Population structure and impact of recurrent selection on popcorn using EST-SSR markers

Amanda Gonçalves Guimarães*, Antônio Teixeira do Amaral Júnior, Janeo Eustáquio de Almeida Filho, Guilherme Ferreira Pena, Cássio Vittorazzi and Messias Gonzaga Pereira

Centro de Ciências e Tecnologia Agropecuária, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego, 2000, 28013-602, Parque Califórnia, Campos dos Goytacazes, Rio de Janeiro, Brazil. *Author for correspondence. E-mail: amandaggumaraes@yahoo.com.br

ABSTRACT. The success of any recurrent selection program depends on the genetic variability of the evaluated population, which is used to refer to the diversity of existing alleles at many genetic loci. Thus, the goal of the present study was to investigate the impact of recurrent selection across nine cycles of a UENF-14 popcorn population through the analysis of genetic diversity and structure using microsatellite markers (EST-SSRs). Genomic DNA was extracted from young leaves of 25 individuals from each cycle (C0, C1, C2, C3, C4, C5, C6, C7, and C8), totaling 225 samples from the UENF-14 population. Fifty EST-SSR markers were used for the analysis of genetic diversity across the recurrent selection cycles, 16 of which were polymorphic. Thirty-four alleles were detected, with an average of 2.13 alleles per locus. Throughout all the recurrent selection cycles, there was a reduction in heterozygosity and an increase in inbreeding. The population structure showed a sharing of alleles, inferring that some may be directly related to the main selection characteristics.

Keywords: Zea mays L. var. Everta, variability, microsatellite markers, heterozygosity, equilibrium.

Estrutura populacional e impacto da seleção recorrente em milho-pipoca por marcadores SSR-EST

RESUMO. O êxito de qualquer programa de seleção recorrente depende da variabilidade genética da população avaliada, sendo utilizada para se referir à diversidade de alelos existentes nos vários locos genéticos. Assim, o objetivo do presente estudo foi investigar o impacto da seleção recorrente em nove ciclos da população UENF-14 de milho-pipoca através da análise da diversidade genética e da estrutura utilizando marcadores microssatélites (SSR-ESTs). O DNA genômico foi extraído de folhas jovens de 25 indivíduos de cada ciclo (C0, C1, C2, C3, C4, C5, C6, C7 e C8), totalizando 225 amostras da população UENF-14. Para a análise da diversidade genética entre os ciclos de seleção recorrentes foram utilizados cinquenta marcadores SSR-ESTs, dos quais 16 revelaram ser polimórficos. Trinta e quatro alelos foram detectados, com uma média de 2,13 alelos por loco. Ao longo de todos os ciclos de seleção recorrentes houve uma redução na heterozigosidade e aumento na endogamia. A estrutura da população mostrou um compartilhamento de alelos inferindo que alguns podem ser direcionados para as principais características de seleção.

Palavras-chave: Zea mays L. var. Everta, variabilidade, marcadores microssatélites, heterozigosidade, equilíbrio.

Introduction

Investment in the breeding of popcorn in Brazil has grown considerably among public and private institutions, which allows for a reduction in the importation of seeds and an increase in the development of new varieties adapted to different regions of the country (Ribeiro et al., 2016). Recurrent selection is a methodology used in popcorn breeding programs aimed at the production of varieties in which the frequency of favorable alleles in original populations is increased, providing successive genetic gains (Hallauer & Carena, 2009; Pereira & Amaral Junior, 2001). However, success depends on the genetic variability of the involved population.

Genetic variability is used to refer to the diversity of existing alleles at genetic loci (Solomon, Martin, & Zeppa, 2010). To understand the genetic variation during selection cycles, estimating the proportion of heterozygotes in the populations is important to ensure the progress of the selection of traits of interest for the crop, as alterations in allele frequencies due to selection may lead to biased estimates and reduce the degree of polymorphism and expected heterozygosis (Choudhary et al., 2012; Sarcevic, Pejc, Baric, & Kozumplik, 2007). Therefore, knowing the variability and genetic structure of a population can contribute to the long-term success of breeding programs.

Molecular markers have yielded satisfactory results in the studies of alterations of the genetic
structure of populations subjected to several selection cycles (Solomon et al., 2010). Of the many types of molecular markers, microsatellite markers (simple sequence repeats (SSRs)) are the most widely used to determine the genetic diversity of plants due to their highly polymorphic nature, co-dominance, easy reproducibility and high specificity (Dandolini et al., 2008; Pinto, Vieira, Souza Júnior, & Souzam, 2003).

Despite the relevance of the use of recurrent selection for the release of better adapted, productive cultivars with higher grain-popping expansion, the study of the impact of genetic diversity throughout selective cycles at the molecular marker level has not been fully exploited. Therefore, the goal of the present study was to analyze the genetic diversity and structure from the UENF-14 popcorn population during nine cycles of recurrent selection using microsatellite markers (EST-SSR).

Material and methods

Studied population and genetic gains through recurrent selection in successive cycles

The studied population, UENF-14, is an open-pollinated variety of popcorn. Initially, it consisted of an indigenous compound named UNB-1 donated to the University of Brasilia (UNB) by ESALQ/USP. Later, UNB-1 was brought to the State University of Northern Rio de Janeiro (UENF) by Professor Joachim Friedrich Wilhelm Von in the year 1993 and crossed with popcorn variety SAM (South American Mushroom). The first filial generation was then crossed with a popcorn variety resistant to Exserohilum turcicum (spot blotch). After two cycles of massal selection, three backcrosses were performed with the variety SAM, producing UNB-2. After another two cycles of massal selection, UNB-2 became population UNB-2U (Pereira & Amaral Júnior, 2001), which has been genetically improved via intrapopulation recurrent selection for the north and northwest regions of Rio de Janeiro State, Brazil.

Aiming to promote strategies to obtain genetic gains in the popcorn population UNB-2U for the main traits – grain yield and popping expansion – Design I of Comstock and Robinson (1948) was adopted for being suitable for studying the genetic structure of a population. In 1997, full- and half-sibling progenies of UNB-2U were obtained, from which one plant used as a male was crossed with four female plants, resulting in a set of 20 progenies of full siblings, developed from five males. In the 1997/1998 cropping season, 92 progenies of full siblings were evaluated in the north and northwest regions of Rio de Janeiro State using the predictive model according to Eberhart (1970), with genetic gains estimated according to different methods of intrapopulation recurrent selection. Based on the results, the largest genetic gains with population UNB-2U for grain yield and popping expansion, respectively, were from strategies involving full-sibling families (9 and 27%); inbred S1 families (8 and 19%); half-siblings (6 and 18%); stratified massal selection (2 and 12%); and massal selection (2 and 12%) (Pereira & Amaral Júnior, 2001).

Then, in the first cycle (C1) of UNB-2U, seventy-five full-sibling families were obtained, and their respective plants were self-pollinated. The full-sibling families were evaluated in two environments in Rio de Janeiro State in the 1998/1999 cropping season. To estimate the genetic gain, 30 superior families were selected, resulting in a 10% gain in popping expansion and a 5% increase in grain yield (Daros, Amaral Júnior, & Pereira, 2002). The recombination of these 30 superior families of full siblings consisted of planting 60 S1 families corresponding to the self-pollinated parents.

Daros et al. (2004) implemented the second (C2) recurrent selection cycle in population UNB-2U using 22 inbred families (S1) evaluated in the same two environments in the 2001/2002 cropping season. Forty superior families were selected and used to estimate genetic gain, which reached 27% for grain yield and 18% for popping expansion. For recombination, seeds remaining from the 40 selected S1 inbred families were used.

In the third cycle (C3) of UNB-2U, Santos, Amaral Júnior, Freitas Júnior, Rangel, and Pereira (2007) obtained 192 families of half-siblings, with their respective self-pollinated plants. For the evaluation in the two environments during 2004/2005, 192 half-sibling families were used, from which the 30 superior ones were selected that provided genetic gains of 7% for popping expansion and 10% for grain yield. The recombination of superior progenies was performed using the respective self-pollinated seeds.

Consequently, from the fourth (C4) to the eighth (C8) cycles, 200 families of full siblings were used for evaluation in the two environments, and selection was performed based on the 30 best families for the estimate of the genetic gains for grain yield and popping expansion, respectively, as follows: 8 and 10% in C4 (Freitas Júnior, Amaral Júnior, Rangel, & Viana, 2009); 8 and 6% in C5 (Rangel, Amaral Júnior, Gonçalves, Freitas Júnior, & Candido, 2011); 15 and 11% in C6 (Ribeiro et al., 2012); 8 and 5% in C7 (Freitas et al., 2014); and 5 and 4% in C8 (unpublished data). The remaining seeds from each cycle were used in the recombination process of the respective cycle. During the fifth cycle, the improved population was registered in the Brazilian Ministry of Agriculture, Livestock, and Food Supply (Ministério da
Agricultura, Pecuária e Abastecimento (MAPA)) and released as a new cultivar under the name of UENF-14 for producers and consumers of the north and northwest regions of Rio de Janeiro State (Amaral Junior et al., 2013).

DNA extraction

For the evaluation of the diversity and genetic structure of population UENF-14 at the EST-SSR molecular marker level in the successive recurrent selection cycles, 25 seeds from each cycle (C0, C1, C2, C3, C4, C5, C6, C7, and C8) were used, totaling 225 samples. The seeds were germinated in styrofoam trays containing substrate and maintained in the greenhouse.

DNA was extracted from young leaves of the 225 samples using the standard CTAB method with modifications (Doyle & Doyle, 1990). Next, the DNA was quantified by analysis in 1% agarose gel with 1X TAE buffer (Tris, sodium acetate, EDTA, pH 8.0) using the 100 pb (100 ng μL⁻¹) Lambda (λ) marker (Invitrogen, USA) by comparing bands. For this procedure, the samples were stained with a Gel Red™ and Blue Juice (1:1) mixture, and the image was captured using a MiniBis Pro gel documentation system (Bio-Imaging Systems). Subsequently, the DNA samples were diluted to a working concentration of 5 ng μL⁻¹ for use in the amplification reactions (PCR).

Microsatellite markers and polymerase chain reaction

Microsatellite markers (EST-SSRs) were identified in the literature based on the sequences developed for corn (Zea mays) located in the MaizeGDB database (http://www.maizegdb.org.php). Fifty EST-SSR primers were selected to cover the ten popcorn linkage groups. Subsequently, the primers were tested to determine which would present polymorphic bands through a DNA bulk of the 225 samples. To test the polymerase chain reaction (PCR) conditions, temperature gradients of 59, 61, and 63°C were used to define the optimal annealing temperatures, which were provided by the manufacturer, for each one of the synthesized primers. Afterwards, screening was performed to select the primers based on the degree of polymorphism and quality of amplification.

The PCRs were performed on an Applied Biosystems/Veriti 96-well thermal cycler. For PCR, a 35-cycle program was used consisting of the following temperatures and times: 94°C for 5 min. (initial denaturation); 94°C for 1 min. (cyclic denaturation); specific temperature of each marker for 1 min. (annealing); 72°C for 2 min. (cyclic extension); 72°C for 10 min. (final extension); and 4°C until termination. A final volume of 11 μL per sample was achieved as follows: 2 μL of DNA (5 ng μL⁻¹), 1.30 μL of 10X buffer (NH₄SO₄), 1 μL of M₇Cl₂ (25 mM), 1.5 μL of dNTPs (2 mM), 1 μL of primers (R+F) (5 μM), and 0.12 μL of Taq DNA polymerase (5 U μL⁻¹) (Invitrogen, Carlsbad, California, USA). The amplification products were separated in MetaPhor 4% agarose gel, immersed in TAE buffer [90 mM Tris-acetate (pH 8.0) + 10 mM EDTA], stained with Gel Red™ and Blue Juice (1:1), visualized using the MiniBis Pro (Bio-Imaging Systems) gel documentation system, and compared with the 100 pb High DNA Mass Ladder marker (0.5 ng μL⁻¹) (Invitrogen, USA) during the runs to determine the amplified fragments.

Analyses of genetic diversity of EST-SSR markers of the nine recurrent selection cycles

Polymorphic primers were used to estimate the genetic diversity among the recurrent selection cycles of the UENF-14 popcorn population using Genalex software version 6.3 (Peakall & Smouse, 2009). As such, the following variables were estimated: number of alleles per locus (NA), observed (OH) and expected (EH) heterozygosities, Hardy-Weinberg equilibrium (HWE), inbreeding coefficient (IC) and allele frequency.

Analysis of structure across recurrent selection cycles

Based on the information obtained from the polymorphic primers, the 25 samples from each cycle of the UENF-14 popcorn population were evaluated for genetic structuring by the method based on Bayesian clustering algorithms using STRUCTURE software version 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). For this purpose, the “no admixture model” was employed, and allele frequencies were correlated using a 10,000 “burn-in period”, followed by an extension (Markov Chain Monte Carlo) of 50,000 repetitions, with the number of subpopulations (k) ranging from 1 to 10.

Results

Reduction in heterozygosity and increase in inbreeding of population UENF-14 throughout recurrent selection cycles

To evaluate the genetic structure at the EST-SSR marker level of the UENF-14 popcorn population subjected to nine selection cycles, of the first 50 initially tested primers, 34 produced amplified DNA fragments, 16 of which were polymorphic (Table 1). Of the 16 primers, only two showed three alleles (Umc1393 and Umc1448), whereas the others had two alleles, totaling 34 alleles, with an average of 2.13 alleles per locus (Table 1).
Table 1. Sequence, motif, annealing temperature (°C) and number of alleles (NA) of 16 microsatellite primer pairs evaluated in nine selection cycles (C0-C8) in popcorn population UENF-14.

| Locus   | Primer forward | Primer reverse | Motif | T °C | NA |
|---------|----------------|----------------|-------|------|----|
| Umc1948 | tgtgtaaatgggacaccc | atctctgcgtttaacgtgc | (TACGA)3 | 63   | 2  |
| Umc1071 | agggaagacagpapacactag | pggtggttcctccgttgggtt | (GCT)2   | 61   | 2  |
| Umc1488 | atctctgcgttccttggact | catggagctggttgcagtc | (CGA)1   | 61   | 2  |
| Umc2174 | gtaactgacaccacgtgtagc | acaaataaaatggygtgcccag | (TGAC)3   | 59   | 2  |
| Umc1589 | aacacacgcggtggacacac | ggtgttttcgcscttta | (AG)2   | 61   | 2  |
| Umc1135 | ttttaatgcgcctgggtgtgtt | cctcgggcttgctctctct | (TC)2   | 59   | 2  |
| Umc1680 | ttgaagagcgggagggagac | gggcttttcgctggtgactc | (CT)2   | 61   | 2  |
| Umc1221 | aacacgcaacagtacactgcag | gcaguacgcctggacgagcag | (GCT)4   | 59   | 2  |
| Umc1336 | gtaactgacaccacgtgtagc | gctctgggttggctcttttg | (ACCAG)5  | 61   | 2  |
| y1     | caagataaggaccaggacttga | tgttgcaaggagcacttg | (CCA)3   | 61   | 2  |
| Umc1393 | cctctcttttcgcgcacgac | gcgcagctgcttcacggcag | (GTC)2   | 59   | 2  |
| Umc1527 | aggagctctgcggtagctgcc | gcggagcttggtgcgtggt | (GTC)2   | 61   | 2  |
| Umc1415 | gtgagtttaaccgccttccc | gcagctctgctcagcttcttc | (GAC)2   | 61   | 2  |
| Umc1714 | caagctctgcttggctacta | ccgcctctcagttgctcttt | (AGG)3   | 63   | 2  |
| Umc1196 | cgctctacactgctcacaagcag | agctgggtggtgctggcact | CACACG   | 61   | 2  |
| Umc1506 | aacacgcaacagtacactgcag | atcagctctgctcagcttcttc | (AACA)3   | 63   | 2  |

To evaluate the impact of recurrent selection on the genetic diversity between cycles of the UENF-14 popcorn population, with the use of the 16 EST-SSR primers, on average, the parameters indicated a loss of 62% of observed heterozygosity (OH) from the initial (C0) to the final (C8) cycle, which can be considered relatively large in comparison with the expected heterozygosity (EH), which decreased by 18%. The frequency levels of OH at each locus, over the cycles, were all significant (p < 0.01) (Figure 1).

Figure 1. Frequency of observed (OH) and expected (EH) heterozygosity and Hardy-Weinberg equilibrium (HWE) - (** p < 0.01, * p < 0.05) - of 16 EST-SSR primers evaluated in nine recurrent selection cycles (C0-C8) in popcorn population UENF-14. $r^2$ = coefficient of determination, b = regression coefficient.
It is of fundamental importance for initial studies and sequential generations to study the genetic basis of the population by performing the Hardy-Weinberg Equilibrium (EHW) test, as it allows the breeder to know the allele and genotypic frequencies that will occur in the future generations as well as to manipulate the population and its evolutionary dynamics (Cruz, Ferreira, & Pessoni, 2011). The Hardy-Weinberg equilibrium (HWE) was calculated using the $\chi^2$ test between the 16 EST-SSR loci evaluated throughout the selection cycles of population UENF-14 (Figure 1). Six loci (Umc1948, Umc1389, Umc1506, Umc1327, Umc1221, and Umc1448) showed to be in HWE in all cycles.

The inbreeding coefficient (IC) parameter is an estimate that allows references of the level of homozygosity in individuals at each recurrent selection cycle. According to the result estimates, on average, the IC increased by 2% between the initial and final cycles. Variations throughout the cycles for the IC were obtained for all loci ($p < 0.01$), which reveals that the alleles of the loci are tending to increase in homozygosity at every cycle. The loci that showed the highest values in the last cycle were Umc2174 and Umc1196 (Figure 2).

Recurrent selection is a method that aims to increase the frequency of alleles related to selected traits; thus, allele frequencies were estimated for EST-SSR markers. Based on the results, for most alleles there was no stable change in allele frequency (Figure 3); only in three markers (9%) was the change in allele frequency significantly adjusted for linear regression (Umc1714 ($p < 0.01$), Umc1506 ($p < 0.01$), and Umc1071 ($p < 0.10$)). A frequency near 0.90 was obtained for the first loci, with increases of 0.03 and 0.06 in allele frequency per cycle, respectively.

Figure 2. Inbreeding coefficient for 16 EST-SSR primers evaluated in nine recurrent selection cycles (C0-C8) in popcorn population UENF-14. $r^2 =$ coefficient of determination, $b =$ regression coefficient.
Figure 3. Allele frequency of 16 EST-SSR primers evaluated in nine recurrent selection cycles (C0-C8) in popcorn population UENF-14. \( r^2 = \) determination coefficient, \( b = \) regression coefficient.

Figure 4. Analysis of genetic structuring by Bayesian inference of nine recurrent selection cycles (C0-C8) of popcorn population UENF-14 represented by 25 samples of each selection cycle for 16 EST-SSR primers. A) Delta K with the respective number of groups (K); B) Probability of subgroups for each recurrent selection cycle.
Structure among the recurrent selection cycles of population UENF-14

To analyze the structure of the UENF-14 population at the level of EST-SSR markers, it is important to gather all alleles of the markers in the 25 individuals of each cycle over the selection cycles in order to verify the probabilities of the allele frequencies. The 34 obtained alleles used in the analysis of genetic structure with the Bayesian approach showed that \( k = 3 \) was the ideal number of genetic groups (\( K \)) with the best fit, as it showed the highest \( \Delta K \) value (10.37), which agreed with the inference of Evanno, Regnaut, and Goudet (2005) (Figure 4a). Thus, it was possible to distinguish the three subgroups based on the sharing of alleles in the individuals from each cycle. Greater variability was obtained in the initial cycles (C0, C1, and C2) due to a better distribution of the probability of each subgroup (Figure 4b). From C3 onwards, there was a higher share of some alleles with higher probability, which are represented by the dark gray color subgroup.

Discussion

Recurrent selection is a breeding method used in several species, but for the use of this procedure it is fundamental that there is genetic variability. Therefore, this study monitored the impact of nine selective cycles on the structure and genetic diversity of the UENF-14 popcorn population. Although the two main selection characteristics are productivity and grain expansion capacity in the population improvement program of the UENF-14 popcorn population, among the 16 EST-SSR primers it was detected that six are associated with important quantitative trait loci (QTL) for popcorn: two for popping expansion (Umc1948 and Umc1153) (Babu et al., 2006; Li, Dong, Niu, & Cui, 2007) and four for nutritional quality: Umc1196 is associated with oil content; Umc1389 with protein and starch content; Umc1327 with the amino acid lysine; and Umc1714 with protein content (Dong et al., 2015).

One of the ways to estimate genetic variability in a population is to analyze the different alleles at the various gene loci with the use of molecular markers (Solomon et al., 2010). The average number of alleles obtained per locus in the present study (2.13) (Table 1) was lower than those in other studies for common corn and popcorn (Dandolini et al., 2008; Eloi, Mangolin, Scapim, Gonçalves, & Machado, 2012; Franzoni, Scapim, Beviláqua, Pacheco, & Mangolin, 2012; Trindade et al., 2010) perhaps due to the type of EST-SSR marker used, as the markers represent expressed regions of origin of common corn and not specifically for popcorn. Also, the process of repeating selection cycles in the popcorn crop prioritizes the selection of specific traits, such as grain yield and popping expansion (Franzoni et al., 2012).

The selection of superior genotypes is of great relevance to the knowledge of genetic variation throughout selection cycles by estimating the proportion of heterozygotes and allele frequencies, which avoids compromising future genetic gains. Considering corn, which is typically a palmitic species, the proportion of observed heterozygotes was expected to be close to that of the expected heterozygotes, corresponding to a panmixia equal to one and, consequently, inbreeding close to zero. However, throughout the cycles, these proportions were found to increasingly differ from one another. Excess heterozygotes were obtained in the initial cycles, while heterozygote deficiency occurred in the more advanced cycles. Agreeing with this variation between successive cycles, Franzoni et al. (2012), in a study with 20 SSR loci in eight recurrent selection cycles of popcorn using compound CMS-43, also reported a reduction in observed and expected heterozygosities during the cycles, which were 31 and 25% from cycles C0 to CVIB, respectively.

For the majority of markers, there was a loss of heterozygotes but without great changes in the allele frequency considered by loci. An excessive amount of heterozygotes in the initial cycles can be interpreted as overdominance. Heterozygote deficiency, in turn, may be due to genetic drift, likely resulting from the recombination of a limited number of individuals selected throughout the cycles; this drift effect could increase the probability of eliminating certain genotypes and consequently contribute to the predominance of certain genotypic combinations of the population (Cruz et al., 2011; Souza Júnior, Geraldi, & Vencovsky, 2000), beyond those that favor crossing between related individuals and a resulting increase in inbreeding (Falconer & Mackay, 1996). This increase in inbreeding can be seen in Figure 2. The zero IC estimate in the initial cycles may be due to the predominance of more heterozygous individuals, and as the cycles advance, the IC value increases, indicating that some alleles from the EST-SSR loci identified among the cycles tend to become homozygous due to the reduced frequency of the heterozygotes. This is a concern that the breeder must prioritize, since the random fixation of alleles due to selection in finite populations may result in loss or reduced variability.
(Solomon et al., 2010) in future generations; thus, genetic progress could be compromised.

In parallel with the decrease in the proportion of heterozygotes in the present research, it is interesting that the genetic gains obtained during the cycles for the characteristics of productivity and capacity of expansion of the grains also tended to decrease (Daros et al., 2002; Daros et al., 2004; Freitas et al., 2014; Freitas Júnior et al., 2009; Rangel et al., 2011; Ribeiro et al., 2012; Santos et al., 2007). Although the initial cycles are expected to have greater gains due to the variability of the origin of the base population, as the generations progress, the management of a limited set of selected genotypes may cause a slight reduction in genetic variability, leading to a reduction in gain by selection (Souza Júnior et al., 2000).

It was expected that in the studied population (UENF-14) all loci were in HWE, as the selected individuals produced the plants that would then be recombined to form a subsequent improved population. However, of the 16 EST-SSR primers, only six loci showed HWE in all cycles; three of them (Umc1948, Umc1327, and Umc1389) were related to quantitative trait loci (QTL) in popcorn (Dong et al., 2015; Li et al., 2007). This confirms how the potential effect of genetic drift could increase the probability of eliminating certain genotypes and consequently contribute to the predominance of certain genotypic combinations in the population (Cruz et al., 2011; Souza Júnior et al., 2000). As in recurrent selection, a finite number of selected individuals are recombined. The impact of drift is inevitable; therefore, it is up to the breeder to monitor the breeding population in order to determine whether the population can continue to be subjected to the same mating system in recurrent selection, or the number of individuals to be recombined could be increased to reduce the drift effect and maintain variability.

It is known that recurrent selection provides a gradual increase in the frequency of favorable alleles for the traits of agronomic interest. Although among all 16 EST-SSR primers only six were related to popcorn, it was observed during the cycles that the structure of the UENF-14 population showed more sharing of alleles from the dark-gray color subgroup, resulting in an increase in its frequency, which infers that the alleles of said loci may be directly related to the main selection characteristics. These results are in agreement with those reported by Ribeiro et al. (2016) in the same study population (UENF-14) in the investigation of phenotypic responses after seven selective cycles (C0-C6), which showed an average cumulative gain for each cycle of recurrent selection of the UENF-14 population for both grain yield and expansion capacity, thus assuming an increase in the frequency of favorable alleles resulting from the selection of the superior genotypes for recombination. Similar results for a gradual increase in allele frequency were also reported in the studies conducted by Pinto et al. (2003) and Franzoni et al. (2012), who used recurrent reciprocal selection in maize and intrapopulation selection in popcorn with 30 and 20 locus SSRs, respectively.

Conclusion

In the present study, despite the use of the 16 EST-SSR primers not developed directly for the productivity and grain expansion capacity characteristics of the UENF-14 popcorn population, it was possible to analyze the parameters of diversity and genetic structure in the population throughout selection cycles. A reduction in the frequency of heterozygotes and, consequently, an increase in inbreeding were then obtained. The population structure showed some sharing of alleles, which infers that the alleles may be directly related to the main selection characteristics.

Acknowledgements

The authors thank the Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)) for financial support and the Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES)) for the Doctoral fellowship granted to the first author.

References

Amaral Júnior, A. T., Gonçalves, L. S. A., Freitas Júnior, S. P., Candido, L. S., Vittorazzi, C., Pena, G. F., ... Carvalho, G. F. (2013). UENF 14: a new popcorn cultivar. Crop Breeding and Applied Biotechnology, 13(3), 218-220. doi:10.1590/S1984-70332013000300013

Babu, R., Nair, S. K., Kumar, A., Rao, H. S., Verma, P., Gahalain, A., ... Gupta, H. S. (2006). Mapping QTLs for popping ability in a popcorn × flint corn cross. Theoretical and Applied Genetics, 112(7), 1392-1399. doi:10.1007/s00122-006-0242-1

Choudhary, P., Khanna, S. M., Jain, P. K., Bharadwaj, C., Kumar, J., Lakhra, P. C., & Srinivasan, S. (2012). Genetic structure and diversity analysis of the primary gene pool of chickpea using SSR markers. Genetics and Molecular Research, 11(2), 891-905. doi:10.4238/2012.April.10.5
Cornstock, R. E., & Robinson, H. F. (1948). The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. *Biometrics, 4*(4), 254-266. doi:10.2307/3001412

Cruz, C. D., Ferreira, F. M., & Pessoni, L. A. (2011). *Biometria aplicada ao estudo da diversidade genética. Visconde do Rio Branco, MG: Suprema.*

Dandolini, T. S., Scapim, C. A., Amaral Júnior, A. T., Mangolin, C. A., Machado, M. F. P. S., Mott, A. S., & Lopes, A. D. (2008). Genetic divergence in popcorn lines detected by microsatellite markers. *Crop Breeding and Applied Biotechnology, 8*(4), 313-320. doi:10.1590/S1984-70332013000300013

Daros M., Amaral Júnior, A. T., & Pereira, M. G. (2002). Genetic gain for grain yield and popping expansion in full-sib recurrent selection in popcorn. *Crop Breeding and Applied Biotechnology, 2*(3), 339-344. doi:10.1590/S0103-90162004000600008

Eloi, I. B., Mangolin, C. A., Gonçalves, C. S., & Machado, M. F. (2012). Selection of high heterozygosity popcorn varieties in Brazil based on SSR markers. *Genetics and Molecular Research, 11*(3), 1851-1860. doi:10.4238/2012.July.19.5

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology, 14*(8), 2611-2620. doi:10.1111/j.1365-294X.2005.02553.x

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics.* London, UK: Longman Group Limited.

Freitas Júnior, S. P., Amaral Júnior, A. T., Rangel, R. M., & Viana, A. P. (2009). Genetic gains in popcorn by full-sib recurrent selection. *Crop Breeding and Applied Biotechnology, 9*(1), 1-7. doi:10.12702/1984-7033.x09n01a01

Franzoni, J., Scapim, C. A., Beviláqua, M. R., Pacheco, C. A., & Mangolin, C. A. (2012). Application of microsatellite markers to evaluate the heterozygosity from the popcorn composite CMS-43 (*Zea mays L.*) during eight cycles of selection. *Plant Breeding, 131*(4), 479-485. doi:10.1111/j.1439-0523.2012.01981.x

Hallauer, R., & Carena, M. J. (2009). *Maize Breeding.* In: M. J. Carena (Ed.), *Cereals: Handbook of Plant Breeding* (p. 3-98). New York, NY: Springer.

Li, Y. L., Dong, Y. B., Niu, S. Z., & Cui, D. Q. (2007). QTL for popping characteristics in popcorn. *Plant Breeding, 126*(5), 509-514. doi:10.1111/j.1439-0523.2007.01372.x

Peckal, R., & Smouse, P. (2009). *GenALEx Tutorials – Part 1: Introduction to Population Genetic Analysis.* Canberra, NSW: Australian National University.

Pereira, M. G, & Amaral Júnior, A. T. (2001). Estimation of genetic components in popcorn based on the nested design. *Crop Breeding and Applied Biotechnology, 1*(1), 3-10. doi:10.13082/1984-7033.v01n01a01

Pinto, L. R., Vieira, M. L. C., Souza Júnior, C. L., & Souzam A. P. (2003). Reciprocal recurrent selection effects on the genetic structure of tropical maize populations assessed at microsatellite loci. *Genetics and Molecular Biology, 26*(3), 355-364. doi:10.1590/S1415-47572003000300022

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

Rangel, R. M., Amaral Júnior, A. T., Gonçalves, L. S. A., Freitas Júnior, S. P., & Candido, L. S. (2011). Biometric analysis of selection gains in popcorn population of the fifth cycle of recurrent selection. *Revista Ciência Agronômica, 42*(2), 473-481. doi:10.1590/S1806-66902011000200029

Ribeiro, R. M., Amaral Júnior, A. T., Gonçalves, L. S. A., Candido, L. S., Silva, T. R., & Pena, G. F. (2012). Genetic progress in the UNB-2U population of popcorn under recurrent selection in Rio de Janeiro. *Genetics and Molecular Research, 11*(2), 1417-1423. doi:10.4238/2012.May.15.12

Ribeiro, R. M., Amaral Júnior, A. T., Pena, G. F., Vivas, M., Kurosawa, R. F. N., & Goncalves, L. S. A. (2016). Effect of recurrent selection on the variability of the UENF-14 popcorn population. *Crop Breeding and Applied Biotechnology, 16*(2), 123-131. doi:10.1590/1984-70332016v16n2a19

Sarcevic, H., Pejic, I., Baric, M., & Kozumplik, V. (2007). Originality of M3S maize population and changes in allele frequencies revealed by SSR markers after two cycles of selfed progeny recurrent selection. *Euphytica, 161*(1), 97-105. doi:10.1007/s10681-007-9450-2

Santos, F. S., Amaral Júnior, A. T., Freitas Júnior, S. P., Rangel, R. M., & Pereira, M. G. (2007). Predição de ganhos genéticos por índices de seleção na população de milho-pipoca UNB-2U sob seleção recorrente. *Bragantia, 66*(3), 389-396. doi:10.1590/S0006-87052007000300004

Solomon, K. F., Martin, L. & Zeppa, A. (2010). Temporal genetic structure patterns in tropical maize populations under reciprocal recurrent selection. *Euphytica, 176*(2),
Souza Júnior, C. L. D., Geraldi, I. O., & Vencovský, R. (2000). Response to recurrent selection under small effective population size. Genetics and Molecular Biology, 23(4), 841-846.

Trindade, A. P. R., Pinto, R. J. B., Amaral Júnior, A. T., Mangolin, C. A., Machado, M. F. P. S., & Scapim, C. A. (2010). Genetic diversity of breeding popcorn lines determined by SSR markers. Electronic Journal of Biotechnology, 13(1), 1-9. doi:10.2225/vol13-issue1-fulltext-11

Received on February 4, 2017.
Accepted on May 10, 2017.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.