Electronic Journal of Biotechnology 18 (2015) 181–187

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Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Population structure and genetic diversity of Brazilian popcorn germplasm inferred by microsatellite markers

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ARTICLE INFO

Article history:
Received 2 June 2014
Accepted 16 December 2014
Available online 30 March 2015

Keywords:
Genetic variability
Maize
Molecular marker

ABSTRACT

Background: The genetic diversity and structure of 31 popcorn accessions of the germplasm bank of the State University of Maringá were assessed using 30 microsatellite primers.
Results: 127 alleles were identified from 30 evaluated loci. The number of alleles per locus ranged from two to eight. The overall mean of the polymorphic loci averaged 79.89%. The primers UMC1549 and UMC1072 detected polymorphism in all accessions analyzed. The mean observed heterozygosity ranged from 0.07 to 0.30 and the highest proportion of heterozygous plants was observed in accession BOZM 260 (Ho = 0.30). The analysis of molecular variance revealed that 60% of the total genetic variation was found within accessions and 40% was found between accessions. The Bayesian clustering approach grouped the 31 accessions into two genetically differentiated clusters. The dendrogram revealed that accessions TATU 2 and ARZM 05 083 are genetically less similar than the others.
Conclusions: The analysis allowed to identify microsatellite loci with high levels of heterozygosity (UMC1549 and UMC1072). These loci can be indicated as promising for detecting polymorphisms in popcorn accessions and in the monitoring of genetic improvement programs. Moreover, allowed to identify heterozygous accessions (BOZM 260), this accession showed allelic variation at all analyzed microsatellite loci and can be recommended for crosses with plants that have desirable agronomic characteristics, with a view to the broadening of the genetic base of popcorn accessions and developing new cultivars.

1. Introduction

The objective of a breeding program is to develop popcorn cultivars that unite good agronomic characteristics with a high popping expansion rate [1,2]. Lima et al. [3] stated that the success of a breeding program depends on the indication of the most promising population for the development of lines and on the choice of contrasting lines for hybrid formation, which can be done by establishing heterotic groups [4].

Thus, genetic divergence studies have become essential as an orientation for the selection of potentially promising parents to generate populations with high variability and adaptation capacity, which, when crossed, increase the chances of obtaining superior genotypes in segregating generations. This allows a concentrated breeding effort on a smaller number of hybrids obtained from the most divergent genotypes [5,6].

The genetic characterization of popcorn populations destined for improvement can be based on agronomic traits and/or molecular markers. Different types of molecular markers have been used to estimates of genetic variability; these markers indicate the similarity between genotypes based on a direct analysis of the genome [7].

The technique of SSR analysis (Simple Sequence Repeats of genomic DNA) has been considered appropriate to estimate the genetic diversity of popcorn accessions [1,8,9,10,11,12]. In spite of these studies, the number of researches addressing the genetic diversity of popcorn in Brazil is still comparably small in view of the number of varieties potentially available to be exploited in breeding programs. The SSR loci in popcorn have been analyzed to a limited extent, based on a restricted number of samples of accessions and plants per accession, and with a little more than a dozen primers. Therefore, the purpose of this study was to analyze 30 SSR loci in 31 popcorn accessions from the germplasm bank of the State University of Maringá, in order to: a) select and relate the promising SSR loci to estimates of genetic

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Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2015.03.005

diversity and to monitor the improvement program, b) analyze the population structures, c) analyze the genetic variability of populations, and d) select promising accessions to guide our breeding program.

2. Materials and methods

2.1. Genetic material and DNA extraction

In this study, we used SSR markers to assess the genetic diversity and structure of 31 popcorn accessions of the germplasm bank of the State University of Maringá (1 — CHZM 13 0134; 2 — ARZM 13 050; 3 — URUG 298; 4 — ARZM 05 083; 5 — PARA 172; 6 — ARZM 07 049; 7 — BOZM 260; 8 — BOYA 462; 9 — IAC-125; 10 — IAC-112; 11 — JADE; 12-Zélia; 13 — ARGENTINA; 14 — SE 013; 15 — Viçosa; 16 — RS-20; 17 — BEIJA-FLOR; 18 — UNB-2UC1; 19 — UNB-2UC2; 20 — UENFV-Explosivo C4; 21 — UNB-2UC3; 22 — PA 091; 23 — PR-023; 24 — BRS ANGELA; 25 — SAM; 26 — PARA 170; 27 — COLOMBIANA; 28 — TATU 1; 29 — UNB-2U C0; 30 — UFVM2-Barão Viçosa; 31 — TATU 2), all popcorn accessions analyzed are hybrids (four accessions, 9, 10, 11 and 12) or open pollination populations (twenty-seven accessions). We analyzed 15 plants of each accession. The DNA was extracted from each plant individually, from leaf tissue by the method described by Hoisington et al. [13], with minor modifications and was quantified with a Qubit™ fluorometer using the Quant-iT assay kit (Invitrogen) and the DNA samples were diluted to a concentration of 10 ng/μL^{-1}, for use in amplification reactions.

2.2. SSR amplification

We tested 159 pairs of SSR primers mapped from common corn, of which 42 were polymorphic; of these, 30 were selected to study popcorn populations (Table 1). All microsatellites were obtained from the website Maize DB at http://www.maizegdb.org/ssr.php.

The PCR amplification was performed with the program "Touchdown" PCR [14] in a total volume of 20 μL containing 25 ng of DNA, with 2.0 μL of 10× reaction buffer, 2.5 mM $MgCl_2$, 0.8 mM of each dNTP, 1 U Taq-DNA-Polymerase (Invitrogen), and 0.4 μM of the specific primers F and R. After amplification, a total of 20 μL of each sample (465 total samples) were separated by electrophoresis on a 4% agarose gel (50% agarose and 50% agarose Metaphor (CAMBREX) containing 0.5× TBE buffer (44.5 mM Tris, 44.5 mM boric acid, and 1 mM EDTA)). All 465 samples amplified for each SSR primer were run at 60 V for 4 h. A 100 pb ladder (Invitrogen) was used as the weight molecular marker. Gels were stained using SYBR® Safe DNA gel stain, and the image was captured using Ultraviolet Transilluminator High Performance-Edas 290 using the Kodak 1D 3.5 program. The allele numbers per locus were determined based on their relative position in the gel.

Table 1
Microsatellite loci; replication block; location of each primer; mean observed H_o; Nei's mean expected H_e; PIC; number of alleles observed in 30 microsatellite loci (Na); and percentage of the most frequent allele per locus.

Loci	Replication	Bin	Ho	He	PIC	Na	% allele Most frequent
Umc2401	(TACGA)5	1.01	0.04	0.39	0.48	3.00	0.48
Umc2108	(ACG)4	1.01	0.03	0.39	0.55	3.00	0.43
Umc1125	(AG)30	1.04	0.31	0.30	0.36	2.00	0.63
Mmc0271	(GAGCA)4	1.11	0.22	0.29	0.51	6.00	0.47
Umc2246	(CCTCCT)4	2.00	0.12	0.35	0.58	5.00	0.42
Bnlg1175	(AG)38	2.04	0.21	0.49	0.79	8.00	0.31
Umc1755	(GAAGG)4	2.05	0.03	0.35	0.61	5.00	0.41
Umc2166	(ACA)17	2.05	0.12	0.43	0.70	5.00	0.30
Umc1118	(CACGAG)4	2.06	0.10	0.32	0.54	5.00	0.61
Umc1065	(GA)39	2.07	0.28	0.50	0.76	6.00	0.27
Umc1642	(CTCTCTCTCT)4	2.07	0.04	0.37	0.59	5.00	0.56
Umc2118	(CTTT)4	3.00	0.07	0.28	0.54	3.00	0.47
Umc1137	(CTGCA)4	3.00	0.22	0.22	0.26	4.00	0.84
Umc2059	(GCCTC)4	3.05	0.21	0.33	0.49	3.00	0.46
Umc1635	(CGC)6	4.07	0.27	0.42	0.64	5.00	0.38
Umc1071	(AGAAAGAA)4	5.04	0.33	0.45	0.64	4.00	0.46
Umc1415	(CGGC)4	5.05	0.06	0.05	0.06	4.00	0.97
Umc1336	(GGACTG)8	5.06	0.21	0.29	0.37	2.00	0.58
Umc1363	(GGA)10	5.07	0.15	0.32	0.45	3.00	0.58
Umc2205	(TTC)12	6.07	0.34	0.48	0.67	6.00	0.37
Umc1549	(GAAA)24	6.07	0.77	0.46	0.48	4.00	0.61
Bnlg2295	(CAG)8	6.08	0.23	0.32	0.41	4.00	0.73
Umc2302	(GCTA)6	7.00	0.09	0.23	0.40	3.00	0.70
Umc1072	(GCCTCT)4	7.02	0.99	0.56	0.55	4.00	0.47
Umc2165	(CTCG)5	7.04	0.14	0.33	0.57	3.00	0.45
Umc1847	(GAC)10	8.03	0.04	0.21	0.32	2.00	0.73
Umc1653	(CTCTCT)4	8.05	0.27	0.38	0.63	8.00	0.53
Mmc0501	(CT)15	9.08	0.19	0.42	0.68	4.00	0.33
Umc1524	(GA)36	10.02	0.34	0.47	0.66	4.00	0.42
Umc2164	(ACCAG)4	10.03	0.10	0.40	0.63	4.00	0.39
Mean			0.22	0.36	0.53	4.23	

2.3. Genetic diversity and population structure analysis

To analyze the genetic diversity in popcorn populations, each amplified DNA segment, identified as a band in the gel, was considered a distinct phenotype and independent of the others, determining the alleles of each SSR locus. Basic statistics were calculated using the software GENALEX 6.1 [15] to determine the allele frequencies, the mean observed heterozygosity (H_o) and Nei's expected heterozygosity (H_e), the number of alleles at each SSR loci, and the percentage of polymorphic alleles for each population. The polymorphic information content (PIC), and the allele with the highest frequency were calculated using PowerMarker 3.25 [16]. The analysis of molecular variance (AMOVA) and the principal component analysis (PCA) were also performed using the software GENALEX 6.1 The genetic population structure of the 31 popcorn accessions was analyzed by the program STRUCTURE [17], which assigns individuals to a number K of genetically homogeneous groups, based on the Bayesian estimate in accordance to the expected Hardy–Weinberg equilibrium and absence of linkage disequilibrium between the loci analyzed in each population. For the analyses with the program STRUCTURE, a burn-in period of 50,000 and a posterior number of Markov Chain Monte Carlo (MCMC) of 100,000 permutations was used. Fifteen replications (runs) were performed for each possible value of K (K = 1 to K = 10). An admixture and allele frequencies correlated model was used.

Two different approaches were used to detect the most likely K value: the first was the proposed by Pritchard et al. [17] based on the rate of change of LnP(D) for each K between 1 and 10 and the second was the criterion proposed by Evanno et al. [18], which is based on the second order rate of change of the likelihood function with respect to K (ΔK) (the ad hoc ΔK test). The results from STRUCTURE were processed with the software STRUCTURE HARVESTER v.0.6.1 [19]. The convergence of the Gibbs chains was determined using the test proposed by Heidelberger and Welch [20], which was performed in the R program with the convergence diagnosis and output analysis (CODA) library. The program CLUMPP [21] was used to analyze the stability among the fifteen runs for the real value of K determined. The barplot of the probability of membership from the results of Q-matrix were visualized by DISTRUCT software [22]. In order to assign accessions into groups, accessions with probability of membership ≥0.70 were considered to belong to discrete groups, whereas accessions with probabilities <0.70 were considered as a mixture.

From the matrix of Rogers' genetic distance [23], calculated by the program TOOLS FOR POPULATION GENETIC ANALYSES, a dendrogram was constructed by UPGMA cluster analysis (Unweighted Pair — Group Method Using the Arithmetic Average) using the program MEGA v.5.05 [24].