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Molecular analysis of apomixis in cassava

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ABSTRACT. Cassava is the main staple for more than 800 million people in the tropics. It is propagated vegetatively by stem cuttings, which maintains superior genotypes but favors disease accumulation and spread. In this report, we present the results of the screening of the progeny and the second generation of the clone UnB 307 for apomixes using microsatellites. A total of 29 plants were screened, representing the maternal plant, its first and second generations that were left to open pollination. About 20% of the offspring were rated as genetically identical plants. This result confirms the facultative apomictic nature of cassava, with high environmental effect.

Key words: Apomixis, Microsatellite, Second generation progeny, Open pollination, Protogeny

INTRODUCTION

Cassava is the main staple for more than 800 million people in the tropics and an important crop for Brazil. It is propagated vegetatively by stem cuttings, which maintains superior genotypes. However, such asexual propagation system favors the accumulation of viral and bacterial diseases, which reduces productivity and leads to the degeneration of many excellent cultivars. If seeds were used to propagate the crop, systemic pathogen contamination could be avoided. However, the breakdown of selected heterozygous genotypes due to genetic segregation in the progeny has always excluded this approach.

If apomictic seeds were available it could resolve this problem and would maintain heterozygosity. Apomixis refers to a process in which plants produce seeds without fertilization through female syngamy, which produces embryos genetically identical to the maternal parent. Apomixis genes were found in the wild *Manihot* species and transferred successfully to the cultigen (Nassar, 1995, 1997, 2000; Nassar and Collevatti, 2005).

In previous research (Nassar and Collevatti, 2005), the progeny of the clone UnB 307 was screened for apomixes using microsatellite markers. However, since a small progeny was available in the previous analysis, in this study we present the results of the screening on larger number of offspring, particularly those of second generation.

MATERIAL AND METHODS

Expanded leaves from the mother plant 307/M (UnB 307 clone), and from 307/2M, 307/3M, 307/4M, and 307/5M, which are also UnB 307 clone progeny, and from their progeny, were collected and stored at -80°C for the microsatellite analysis. Two individuals of *Manihot esculenta* were used for amplification control. Genomic DNA was extracted following a standard CTAB procedure (Doyle and Doyle, 1990).

Six microsatellite loci developed for *M. esculenta* (Chavarriaga-Aguirre et al., 1998), which were previously tested and transferred to UnB 307 clone (Nassar and Collevatti, 2005), were used to genotype all individuals (GA-12, GA-13, GA-16, GA-21, GA-126, GA-131). Microsatellite amplification was performed in a 15-µL volume containing 0.3 µM of each primer, 1 unit Taq DNA polymerase (Phoneutria, BR), 250 µM of each dNTP, 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 0.25 mg BSA, and 10.0 ng of template DNA. Amplifications were performed using a Gene Amp PCR System 9700 (Applied Biosystems, CA) with the following conditions: 96°C for 2 min (1 cycle), 94°C for 1 min, 45 to 55°C for 1 min (according to each locus), 72°C for 1 min (30 cycles), and a final elongation of 72°C for 10 min (1 cycle). The amplified products were separated on 4% denaturing polyacrylamide gels, stained with silver nitrate (Bassam et al., 1991) and sized by comparison to a 10-bp DNA ladder standard (Invitrogen, MD). The DNA from two individuals of *M. esculenta* was used as positive control.

For each locus, the number of alleles and expected and observed heterozygosities under Hardy-Weinberg equilibrium and probability exclusion of the first and second parents were estimated (Nei, 1978; Marshall et al., 1998). Other analysis of departure from Hardy-Weinberg could not be performed, because of the low number of heterozygotes. For the same reason, it was not possible to estimate the likelihood of observing at least identical multilocus genotypes by simulation (Stenberg et al., 2003).

RESULTS

The six loci used in this study presented one to four alleles, considering the 29 individuals analyzed (Table 1). Observed and expected heterozygosities were low for all loci (Table 1). Thus, because of the low number of alleles per loci and the low frequency of heterozygotes, all loci showed low-combined paternity exclusion probability (Table 1).

Table 1 Characterization of six microsatellite loci transferred from *Manihot esculenta* to the UnB 307 clone, based on a sample of 29 related individuals.

Locus	*A*	H_e	H_o	*Q1*	*Q2*
GA-12	02	0.131	0.000	0.008	0.060
GA-13	01	0.000	0.000	0.000	0.000
GA-16	02	0.100	0.103	0.005	0.047
GA-21	03	0.194	0.207	0.018	0.098
GA-126	03	0.194	0.207	0.018	0.096
GA-131	04	0.252	0.276	0.031	0.129
Mean	2.5	0.145	0.132	*QC1* = 0.078	*QC2* = 0.364

A, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; *Q1*, probability of paternity exclusion of the first parent; *Q2*, probability of paternity exclusion of the second parent; *QC*, combined probability of paternity exclusion.

The two individuals of *M. esculenta* (positive control) showed clear amplification for all loci. For the six loci, both individuals of *M. esculenta* had the same genotype as the UnB 307 clone. For GA-131, both individuals of *M. esculenta* had the genotype 98/116 bp.

From the progeny of the UnB 307 clone (307/M), four sibs were not identical to the mother plant (307/12, 307/13, 307/14, 307/15) for at least one locus, showing that sibs were sired by cross-pollination (Table 2). For the mother plant 307/2M, two of four sibs were sired by cross-pollination (Table 2). For 307/3M and 307/4M, all sibs were sired by cross-pollination. For 307/5M, only one sib of six was identical to the mother plant.

DISCUSSION

The results of this research indicate that some of the offspring from the UnB 307 clone may be the outcome of apomixes, because some sibs had the same genotype as the mother plant for the six loci (Table 2). Apomixes could be inferred for all the progeny of UnB 307 clone analyzed, but not for 307/3M and 307/4M, since neither of these sibs had the genotype identical to the mother plant (Table 2). Some sibs from UnB 307 clone (307/2, 307/3, 307/4, and 307/5) were previously analyzed using five microsatellite loci from the six used in the present study (Nassar and Collevatti, 2005). When one more locus was included in this research, apomixis was confirmed in the clone 307, since the offspring showed the same genotype as the mother plant.

The loci used in this research showed a medium to high number of alleles in previous studies: 5 to 15 (Chavarriaga-Aguirre et al., 1998) and 4 to 9 alleles (Elias et al., 2001). Additi-

Table 2. Genotype of the mother plants and their progeny, based on six microsatellite loci (allele size in bp) transferred from *Manihot. esculenta*.

Individual	GA-12	GA-13	GA-16	GA-21	GA-126	GA-131
	140/140	140/140	104/104	114/114	180/180	116/116
307/M						
307/10	140/140	140/140	104/104	114/114	180/180	116/116
307/11	140/140	140/140	104/104	114/114	180/180	116/116
307/12	140/140	140/140	104/104	114/114	180/180	98/116
307/13	140/140	140/140	104/112	114/114	180/180	98/116
307/14	140/140	140/140	104/112	114/114	180/224	116/116
307/15	140/140	140/140	104/104	114/114	180/180	98/116
307/2M						
307/2-1	140/140	140/140	104/104	114/114	180/180	116/116
307/2-2	140/140	140/140	104/104	114/114	180/180	116/116
307/2-3	140/140	140/140	104/104	98/114	180/180	116/116
307/2-4	140/140	140/140	104/104	98/114	180/180	116/116
307/3M						
307/3-1	140/140	140/140	104/104	114/118	180/224	104/116
307/3-2	140/140	140/140	104/104	114/114	180/180	104/116
307/3-3	140/140	140/140	104/104	114/114	180/180	98/116
307/3-4	140/140	140/140	104/104	114/114	180/224	116/116
307/3-5	140/140	140/140	104/112	114/118	180/180	116/116
307/3-6	140/140	140/140	104/104	114/118	180/224	116/116
307/4M						
307/4-1	140/140	140/140	104/104	114/114	180/180	108/116
307/4-2	140/140	140/140	104/104	98/114	180/180	116/116
307/5M						
307/5-1	140/140	140/140	104/104	114/114	180/218	116/116
307/5-2	140/140	140/140	104/104	114/114	180/180	98/116
307/5-3	140/140	140/140	104/104	114/114	180/180	116/116
307/5-5	140/140	140/140	104/104	114/114	180/218	116/116
307/5-6	132/132	140/140	104/104	114/114	180/180	116/116
307/5-7	132/132	140/140	104/104	114/114	180/180	116/116

307/M - UnB 307 clone - mother plant; 307/2M to 307/15 - 307/M progeny; 307/2-1 to 307/2-4 - 307/2 progeny; 307/3-1 to 307/2-6 - 307/3 progeny; 307/4-1 and 307/4-2 - 307/4 progeny; 307/5-1 to 307/5-7 - 307/5 progeny.

onally, the battery of loci used displayed a medium power of individual distinction, as a result of the breeding design, shown by the probability of genetic identity ($1.56527.10^{-5}$ and $1.711202.10^{-6}$; Nassar and Collevatti, 2005). Considering that each progeny was exposed to open pollination in each generation, the low number of alleles may be the outcome of the low diversity of the original population used as the parental source at the beginning of the breeding program, genetic drift during the breeding undertaking, or the occurrence of apomixes.

The genetic uniformity of the UnB 307 clone and its progeny detected by microsatellite showed the apomictic nature of the maternal plant. In the present study, we used a larger number of progeny than that in previous research (Nassar and Collevatti, 2005). This research

may confer more validity to the assumption of the apomictic nature of the UnB 307 clone. In previous research, all the progeny were shown to be identical to the mother plant with the same microsatellite loci included in the analysis (except one, GA-16), while in this study, such individuals that revealed identical alleles were only about 20%, which may be explained by the larger number of individuals used in this study which permitted a higher chance for outcrossing. Another hypothesis is that apomixis in cassava is highly affected by the environment and its incidence may drop drastically from one generation to another (Nassar, 1995). The embryonic investigation in previous research that proved a high incidence of multiembryonic sacs in ovules of this clone supports clearly its apomictic nature. Moreover, the high sterility of the clone, which reaches 11% of viable pollen, and the prominent protogeny of its flowers, with the maturation of staminate and pistillate flowers separated by three weeks, exclude self pollination as a cause for identical alleles in its offspring.

Cassava has a nucellar apomixis of the aposporic category (Asker, 1979). This type is characterized by the presence of more than one embryo in the embryonic sac (Nassar, 2001). Normally one embryo is sexual and the other(s) are vegetatively grown. The apomixis gene in cassava is different from apomixis genes in other crops, because it causes a low percentage of apomictic seeds (10 to 20%). It is also highly modified by environmental conditions, which affect the percentage of apomictic seeds and plants raised. Thus, a variable percentage of apomictic plants can be obtained under different environmental conditions.

It seems that apomixis has played an important role in the whole *Manihot* genus speciation. Apparently, polyploidy provided the wide genetic variability of the genus and apomixes maintained the genotypes that may be favored in certain niches. This facultative apomixis may keep the genetic variability through sexual reproduction, allowing new genotypes to undergo another cycle of speciation in new environmental conditions.

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