Effect of recurrent selection on the genetic variability of the UNB-2U popcorn population using RAPD markers

Felipe Oliveira Vilela[1], Antonio Teixeira do Amaral Júnior[1*], Messias Gonzaga Pereira[1], Carlos Alberto Scapim[2], Alexandre Pio Viana[1] and Silvério de Paiva Freitas Júnior[1]

*[1]Laboratório de Melhoramentos Genético Vegetal, Centro de Ciências e Tecnologias Agropecuárias, Universidade Estadual do Norte Fluminense, Av. Alberto Lamego, 2000, 28013-602, Parque Califórnia, Campos dos Goytacazes, Rio de Janeiro, Brazil. [2]Departamento de Agronomia, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: amaraljr@uenf.br*

ABSTRACT. The aim of this research was to study the effects of recurrent selection on the genetic variability of UNB-2U popcorn population after three cycles of recurrent selection (mass selection, full-sib selection and S_1 families) based on RAPD markers in 30 progenies from each selection cycle. There was no significant variation between the C0 and C2 cycles based on RAPD, showing that the use of different recurrent selection strategies in the cycles did not decrease genetic variability, due to the size of the population selected in the different cycles. The significant difference observed between mean values of C1 and C2 cycles was attributed to the smaller population size in C1 generation. Individuals were distributed into three large clusters and 20% of the individuals were placed in a group different from their original cycle. This can be explained by alleles' transference from one generation to another and by the relationship between cycles.

Key words: genetic variability, population size, selection pressure, *Zea mays,* RAPD.

RESUMO. Efeito da seleção recorrente na população UNB-2U de milho pipoca por marcadores RAPD. Com o objetivo de averiguar o impacto da seleção recorrente na variabilidade genética de progênies da população de milho pipoca UNB-2U, após 3 ciclos de seleção recorrente por diferentes métodos (massal, irmãos completos e famílias S_1), 30 progênies de cada ciclo foram avaliadas por marcadores RAPD. Constatou-se que não houve variação molecular significativa entre os ciclos C0 e C2, revelando que o uso de diferentes estratégias de seleção recorrente não promoveu estreitamento genético, em razão do tamanho populacional selecionado nos ciclos. A diferença significativa na média entre os ciclos C1 e C2 é atribuída ao menor tamanho populacional da geração C1. A distribuição dendrogrâmica dos indivíduos revelou a formação de 3 grandes grupos, sendo que 20% dos indivíduos foram alocados em grupo distinto do ciclo a que pertenciam, em razão da transferência de alelos nas subseqüentes gerações, bem como da própria semelhança entre os ciclos.

Palavras-chave: variabilidade genética, tamanho populacional, pressão de seleção, *Zea mays,* RAPD.

Introduction

Genetic variability is essential for a breeding program success, especially when the recurrent selection method is used. This method ensures the gradual increase of favorable alleles frequency without reducing the population's genetic variability (Hallauer and Miranda Filho, 1981; Paterniani and Miranda Filho, 1987). However, it has been reported by several authors that there is reduction in maize genetic variability after recurrent selection cycles, usually due to reduced population size. Hallauer (1971), for example, studied the BSSS population and verified a reduction in the estimates of variance components after four selection cycles, while Helms *et al*. (1989) reported a likesome reduction after nine selection cycles in the same population.

Reeder Jr. *et al*. (1987) assessed the effects of six reciprocal recurrent selection cycles of full sibs in the BS10 and BS11 populations and reported that the genetic variability was reduced after six recurrent selection cycles. Guimarães and Lamkey (2003) also studied the effects of recurrent selection on the genetic structure of BSSS corn population, using RFLP markers. After 14 selection cycles, it was observed that genetic variability decreased in the plant cycles and the genetic divergence increased among the selection cycles.

Although there are studies on the effect of recurrent selection in maize, such studies are still

rare for popcorn (Granate *et al*., 2001), especially ones using molecular markers. In order to develop a popcorn breeding program, Pereira and Amaral Júnior (2001) analyzed the genetic structure of UNB-2U population (C0) with design I by Comstock and Robinson (1948), and found the possibility of 9.42% genetic gains for yield and 27.09% for expansion capacity, per year, using the full sib families recurrent selection strategy; and 19.54% and 7.93% for the S_1 progeny, respectively.

Daros *et al.* (2004) studied the population's genetic progress in the first cycle of recurrent selection with full sib families (C1) and in the second cycle with S_1 families (C2), using the UNB-2U base population. Although genetic gains in both cycles were reported for expansion capacity and yield, C2 showed decrease in the mean of the selected families when compared to C1 for yield. Such reduction may be due to the inbreeding depression (S_1 families) and probably are due to a possible reduction in the population's genetic variability.

Thus, the aim of the present study was to study the effect of recurrent selection on the genetic variability of UNB-2U popcorn population after three cycles of selection by RAPD markers.

Material and methods

Three populations were analyzed in the present study: the base population derived from the second mass selection cycle (C0); the population derived from the first recurrent selection of full sib families (C1); and the population derived from the second recurrent selection of S_1 families (C2). The first cycle (C1) consisted of the evaluation of 75 full-sib families and the selection and recombination of 30 (40%) superior families. The second cycle (C2) consisted of the evaluation of 222 S_1 families and the selection and recombination of 40 (18.01%) superior families. Thirty individuals per population (C0, C1 and C2), making a total of 90 individuals, were used in this study.

The seeds for each genotype were sown in the greenhouse. Ten-liter pots containing organic substrate without fertilizer were used and two seeds were sown in each pot. Young and healthy leaves were collected at development stage 2, which usually occurs one month after germination (Fancelli and Dourado Neto, 2000). The leaves were collected from each of the 90 individuals, without the middle nervure.

The leaf samples were frozen in liquid nitrogen and stored in a -70°C ultra freezer. The leaf tissue samples were squashed in a porcelain mortar with liquid nitrogen until a fine powder was formed. The DNA was isolated through Doyle and Doyle (1990) protocol. The isolated DNA was quantified in a spectrophotometer (Spekol UV VIS, Zeis) with ultraviolet light at 260 nm length.

The DNA polymorphism was analyzed following Williams *et al*. (1990) protocol. The amplification process was carried out in a Perkin Elmer thermocycler, model 9700. The DNA was initially submitted to 95°C for 1 minute, and then for 45 one-minute cycles at 94°C, 1 minute at 36°C and two minutes at 72°C. After the last cycle, the last step of seven minutes was performed at 72°C.

A hundred 10-mer primers from Operon Technologies were initially screened using a sample per each cycle to determine the suitability of each primer for the study. Fourteen (OPAA 11, OPAA 14, OPAB 2, OPAB 5, OPAB 6, OPAB 19, OPAE 9, OPAE 10, OPAE 11, OPAE 18, OPAE 20, OPAF 2, OPAF14 and OPI 7) were selected based on the ability to detect distinct, clearly resolved and polymorphic amplified products within samples. To ensure reproducibility, the primers generating weak or complex patterns were discarded.

DNA fragments obtained after amplification were separated through electrophoresis on 1.4% agarose gel, at 60 volts, for approximately 4 hours, stained with ethidium bromide, visualized under UV light, and photo documented in an Eagle Eye II appliance.

The RAPD polymorphic bands were used to construct a binary data matrix, attributing values of one to the presence and zero to the absence of band. The distance between the accession pairs was calculated based on the Jaccard Index arithmetic complements (Dudley, 1994), expressed by: $C_{ij} = 1 - (a/a + b + c)$, where **a** represents the number of DNA fragments, codified with one (positive agreement), common to both individuals; and **b** and **c** register the number of DNA fragments where both the individuals disagree, represented, respectively, by 1-0 and 0-1.

The binary distance matrix was used to group the genotypes from cycles C0, C1 and C2, by the Ward's and Tocher's methods using the Genes software (Cruz, 2001). When the Tocher cluster was constructed, the first group was formed by the pair with the lowest distance value (C_{ij}). Then new groups were formed, adopting the criteria that the mean intragroup distance was smaller than any intergroup distance (Cruz and Regazzi, 2001).

The clusters obtained with the genotypes from the C0, C1 and C2 cycles were used to evaluate the effects of recurrent selection on populations genetic

variability by comparing the mean distances obtained by Jaccard's Index arithmetical complements.

The mean value of the dissimilarity coefficient was estimated from the sum of values obtained in the Jaccard's Index arithmetical complements, which was divided by the number of total observations, according to Lübberstedt *et al*. (2000).

Results and discussion

The analyses of the 90 genotypes generated 93 bands, 83 polymorphics and 10 monomorphics. The total number of bands by primer varied from three to 12 and 89.24% of the bands were polymorphic (Table 1).

Figure 1 shows an example of the bands generated by the RAPD markers. The primer OPAE 11 revealed clear polymorphic bands. The resolution of the bands was confident for scoring, indicating trustable results produced by this technique to evaluate genetic variability in popcorn populations.

Comparing the mean distance values for each cycle (Table 2) obtained by RAPD markers, C1 was statistically smaller than C0 and C2 by the t-test at 1% of probability level. The similarity of the mean distance between C0 and C2 is a good indication of genetic variability permanence in the base population, which was being bred. In general, breeders recommend the evaluation of about 200 families and selection and recombination of about 20% of the superior families (Hallauer and Miranda Filho, 1981; Guzman and Lamkey, 2000). This means that the ideal situation should be the selection and recombination of about 40 superior families. Thirty families in C1 cycle were selected and recombined; this may be an explanation of the reduction of mean distance from C0 to C1 observed.

Table 1. Primers, number of monomorphic bands, number and size of polymorphic bands and total number of bands per primers.

Primers	Number of Monomorphic Bands	Number of Polymorphic Bands	Total Number of Bands per Primers	Size (pb) of Polymorphic Bands
OPAA 11	3	5	8	1900, 1700, 1000, 600, 300
OPAA 14	0	5	5	2100, 1700, 1200, 800, 600
OPAB 2	1	6	7	1500, 1250, 700, 500, 450, 400
OPAB 5	0	7	7	2400, 2000, 1900, 1800, 1600, 1100, 1000
OPAB 6	0	4	4	2140, 1980, 1700, 1350
OPAB 19	1	5	6	2100, 1700, 1650, 450, 350
OPAE 9	0	4	4	1700, 1400, 1300, 600
OPAE 10	0	8	8	1900, 1800, 1600, 1200, 1100, 700, 400, 300
OPAE 11	0	9	9	2400, 1900, 1700, 1400, 1200, 800, 500, 400, 300
OPAE 18	1	6	7	1900, 1700, 1200, 800, 400, 300
OPAE 20	1	5	6	1700, 1400, 600, 500, 400
OPAF 2	1	4	5	1600, 700, 500, 350
OPAF 14	2	3	5	1600, 800, 600
OPI 7	0	12	12	1600, 1500, 1050, 700, 600, 550, 520, 480, 430, 400, 350, 300
Total	10	83	93	-

Although different strategies were used to conduct the recurrent selection cycles, results show that the breeder's experience in recombining a minimum of 30 individuals in each cycle under selection is very important to maintain the longevity of the breeding program.

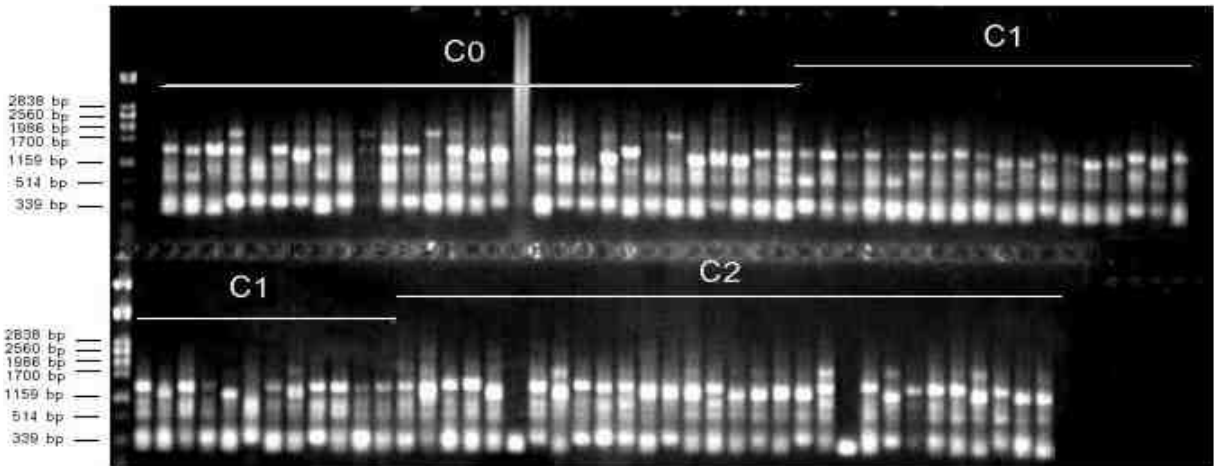

Figure 1. Band patterns amplified by the primer OPAE 11. The line 1 shows the "Kb ladder" and the other lines indicate individuals from the C0, C1 and C2 cycles of recurrent selection from the worked populations.

Table 2. Estimates of mean dissimilarity coefficient of each cycle obtained by Jaccard's index arithmetical complement based on the RAPD markers.

Cycle	Means[1/]
C0	0.3801 a
C1	0.3481 b
C2	0.3786 a

[1/]Means followed by the same letter do not differ by the t-test at 1% of probability level.

Labate *et al*. (1999) analyzed genetic diversity in maize populations, before and after 12 reciprocal recurrent selection cycles and concluded that genetic variation decreased in both studied populations. However, only eight lines were recombined in the first eight selection cycles; after the eighth cycle, 20 lines were recombined.

Guzman and Lamkey (2000) worked with the same selection intensity of 20% but the population size varied from five to 30 families to be recombined. They observed that a small population size increased the genetic uniformity as a consequence of allele loss.

Studies by Labate *et al*. (1999) and Guzman and Lamkey (2000) showed that the main concern of the breeder in recurrent selection programs should be the population size and not the percentage of individuals selected, which corroborates the results found in the present study.

Three clusters were formed by the Ward's method using RAPD markers (Figure 2). The first cluster represents the C1 cycle, the second cluster, the largest one, with 37 individuals, represents the C2 cycle and the third one represents the C0 cycle. Some individuals were placed in a different group than expected considering their original cycle. Specifically, the individuals 3 and 23 from C0 were placed into C1; the individual 89 from C2 to C1; the individuals 10, 16, 28 and 29 from C0 to C2; the individuals 40, 41, 44, 49, 51 and 54 from C1 to C2; the individuals 47 and 48 from C1 to C0; and the individuals 66, 84 and 90 from C2 to C0.

The allocation of a few individuals from one cycle to another is not unexpected, since it is the same population in different cycles. Therefore, cycles overlapping may be expected, since the different cycles are from the same gene pool.

The Tocher's method formed 29 groups (Table 3). The individuals from C0 cycle showed the best distribution and did not form any large group, unlike cycles C1 and C2. The largest group of cycle C0 contained only six individuals corresponding to only 20% of the genotypes.

In cycle C1, 53.33% of the individuals were grouped into a single group (group 1) and 26.67% were distributed into other two groups (groups 3 and 11). This cycle contained one group (22) with only one individual (number 31). The similarity of the different groups from this cycle is greater than the similarity of the groups from other cycles. The individuals in C2 cycle also followed this tendency in clustering, but containing five groups (numbers 20, 26, 27, 28 and 29) with just one individual, indicating that there is genetic variability to be explored in future cycles.

Table 3. Clustering of 90 individuals by the Tocher method, based on Jaccard Index arithmetical complement, using 83 polymorphic bands.

Groups	Genotypes[1/]
1	34 53 36 50 35 39 3 33 46 23 37 60 56 43 57 58 12 48 45
2	71 80 68 63 69 64 61 62 72 67
3	44 49 28 26 54 41 40
4	9 29 2 10 85 1 21
5	11 15 20
6	38 42
7	74 78 76 66 86 77
8	7 8 6
9	75 47 84
10	87 88
11	52 59 55
12	4 19
13	22 27
14	70 82
15	17 18
16	51 32
17	81 83
18	30 24
19	16
20	89
21	25
22	31
23	5
24	13
25	14
26	79
27	65
28	73
29	90

[1/]Genotypes: one to 30: C0 cycle, 31 to 60: C1 cycle, 61 to 90: C2 cycle.

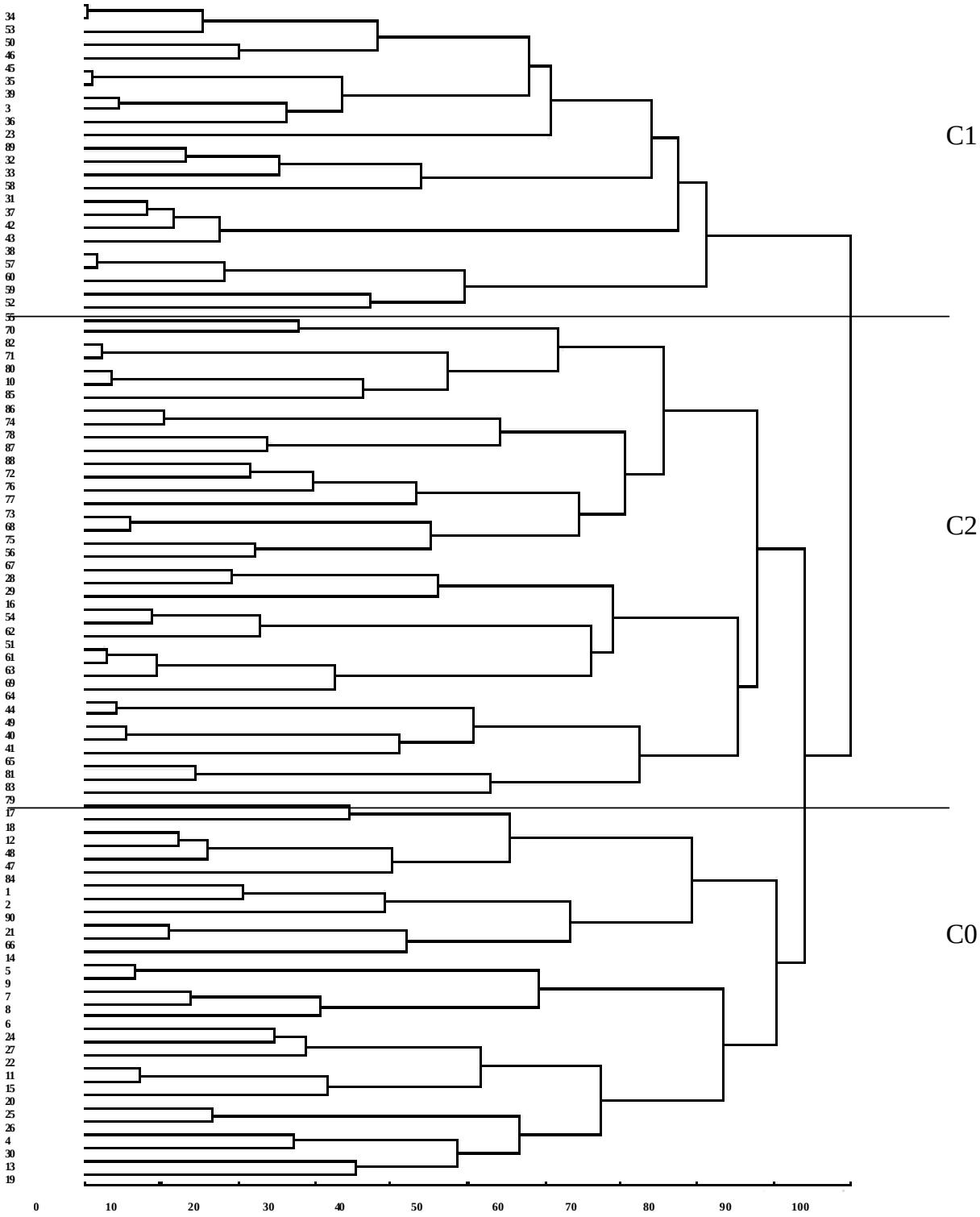

Figure 2. Dendrogram of genetic dissimilarity among the 90 popcorn genotypes obtained by the Ward method.

Conclusion

The utilization of different recurrent selection strategies in the cycles did not decrease the genetic variability, due to the minimum of thirty individuals for recombination.

Together with other researches, the present study confirms that the main concern of the breeder in recurrent selection programs should be the population size and not the percentage of individuals selected.

The RAPD markers proved to be effective in detecting the genetic variability maintained during different recurrent selection cycles.

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