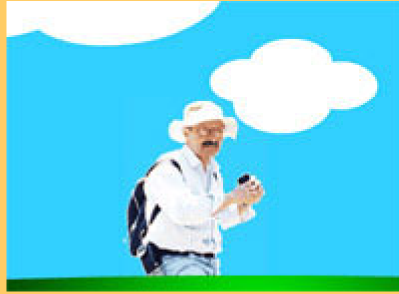

A further study of microsatillite on apomixis in cassava

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Abstract

Cassava is the main staple crop in the tropics. It is vegetatively propagated by stem cuttings that maintain superior genotypes, but favors disease accumulation . 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 environment effect.

Key words

Apomixes, 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 that maintain superior genotypes. However, such asexual propagation system favors the accumulation of viral and bacterial diseases that reduces productivity and leads to the degeneration of many excellent

cultivars. Should seeds were used to propagate the crop, systemic pathogen contamination may 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. Apomixes refers to a process in which plants produce seed without fertilization through female syngamy that produces embryos genetically identical to the maternal parent. Apomixes genes were found in the wild *Manihot* species and transferred successfully to the cultigen (Nassar, 1995; Nassar et al. 1997, 2000; Nassar and Collevatti, 2005).

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

Materials and Methods

Expanded leaves from the mother plant 307/M (UnB 307 clone) , and from 307/2M, 307/03M, 307/4M and 307/5M, which are also UnB 307 clone progeny, and from their progeny, were collected and stored at –80ºC for the microsattelite analysis. Two individuals of *M. esculenta* were used for amplification control. Genomic DNA was extracted following a standard CTAB procedure (Doyle and Doyle, 1987).

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 u Taq DNA polymerase (Phoneutria, BR), 250 µM of each dNTP, 1× 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, probability exclusion of the first and second parents were estimated (Nei, 1978; Marshall et al., 1998). Other analysis of departure of 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 n identical multilocus genotypes by simulation (Stenberg et al., 2003)

Results

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

The two individuals of *M. esculenta* (positive control) presented clear amplification for all loci. For the six loci, both individuals of *M. esculenta* presented the same genotype as the UNB 307 clone. For GA131, both individuals of *M. esculenta* presented 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 out 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 out 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 presented 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 analysed, but not for 307/3M and 307/4M, since none of these sibs presented 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 analysed using five microsatellite loci from the six used in the present work (Nassar and Collevatti, 2005). When one more locus was included in this research, apomixes was confirmed in the clone 307, since the offspring showed the same genotype as the mother plant.

The loci used in this research showed medium to high number of alleles in previous works: 5 to 15 (Chavarriaga-Aguirre et al., 1998) and 4 to 9 alleles (Elias et al., 2001). Additionally, the battery of loci used displayed a medium power of individual distinction, as a result of the breeding design, showed by the probability of genetic identity ($1.56527.10^{-5}$ and $1.711202.10^{-6}$, Nassar and Collevatti, 2005). Considering that each progeny were 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 showed to be identical as the mother plant with the same microsatellite loci included in the analysis (except one, GA16), while in this study, such individuals that reveal identical alleles are only about 20%, which may be explained by the larger number of individuals used in this study that permitted a higher chance for outcrossing. Another hypothesis is that apomixes in cassava is highly affected by the environment and its incidence may drop drastically from a generation to another (Nassar, 1995). The embryonic investigation in previous research that proved 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, excludes selfing pollination as a cause for identical alleles in its offspring.

Cassava has a nucellar apomixis of the aposporic category (Asker, 1980). 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 apomixes gene in cassava is different from apomixes gene in other crops, because it causes a low percentage of apomitic seeds (10 to 20%). It is also highly modified by environmental conditions, which affect the percentage of apomitic seeds and raised plants. Thus, a variable percentage of apomictic plants can be obtained under different environmental conditions.

It seems that apomixes 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 favoured in certain niches. This facultative apomixes 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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Table 1 Characterization of six microsatellite loci transferred from *M. esculenta* to the UnB 307 clone, based on a sample of 29 related individuals. [*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]

Locus	***A***	H_e	H_o	***Q1***	***Q2***
GA12	02	0.131	0.000	0.008	0.060
GA13	01	0.000	0.000	0.000	0.000
GA16	02	0.100	0.103	0.005	0.047
GA21	03	0.194	0.207	0.018	0.098
GA126	03	0.194	0.207	0.018	0.096
GA131	04	0.252	0.276	0.031	0.129
Mean	2.5	0.145	0.132	*QC1* = 0.078	*QC2* = 0.364

Table 2. Genotype of the mother plants and their progeny, based on six microsatellite loci (allele size in base pair) transferred from *M. esculenta*. [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; }

Individual	GA12	GA13	GA16	GA21	GA126	GA131
307/M	**140/140**	**140/140**	**104/104**	**114/114**	**180/180**	**116/116**
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	**140/140**	**140/140**	**104/104**	**114/114**	**180/180**	**116/116**
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	**140/140**	**140/140**	**104/104**	**114/114**	**180/180**	**116/116**
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	**140/140**	**140/140**	**104/104**	**114/114**	**180/180**	**116/116**
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	**140/140**	**140/140**	**104/104**	**114/114**	**180/180**	**116/116**
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

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