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Full-sib reciprocal recurrent selection in the maize populations Cimmyt and Piranão

K.S. da Cunha[1], M.G. Pereira[1], L.S.A. Gonçalves[1], A.P.C.G. Berilli[2], E.C. de Oliveira[1], H.C.C. Ramos[1] and A.T. do Amaral Júnior[1]

[1]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brasil
[2]Instituto Federal do Espírito Santo, Campus Itapina, Colatina, ES, Brasil

Corresponding author: M.G. Pereira
E-mail: messias@uenf.br

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ABSTRACT. We estimated the genetic gains of the 12th cycle of reciprocal recurrent selection for maize traits of agronomic interest. We used 23 ISSR molecular markers in an attempt to maximize genetic variability among and within populations based on selection of S_1 progenies. To this end, 138 full-sib families were evaluated in a randomized block design in two environments (the municipalities of Campos dos Goytacazes and Itaocara, in the State of Rio de Janeiro, Brazil), with replications within sets. Direct selection for grain yield was used for the selection of the families. To assess genetic diversity among and within populations, we examined plants produced from part of the S_{1s} seeds from the parents that originated the 42 full-sib families that were selected from the agronomic traits. Direct selection for grain yield provided good gains for the traits evaluated, with estimated improvement of -0.87 days for days to flowering, 0.35 plants, 1.79 ears per plot, 0.58 g per 100-grain weight, 308.21 g ear weight per plot, and 261.83 kg/ha grain yield. Application of molecular markers at the stage of superior progeny selection led to increased genetic distance among populations, which is a very important factor for maximizing the

utilization of heterosis and providing greater longevity to the reciprocal recurrent selection program.

Key words: *Zea mays* L.; Direct selection; Genetic gains; Full-sib families

INTRODUCTION

Maize (*Zea mays* L.) plays an important role in global agribusiness. It is estimated that worldwide production was around 818 million tons in the 2009/2010 harvest. The United States, China and Brazil are the largest producers, responsible for 41, 20 and 7% of the world's production, respectively (Food and Agriculture Organization of the United Nations, FAO, 2011).

Genetic breeding, with the exploitation of heterosis or hybrid vigor, is considered one of the main factors that have affected the progress of maize crops in the world (Springer and Stupar, 2007). However, most seeds of maize hybrids available for commercial breeding show a reduced genetic base, mainly due to strong commercial pressures that force breeders to extract a narrow range of the elite germplasm tested (Goodman, 2005; Mikel and Dudley, 2006; Hartings et al., 2008; Fan et al., 2010; Reif et al., 2010). Moreover, elite lines developed in breeding programs are recycled repeatedly to join their favorable alleles in new inbred lines and hybrids (Bernado, 2008; Souza Jr. et al., 2010).

Although these procedures may be considered effective for the development of hybrids, in the long term, this reduced genetic variability can lead to genetic vulnerability to biotic and abiotic stresses and limited future selection gains (Smith et al., 2004; Reif et al., 2010; Souza Jr. et al., 2010; Romay et al., 2011). Therefore, the exploration of new sources of germplasm and the adoption of other breeding strategies can be considered an alternative for long-term maize breeding progress.

Reciprocal recurrent selection (RRS), proposed by Comstock et al. (1949), is a method to improve the performance of the crossing of two populations of different heterotic groups. The main focus of the method is the simultaneous selection for general and specific ability, exploring various mechanisms of gene action (additivity, dominance, overdominance, and epistasis; Hallauer and Miranda Filho, 1988).

According to Hallauer et al. (2009), RRS is considered to be well suited for extracting lines aimed at the formation of hybrids, because populations become increasingly productive *per se* and in crosses between them. Meanwhile, the crosses of the lines extracted from improved populations become increasingly heterotic as the number of selection cycles increases. Furthermore, the strains developed in RRS programs are not related to the strains developed in recycling programs. Therefore, these lineages could be incorporated into these programs to maintain the rate of development and launch of new hybrids (Souza Jr. et al., 2010).

The Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) has developed a program aimed at increasing RRS yield and other agronomic traits of economic interest of the populations Cimmyt (flint type) and Piranão (dent type). To increase the efficiency of the RRS, the program has adopted the use of marker-assisted selection aimed at monitoring the genetic variability of the populations that are being worked and selecting families to be recombined to maximize heterosis among populations (Tardin et al., 2007; Berilli et al., 2011).

This study aimed to estimate the genetic gains planned for the 12th cycle of reciprocal recurrent selection for traits of agronomic interest and to maximize, by means of inter-simple

sequence repeat (ISSR) molecular markers, the variability of the populations Cimmyt and Piranão, based on the S_1 progeny selected by selection index.

MATERIAL AND METHODS

Plant material

Two populations belonging to different heterotic groups, Cimmyt (flint type) and Piranão (dent type), were evaluated. Both populations were subjected to 11 cycles of RRS. The five initial cycles were carried out by the Universidade Federal de Viçosa (UFV) and the remaining cycles, by the UENF.

Achievement of S_{1s} and full-sib families

In March 2008, the populations Cimmyt and Piranão were sown in the UENF experimental field (Campos dos Goytacazes, RJ, Brazil) to obtain interpopulation full-sib and S_1 families. For this purpose, a sample of two populations from the previous recurrent selection cycle was sown in alternate 6.00-m rows, spaced 1.00 m between rows and 0.40 m between furrows. Thirty days after emergence, thinning was performed, with one plant per hole.

To obtain the interpopulation full-sib and S_{1s} families, the following procedures were adopted: the ears were covered with transparent plastic bags before stigmata release. Simultaneously, the tassels were covered to prevent contamination. The crosses were carried out in prolific plants selected within each pair of rows, so that the first ear (top) was the product of self-fertilization and the second ear was produced by inter-population crosses. Thus, we obtained 138 full-sib families and 276 self-fertilized progenies (S_1). The S_1 seeds were stored in a cold chamber, and the interpopulation full-sib families were used for evaluation trials.

Evaluation and selection of full-sib progenies

The 138 interpopulation full-sib families were evaluated in the 2008/2009 agricultural year, in a randomized block design in two environments (Campos dos Goytacazes and Itaocara, in the State of Rio de Janeiro), with replications within sets. The experimental plots consisted of single 5.00-m rows, with spacing of 1.00 m between lines and 0.20 m between plants. Three seeds were sown per hole at a depth of 0.05 m. Thinning was performed 21 days after emergence and one plant was placed in each hole.

The following agronomic traits were evaluated:

- number of days for flowering (NDF) - number of days from planting until the emergence of style-stigma of the ear of 50% of the plants of the experimental unit;
- number of plants (NP) - total number of plants during harvest;
- number of ears (NE) - total number of harvested ears;
- weight of 100 grains (W100) - weight, in grams, of a sample of 100 healthy grains.
- weight of ears (WE) - weight, in grams per plot, of the husked ears, and
- grain yield (GY) - achieved by weighing the grains in each plot, after threshing, and expressed in kg/ha.

The data of the agronomic traits were used for the analysis of variance, according to

the statistical model

$$Y_{ijkl} = \mu + E_i + S_j + ES_{ij} + R/ES_{ijk} + F/S_{jl} + EF/S_{ijl} + \xi_{ijkl},$$

where Y_{ijkl} is the average phenotypic value of the plot; μ is the average; E_i is the fixed effect of the i-th environment; S_j is the effect of the j-th set; ES_{ij} is the effect of the interaction between environments and sets; R/ES_{ijk} is the effect of the k-th replication within the interaction between the i-th environment and the j-th set; F/S_{jl} is the random effect of the i-th genotype within the j-th set; EF/S_{ijl} is the effect of the interaction between environments and genotypes within the j-th "set", and ξ_{ijkl} is the experimental error. The estimates of the components of variance were determined. The estimator of the genotypic variance among families was expressed by:

$$\hat{\sigma}_G^2 = \frac{MSF/S - MSR}{er},$$

where *MSF/S* = mean square of families within "sets"; *MSR* = mean square of the residue; *r* = replication, and *e* = environment. The phenotypic variance was expressed by the following equation:

$$\hat{\sigma}_p^2 = \frac{MSF/S}{er}.$$

The variance of the genotype versus environment interaction was determined by the equation:

$$\hat{\sigma}_{GE}^2 = \frac{\frac{MS(E*F)}{S} - MSR}{r} \frac{e-1}{e},$$

where *MS(ExF) / S* = mean square of the families versus environment interaction within sets. Heritability based on the average of families was estimated by the expression:

$$\hat{h}_X^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_F^2} = \frac{\frac{MSF}{S} - MSR}{\frac{MSF}{S}}.$$

The percent genetic variation coefficient (CV_g) was determined by:

$$CV_g = 100\sqrt{\frac{\sigma_g^2}{MSR}}, \text{ and the index of variation, by: } I_v = \frac{CV_g}{CV_e}.$$

The direct gains for grain yield were predicted by the following expression: $\Delta M = p \times DS \times h^2$ (Lynch and Walsh, 1998), where *p* is the parent control and *DS*, the selection differential. The indirect gains were calculated by the expression

$$\Delta M_{p(GY)} = p \times DS_{p(GY)} \times h^2 \text{ (Lynch and Walsh, 1988)},$$

where $DS_{p(GY)}$ is the differential of indirect selection, determined by the difference between the averages of the characteristics of the individuals selected based on the average grain yield and general average.

Assessment of genetic diversity by the ISSR technique

Plants from part of the S_{1s} seeds of the parents that originated the 42 full-sib families selected by the selection index were used to assess the genetic diversity within and between the populations Cimmyt and Piranão.

Total cell DNA was extracted according to Doyle and Doyle (1990), with modifications suggested by Daher et al. (2002). After DNA extraction, DNA was quantified on 1.0% agarose gels. The DNA High Mass Ladder (Invitrogen, USA) was used. The gel was stained with a mixture of 6X Blue Juice (0.4 mL 0.5 M 10X TAE, 0.4 M Tris-base, 34.8 M acetic acid and 0.02 M EDTA; 0.2 mL 10% SDS; 0.2 mL bromophenol blue; 7.0 mL glycerol; 1.7 mL sterile water) with 5X GelRed (1 mL 10,000X GelRed in 0.5 mL dimethyl sulfoxide (DMSO); 2 mL ultrapure water), at a 1:1 ratio. The image was revealed by the MiniBis Pro photo-documentation system.

The selection of primers (Table 1) was carried out, and amplification reactions were performed according to the protocol proposed by Williams et al. (1990) to obtain ISSR fragments, using a final volume of 20 µL. Each reaction contained the following: 2 µL 10X buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.4), 2 µL 25 mM $MgCl_2$, 1.6 µL 2 mM dNTPs, 1 µL DMSO, 1.8 µL 0.5 mM primer, 0.12 µL 5 U Taq DNA polymerase and 2 µL 5 ng genomic DNA. The final volume was completed with ultrapure water. PCR for the marker was conducted as follows: 4 min at 94°C, followed by 37 cycles [94°C for 1 min, 46° to 50°C for 2 min (depending on the primer used) and 72°C for 2 min], and a final extension at 72°C for 7 min.

The products of the amplifications were separated on a 2.0% agarose gel. The marker used was 1-kb DNA Ladder (Invitrogen). The gel was stained with a mixture of 6X Blue Juice (0.4 mL 10X TAE, 0.5 M; 0.2 mL 10% SDS; 0.2 mL bromophenol blue, 7.0 mL glycerol; 1.7 mL sterile water) with 5X GelRed (1 µL 10,000X GelRed in 0.5 mL DMSO, 2 mL ultrapure water) at a 1:1 ratio. The image was obtained by the MiniBis Pro photo-documentation system.

For the analysis of ISSR markers, the revealed gels were visualized and subsequently interpreted according to the presence and absence of bands, generating a binary matrix. The complement of Jaccard's similarity coefficient was used to estimate the genetic distances among the genotypes. A simplified representation of the genetic distances among the accessions was then carried out using the UPGMA (unweighted pair-group method using arithmetic average) clustering method and the principal coordinate analysis. The variability between and within the populations Piranão and Cimmyt was also assessed by analysis of molecular variance (MANOVA).

RESULTS AND DISCUSSION

Assessment of full-sib progenies

All traits were significant for the environments, demonstrating that the environments differed enough to allow differences among the traits (Table 1). Regarding the set effect, a significant effect was observed for most traits, except NE. This result reveals the effectiveness and necessity of using a block design split into sets. All traits revealed significant differences for the environment x set interaction, excepting NP and W100, indicating that the families were randomly distributed into the "sets", showing phenotypic changes stimulated by soil and climatic changes in the environments.

Table 1. Estimate of the mean squares, averages and genetic parameters of six traits evaluated in 138 full-sib progenies of maize from the 11th reciprocal recurrent selection cycle.

Source of variation	d.f.	Mean square					
		NDF	NP	NE	W100	WE	GY
Environment (E)	1	336.39**	2196.13**	798.20**	1189.86**	99911345.61**	251532681.1**
Sets (S)	5	21.40**	18.48**	69.97	72.82**	1437249.52*	4669034.2*
E x S	5	23.14**	10.96	83.34*	16.73	2266869.20**	6015917.7**
Replications (R)/E x S	12	33.95**	10.79*	75.89*	14.35	2451020.85**	7438730.6**
Families (F)/S	132	16.38**	13.53**	72.32**	22.91**	1231798.20**	3621298.6**
E x F/S	132	8.34*	5.79	38.13	7.86	890748.43**	2714704.1**
Residual	264	5.95	4.95	34.09	8.19	614004.44	187112.1
General average		72.90	23.11	31.01	30.88	3053.38	5008.14
Genetic parameter							
σ^2_g	-	2.61	2.15	9.56	3.68	154448.40	437544.40
σ^2_f	-	4.09	3.38	18.08	5.73	307945.50	905324.60
CVe (%)	-	3.35	9.69	18.92	9.27	25.66	27.32
CV_g	-	2.21	6.37	9.97	6.21	12.87	13.21
I_v	-	0.65	0.66	0.53	0.67	0.50	0.48
$h^2_{\bar{x}_f}$	-	63.68	63.39	52.87	64.27	50.15	48.33

d.f. = degrees of freedom; NDF = number of days for flowering; NP = average number of plants per plot; NE = average number of ears per plot; W100 = average weight of 100 grains in g; WE = average weight of ears in g/plot; GY = grain yield in kg/ha. σ^2_g = genotypic variance; σ^2_f = phenotypic variance; CVe = experimental variation coefficient; CV_g = genetic variation coefficient; I_v = variation index; $h^2_{\bar{x}_f}$ = heritability coefficient. *P < 0.10 and **P < 0.05 (F-test).

All traits were significant for the source of family variation within set, demonstrating that there is enough genetic variability to be explored in future selection cycles (Table 1). The significance of NDF, WE and GY for the environment x family interaction within the sets demonstrates that the families did not maintain the same phenotypic behavior in the two environments, indicating that the best families in a certain place may not be the best in a different location. However, the difference between the environments evaluated does not preclude the implementation of a single breeding program for the two locations, since, according to Santos (2005), the most important for selection is the expression of the phenotypic averages of the families in both environments. In such context, it is possible to achieve gains per selection, mainly using the potential of selection indices.

Regarding genetic parameters, the traits NDF, NP and W100 showed high estimates of genotypic variances and heritability values higher than 60%, based on the average of the families, and variation rate with magnitudes higher than 0.60 (Table 1). For NE, WE and GY, the variation index was 0.53, 0.50 and 0.48, respectively, whereas for heritability, it was 52.87, 50.15 and 48.33, respectively (Table 1). The results of this study agree with those obtained in previous cycles (Tardin et al., 2007; Gabriel, 2006, 2009).

The direct selection for grain yield provided adequate returns for the set of traits evaluated. The predicted selection gains were -1.2, 1.53, 5.78, 1.89, 10.09, and 10.46 for NDF, NP, NE, W100, WE, and GY, respectively. Figure 1 shows the boxplots for a 95% confidence interval, which represent the dimensions of variation of the traits evaluated for the progeny under study (138 interpopulation full-sib families) and the selected progeny (40 interpopulation full-sib families). For NDF, NP, NE, W100, WE, and GY, the expected gains were -0.87 days, 0.35 plants, 1.79 ears, 0.58 g, 308.21 g/plot, and 261.83 kg/ha, respectively.

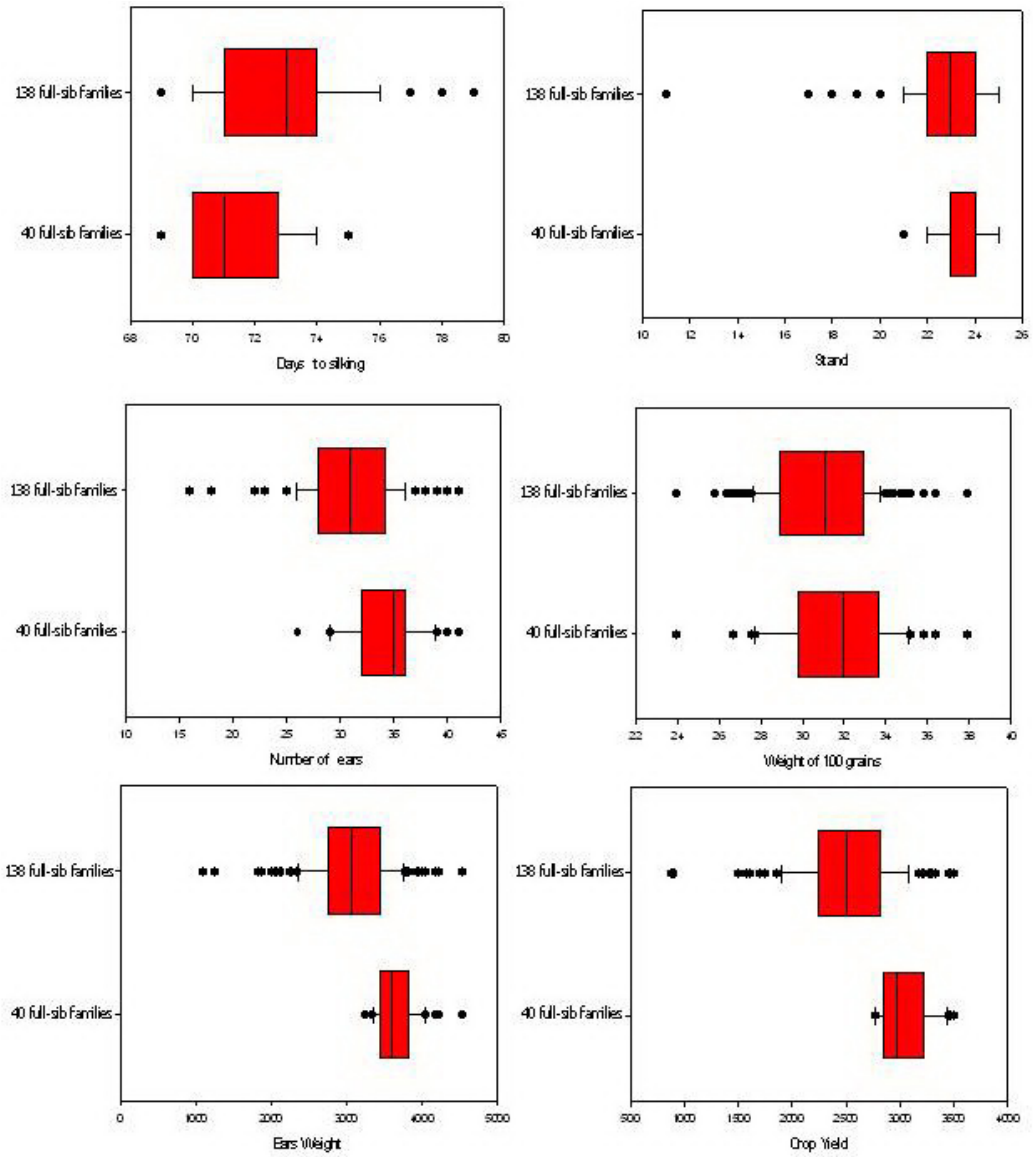

Figure 1. Boxplot for the traits flowering, stand, number of cobs, weight of 100 grains, weight of cobs, and grain yield for the progeny evaluated (138 interpopulation full-sib families) and the progeny selected (42 interpopulation full-sib families).

Analysis of the ISSR markers

By ISSR analysis, each primer produced bands of variable intensity, which could be easily detected, and nonspecific bands, which were discarded. The 23 primers used produced 118 bands (Table 2). Of these, 93 were polymorphic (78.81%) and 25, monomorphic

Table 2. List of primers used and number of amplified fragments and polymorphic ISSR.

Oligonucleotide	Amplified fragments	Polymorphic fragments	Percentage of polymorphism
$(AC)_9$	6	5	83.3
$(GA)_9T$	5	2	40.0
$(GA)_8CA$	4	2	50.0
$(GA)_8GC$	5	2	40.0
$(CAGA)_4$	4	3	75.0
$(CTC)_5RC$*	6	4	66.6
$(GA)_8YC$*	6	5	83.3
$(AC)_8T$	9	8	88.8
$(ATG)_6$	4	4	100.0
$(GT)_8YC$*	4	4	100.0
$(CT)_8RG$*	4	3	75.0
$(AG)_8YA$*	3	2	66.6
$(AC)_8CT$	5	4	80.0
$(AG)_8YT$*	4	3	75.0
$(CT)_8G$	4	4	100.0
$(GTG)_3GC$	4	2	50.0
$(AC)_8CG$	4	2	50.0
$(GT)_6CC$	10	9	90.0
$(CAC)_3GC$	5	5	100.0
$(GGAT)_3GA$	5	4	80.0
$(AC)_8YG$*	5	5	100.0
$(GAA)_6AA$	5	4	80.0
$(AG)_8C$	7	7	100.0
Total	118	93	1773.6
Average	5.1	4.0	77.1

*R = A, G; Y = C, T.

(21.19%). The progenies C36 and P7 were the most distant (0.5657), while P22 and P23 were the most similar (0.2025).

UPGMA hierarchical clustering and principal coordinate analysis demonstrate that the populations Cimmyt and Piranão (Figure 2) were separated. Several subgroups were observed within these populations, demonstrating the existence of variability between and within the populations. These clustering results led to the conclusion that it is possible to achieve gains *per se* by means of selection, and that there are expectations to maximize heterosis in interpopulation crosses or among lines from these different populations. These results are consistent with those obtained by Tardin et al. (2007) and Berilli et al. (2011), who evaluated the genetic divergence between full-sib families of cycles 8 and 10, respectively, of the same populations under SRR.

It is well known that each cycle of recurrent selection basically involves three stages: i) development of progeny, ii) evaluation and selection of progeny, and iii) recombination of superior progeny. Therefore, the progeny selected and recombined must be sufficiently divergent so that the recombination process can restore genetic variability and ensure the continuity of the next cycles. In this context, the inclusion of molecular markers aims to identify reliably the maximum genetic variability within groups and maximize the intergroup genetic distance. Based on this assumption, the intermediate progeny between the populations Cimmyt and Piranão were eliminated, which consequently led to the maximization of the genetic distances between the populations (Figure 2).

A comparison between Figures 2 and 3 revealed that the molecular markers used at the selection stage contributed to the increased genetic distance between the two populations after the elimination of the intermediate genotypes. The populations studied were at the 11th cycle of the reciprocal recurrent selection, and from the 8th selection cycle, they have been monitored by

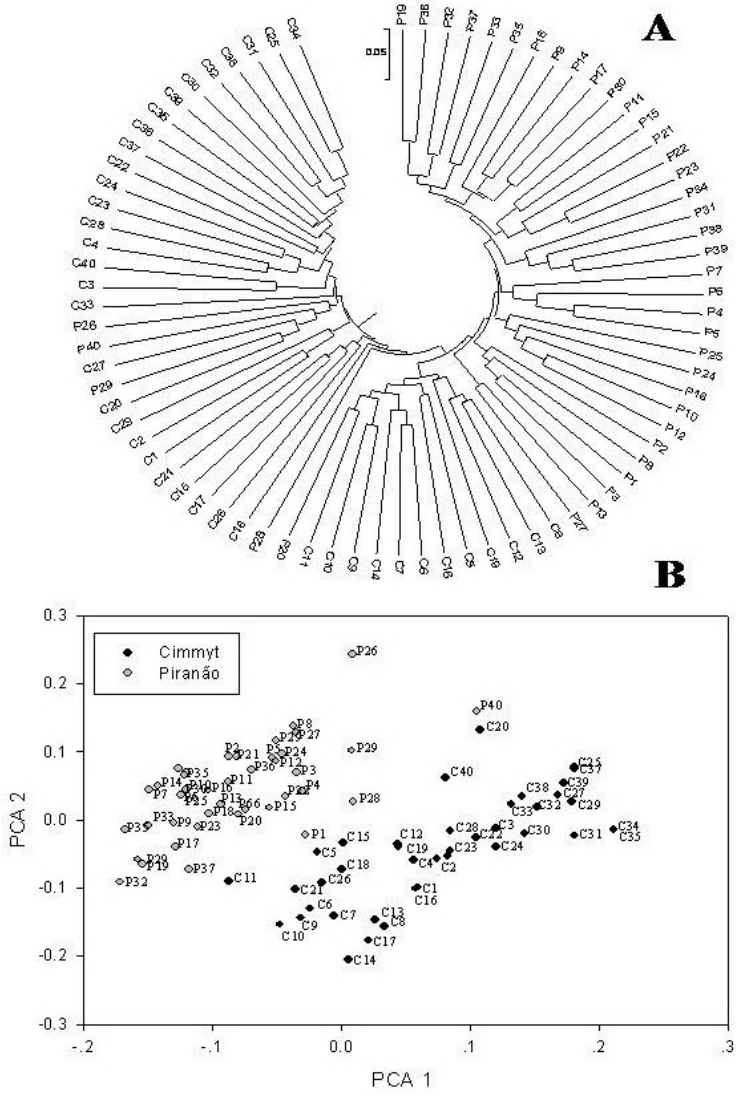

Figure 2. A. UPGMA dendrogram and **B.** principal coordinate analysis (PCA) of the 80 S_1 progenies of the populations Cimmyt and Piranão, genotyped by ISSR markers.

molecular markers. The RAPD technique was applied at the 8th selection cycle (Tardin et al., 2007), AFLP, at the 9th cycle (Gabriel, 2006), and ISSR, at the 10th cycle (Berilli et al., 2011).

AMOVA of the 80 S_1 progenies of the populations Cimmyt and Piranão revealed that the most genetic variation was within populations with 88.77%, while between populations, it was 11.23% (Table 3). After the elimination of the contaminant progeny and selection of the most distant progeny, the genetic divergence between the populations increased to 17.67%, and the distance within populations was 82.33%. Berilli et al. (2011) evaluated the 11th cycle and found the genetic variation of 10.16% between the populations, before the application

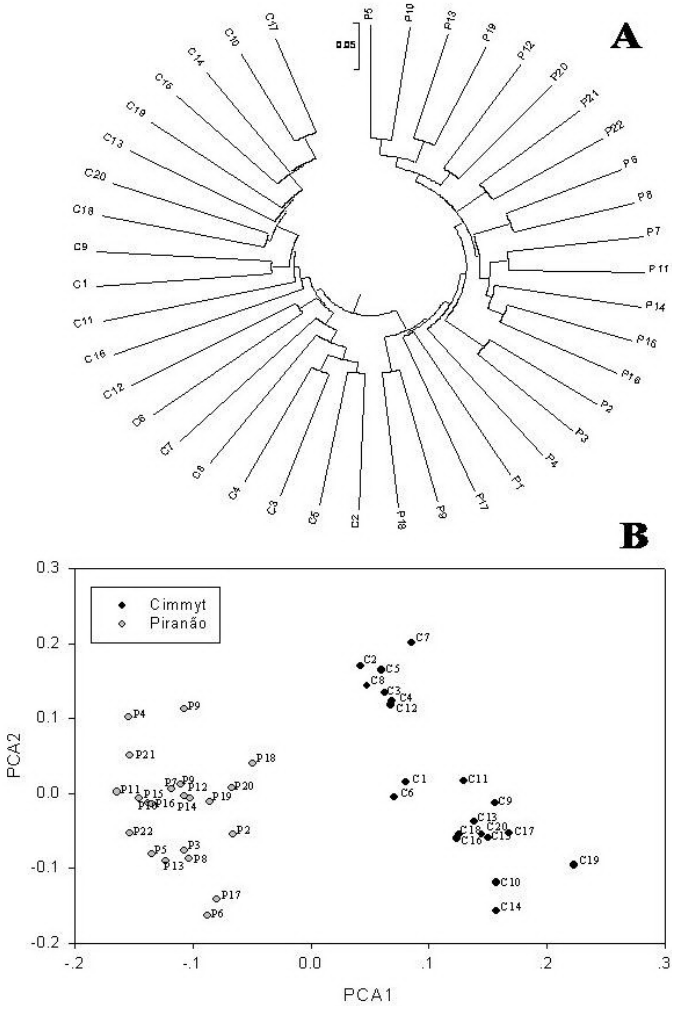

Figure 3. A. UPGMA dendrogram and **B.** principal coordinate analysis (PCA) of the 42 S_1 progenies of the populations Cimmyt and Piranão, genotyped by ISSR markers.

Table 3. Analysis of molecular variance of the maize populations Cimmyt and Piranão before and after molecular genotyping.

	d.f.	MS	Variance components	Percentage	Probability
Before genotyping					
Between populations	1	99.10	2.0687	11.2306	<0.001
Within populations	78	16.3516	16.3516	88.7694	<0.001
Total	79	17.3991	18.4203	100	<0.001
After genotyping					
Between populations	1	86.9584	3.3952	17.6688	<0.001
Within populations	40	15.8207	15.8207	82.3312	<0.001
Total	41	32.7727	19.2159	100	<0.001

d.f. = degrees of freedom; MS = mean squares of traits.

of marker-assisted selection. After the application of marker-assisted selection, the variation between populations was 14:28%. Thus, it is clear that the application of molecular markers at the stage of superior genotype selection allows increased genetic distance between populations, a very important factor when the aim is to maximize the utilization of heterosis and provide higher longevity to the SSR program.

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