shelling in g $plot^{-1}$ and transformed into kg ha^{-1}; m) weight of 100 grains (W100G): expressed by the weight of 100 grains in g; and n) popping expansion (PE): obtained by the ratio between the popped volume and the initial volume of grains.

The morphoagronomic traits were first evaluated by the univariate analysis of variance for the design in complete random blocks, considering the fixed model. Next, the joint analysis was carried out, considering the environment effect as fixed as well.

The multivariate analysis was implemented by the clustering procedures of the optimization method of Tocher and hierarchical clustering of Ward, based on Mahalanobis' generalized distance (Cruz and Regazzi 2001), including all evaluated traits.

The material for the DNA extraction was obtained in bulk by collecting one leaf from every plant in the row to make up a compound sample.

After freezing the leaf tissue samples in an ultrafreezer at -70 ^{o}C, they were ground into a fine powder in a china mortar with liquid nitrogen. The DNA was then isolated according to the DNA extraction protocol proposed by Doyle and Doyle (1990).

The amplification was realized in a 'Perkin Elmer 9700' thermocycler, using a final volume of 25 mL, according to the methodology described by Ferreira and Grattapaglia (1998). The following primers were used: OPAA01, OPAA02, OPAA03, OPAA04, OPAA06, OPAA09,

OPAA11, OPAA12, OPAA14, OPAA15, OPAA16, OPAA19, OPAA20, OPAB02, OPAB03, OPAB04, OPAB05, OPAB06, OPAB07, OPAB08, OPAB09, OPAB11, OPAB12, OPAB13, OPAB14, OPAB17, OPAB18, OPAB19, OPAB20, OPAC01, OPAC03, OPAC05, OPAC06, OPAC07, OPAC11, OPAC12, OPAC14, OPAC17, OPAC20, OPAE02, OPAE03, OPAE05, OPAE06, OPAE07, OPAE08, OPAE09, OPAE10, OPAE11, OPAE12, OPAE13, OPAE14, OPAE15, OPAE18, OPAE19, OPAE20, OPAF02, OPAF10, OPAF14, OPAF16, OPAF19, and OPAF20.

The DNA fragments obtained after the amplification were separated by electrophoresis in 1.4% agarose gel at 60 volts during approximately four hours. The pictures were created by the photo documentation in the ´Eagle Eye II' image system.

Based on the obtained images, a binary data matrix was established, attributing value 1 to the presence of a particular band generated after the amplification and value 0 where the particular band was not found. The distance between the pairs of families was calculated based on the arithmetic complement of the Jaccard index (Ferreira and Grattapaglia 1998). The methods of Tocher and Ward were used to cluster the S_1 families, using the distances obtained with the arithmetic complement of the index of Jaccard (Cruz and Regazzi 2001). Software Genes (Cruz 2001) was used to perform the analyses.

RESULTS AND DISCUSSION

The analysis of variance for the traits evaluated in Campos dos Goytacazes (Table 1) revealed significant difference between the means of the families at 1% probability for 11 of the 14 traits (respectively, PH, EH, W100G, PE, BPL, LPL, PHE, NHE, EW, GY, and NDF). In the experiment evaluated in Itaocara, ten traits revealed significant differences between the means of the families at 1% probability, that is: PH, EH, W100G, PE, BPL, NHE, NDE, EW, GY, and WDE (Table 2). Considering that eight traits were concordantly significant in both environments and that these included those of greatest importance for the crop (PE and GY), we concluded that there is genetic divergence between the 40 selected superior inbred families, which is of fundamental interest for the viability of the succession of the recurrent selection cycles.

The joint analysis showed an absence of significance in the interaction treatments by environment for PE, STAND, LPL, NDE and WDE. The traits EH and W100G were however significant at 5% probability and the traits PH, BPL, PHE, NHE, EW, GY and NDF were significant at 1% probability (Table 3). The absence of interaction for PE is favorable for the implementation of a single program for both evaluated environments; since the same may not be deduced for GY, running proper programs for each environment should be taken into consideration.

The Tocher clustering method, for the families evaluated in the experiment in Campos dos Goytacazes, formed the following eight groups: group I (33, 35, 8, 16, 23, 3, 12, 30, 21, 37, 34, 32, 27, 10, 2, 4, 38, 39, and 15); group II (14, 18, 19, 6, 9, 1, 22, 20, 24, 28, and 29); group III (17, 25 and 36); group IV (7 and 13); group V (31 and 40); group VI (11); group VII (5); and group VIII (26). The first group, consisting of 19 families, agglutinated 47.5% of all studied families. For Itaocara, 16 groups were constituted, which are: group I (3, 37, 4, 36, 11, 24, 22, 13, and 18); group II (1, 15, 14, 28, 26, 38, 30, 6, and 7); group III (12 and 34); group IV (16 and 29); group V (5, 40 and 23); group VI (17 and 35); group VII (8 and 31); group VIII (10 and 39); group IX (19 and 21); group X (32); group XI (9); group XII (33); group XIII (25); group XIV (27); group XV (2); and group XVI (20). The groups of Itaocara were numerous, but there was no concentration of a large number of families in few groups. Still, the two first groups contained nine families each, which correspond to 45% of the total of the families.

A comparison between the two clusters revealed minimal similarities. In the first group for Campos dos Goytacazes and Itaocara only families 3, 37 and 4 were coincident. In the second group of Itaocara the families 1, 14, 28 and 6 were also united in the same group as in Campos dos Goytacazes. The differences presented by the clusters may be explained by the action of the environment on the genotypes, which reflects the occurrence of significance in the interaction treatments x environment for around 64% of the evaluated traits (Table 3). Nevertheless, these significances can justify the occurrence of a greater divergence between the families in the locality of Itaocara.

By Ward's method (Figure 1) with 50% as cutpoint, the formation of seven groups was observed: group I, with the genotypes 33, 35, 8, 16, 23, 4, 37, 7, 12, 21, 3, 30, 2, 32, and 27; group II, formed by the progenies 17, 25, 10, 38, 39, 34, 36, and 31; group III, containing the families 5 and 13; group IV, represented only by genotype 40; group V, formed by 11, 15, 6, and 9; group VI, comprising genotypes 24, 29, 20, and 28; and group VII, uniting the families 22, 26, 14, 18, 1, and 19. Despite the concordance of 73.68% in the groups I between the methods of Ward and Tocher for Campos dos Goytacazes, there was no similarity at all between the other groups formed and, although Tocher's method provided a larger number of groups, Ward's method established a more uniform distribution of the

Table 1. Analysis of variance of 14 traits evaluated in 40 S_1 families in Campos dos Goytacazes, RJ

Traits	Sources of variation (MS[1])		
	Replications	Families	Error
PH	13.3416	633.4030**	89.6126
EH	3.8325	377.3472**	89.9260
W100G	0.0177	3.8310**	1.0285
PE	4.3617	13.0665**	4.9832
STAND	12.0125	4.5355	6.3971
BPL	0.2000	33.9115**	10.1230
LPL	0.1125	0.1509**	0.0612
PHE	1.2499	29.3833**	4.8397
NHE	3.6125	52.0048**	18.2022
NDE	2.1125	27.4663	28.4971
EW	9901.2500	77035.7371**	21372.4038
GY	8611.2500	40163.9743**	12115.0961
NDF	0.2000	7.5897**	2.2512
WDE	52.8125	4764.6714	3638.7099

**P < 0.01; * P < 0.05

[1] PH = Plant height; EH = Ear height; W100G = Weight of 100 grains; PE = Popping expansion; STAND = Number of plants in the plot; BPL = Broken plants; LPL = Lodged plant; PHE = Poorly hulled ears; NHE = Number of healthy ears; NDE = Number of diseased ears; EW = Ear weight; GY = Grain yield; NDF = Number of days to flowering; and WDE = Weight of diseased ears

Table 2. Analysis of variance of 14 traits evaluated in 40 S_1 families in Itaocara, RJ

Traits	Sources of variation (MS[1])		
	Replications	Families	Error
PH	230.5544	367.0230**	109.2017
EH	44.9700	292.1034**	80.6326
W100G	0.0020	4.1949**	0.9268
PE	2.2781	16.3967**	5.4375
STAND	2.8125	7.0099	5.9663
BPL	12.0125	16.0227**	4.5509
LPL	0.2000	0.2353	0.2769
PHE	0.3125	0.6125	0.9022
NHE	4.5125	76.4483**	33.8458
NDE	3.2000	39.8666**	18.8153
EW	18150.3125	106851.2099**	33712.4919
GY	10974.6125	66412.7022**	22221.9201
NDF	5.5125	4.9586	3.4355
WDE	80.0000	4467.1474**	1751.7948

**P < 0.01; * P < 0.05

[1]Coded as in the Table 1

Table 3. Joint analysis of variances of 14 traits evaluated in 40 S_1 families

Traits	Sources of variation (MS[1])				
	Replications/Environment	Families (Fam)	Environments (Env)	Fam x Env	Error
PH	121.9480	779.8834	92731.1220	220.5427**	99.4072
EH	24.4012	522.3418	28470.4948	147.1088*	85.2793
W100G	0.0098	6.3373	131.1345	1.6886*	0.9776
PE	3.3199	22.4885	14.8840	6.9748	5.2104
STAND	7.4125	5.9615	34.2250	5.5839	6.1817
BPL	6.1062	29.1408	709.8062	20.7934**	7.3370
LPL	0.1562	0.1793	0.1562	0.2075	0.1690
PHE	0.7812	16.5109	170.1562	13.4767**	2.8709
NHE	4.0625	51.6993	372.1000	76.7538**	26.0240
NDE	2.6562	42.0959	158.0062	25.2370	23.6562
EW	14025.7812	95125.4447	15307.6562	88761.5024**	27542.4479
GY	9792.9312	52813.5754	58.8062	53763.1011**	17168.5081
NDF	2.8562	6.6331	1870.0562	5.9152**	2.8434
WDE	66.4062	5855.7331	46751.4062	3376.0857	2695.2524

**P < 0.01; * P < 0.05

[1]Coded as in the Table 1

families, indicating, consequently, a greater capacity of detection of variability present among the S_1 families evaluated for the morphoagronomic traits. Along this line, one can infer on the existence of genetic divergence in the S_1 families, confirming the perspective of success with the establishment of superior segregating families in advanced cycles in the continuity of the procedure of recurrent selection.

The dendrogram distribution of the families evaluated in Itaocara (Figure 2), also cut at the level of

50%, showed the formation of a lower number of groups compared to Tocher's method for the same local. By the procedure of Ward, the following groups with the respective families were formed: group I (3, 37, 4, 11, and 36); group II (12, 34 and 27); group III (1, 15, 20, 2, 6, 13, 18, 7, 28, 38, 14, 24, 26, 9, and 30); group IV (17, 35, 19, 22, 21, and 33); group V (10 and 39); group VI (16 and 29); and group VII (8, 31, 5, 40, 23, 25, and 32). There was a minimal concordance between these two methodologies in the distributions of the families in groups; the Method of Tocher was more sensitive to detect morphoagronomic divergence between the families. We stress that differences in the composition of groups between the methods used are acceptable, once they are based on distinct procedures.

In conclusion, one may assume the existence of considerable divergence in the families in Itaocara, of fundamental importance to obtain gains with selection.

For the RAPD markers, 205 polymorphic markers were obtained; the total number per primer varied from one to eight markers.

Based on the RAPD markers by the Tocher Method, 12 groups were formed: group I (7, 9, 5, 3, 6, 16, 13, 39, 11, 8, 20, 14 and 19); group II (24, 28, 10, 27, 31, 32, 17, 12, 36 and 37); group III (29, 30 and 35); group IV (18, 22, 21 and 23); group V (38 and 40); group VI (25 and 26); group VII (33); group VIII (2); group IX (34); group X (1); group XI (4); and group XII (15). Groups I and II contained 23 families, corresponding to 57.5% of the evaluated families. The remaining families were distributed among 10 groups. Six of these groups were consisted of only one individual, which reveals the existence of dissimilar families, of fundamental importance for the selection procedure.

The dendrogram dispersion of the families by Ward's method (Figure 3) established the formation of 18 groups, considering a vertical cut at 50%, based on the abscissa axis. By this analysis, opposite to the results with the Method of Tocher for the RAPD markers, there was no concentration of a large number of families in few groups. The groups formed were: group I (8, 13, 31, and 35), group II (1); group III (11, 32, 26, and 20); group IV (37); group V (39 and 40); group VI (27 and 33); group VII (18); group VIII (4 and 23); group IX (19 and 29); group X (6 and 21), group XI (30, 38 and 10); group XII (9, 14 and 25); group XII (5); group XIV (3, 15 and 16); group XV (2); group XVI (24 and 36); group XVII (22 and 28); and group XVIII (7, 17, 12 and 34). These results show that the RAPD markers, by Ward's method, provided the clearest genotypic discrimination, evidencing the genetic divergence present in the population selected for new cycles of recurrent selection.

In maize, Reeder Jr. et al. (1987) evaluated the effects of the full-sib reciprocal recurrent selection in the populations BS10 and BS11 and verified that the genetic divergence was reduced after six selection cycles. It is assumed that this reduction in the divergence is linked to the reduced population size.

Considering that, in the present study 40 S_1 families derived from 222 cultivated families were selected, one may infer that in spite of the different strategies used in

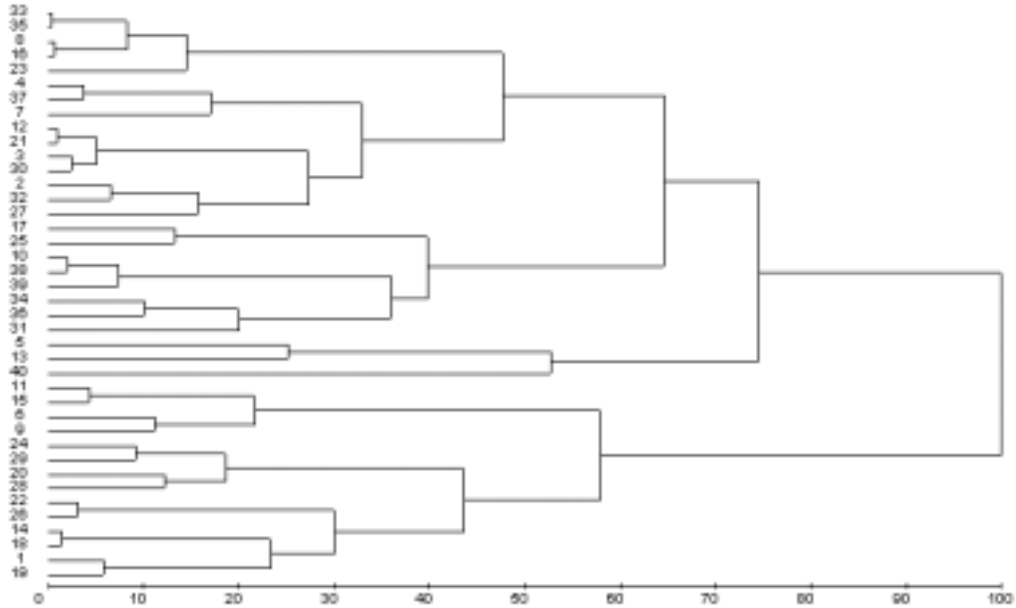

Figure 1. Dendrogram of genetic dissimilarity among 40 S_1 families obtained by Ward's clustering method for Campos dos Goytacazes, RJ

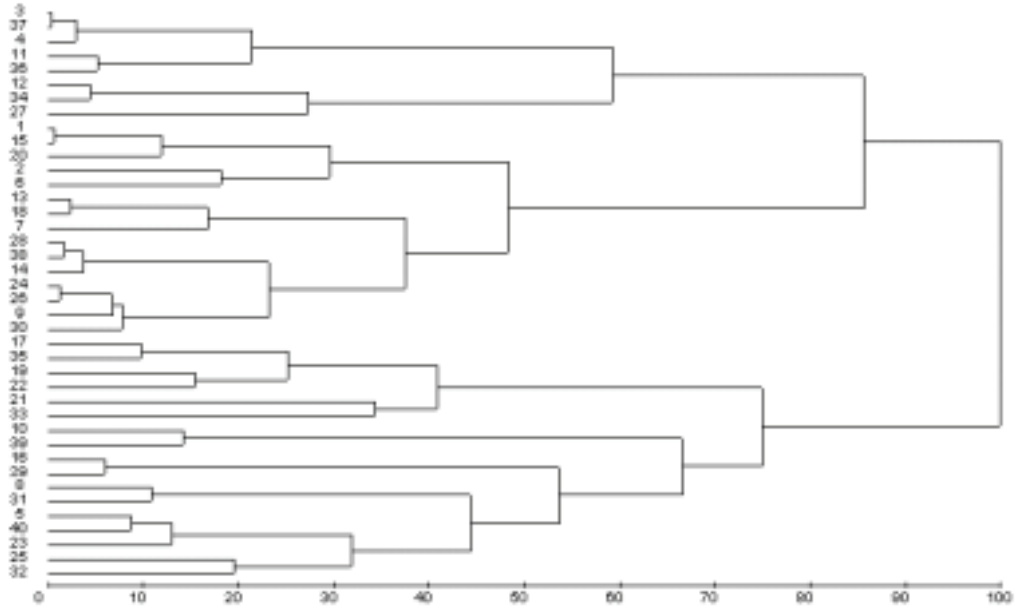

Figure 2. Dendrogram of genetic dissimilarity among 40 S_1 families obtained by Ward's clustering method for Itaocara, RJ

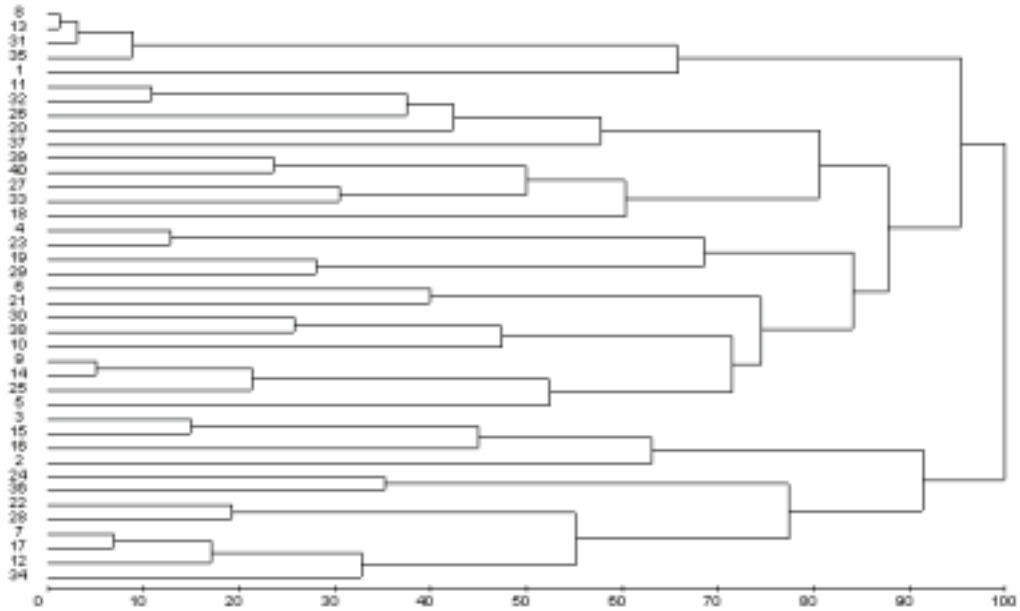

Figure 3. Dendrogram of the 40 S_1 families by Ward's method for RAPD markers

the formation of the recurrent selection cycles, the breeder's know-how to recombine a significant proportion of the population under selection is of fundamental importance in the maintenance of the durability of the improvement program.

The results obtained with the RAPD markers for the 40 selected families confirm the viability of continuation with the improvement program of popcorn of the UENF, with the perspective of concentrating favorable alleles in the successive cycles until achieving a population that can be used as a novel variety by the farmers for the North and Northwest regions of the Rio de Janeiro state, Brazil.

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Viabilidade do programa de melhoramento de milho pipoca da UENF pela divergência de famílias S_1

RESUMO - *Com o objetivo de avaliar a viabilidade do programa de seleção recorrente com milho pipoca da UENF, com base no comportamento de 40 famílias S_1 que deram origem ao segundo ciclo de seleção recorrente intrapopulacional, efetuou-se a análise de variância de quatorze características morfoagronômicas, em Campos dos Goytacazes e Itaocara, RJ; e quantificou-se a divergência morfoagronômica e molecular – por meio de marcadores RAPD –, utilizando-se de procedimentos multivariados de agrupamento. As análises de variância revelaram a existência de variabilidade para a maioria das características morfoagronômicas avaliadas. O agrupamento de Tocher para as características morfoagronômicas proporcionou a formação de oito grupos para Campos dos Goytacazes e dezesseis para Itaocara. Para os marcadores RAPD houve a formação de dezoito grupos de famílias S_1 pelo Método de Ward. Depreende-se que há divergência genética nas famílias selecionadas, permitindo-se concluir por suficiente variabilidade para a continuidade do processo de seleção recorrente na formação de novos ciclos.*

Palavras-chave: milho pipoca, variabilidade genética, seleção recorrente, interação genótipos por ambiente.

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