



# Breeding system of cassava (*Manihot esculenta* Crantz) ethnovarieties from different regions of Brazil



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## Introduction

One of the most important sources of carbohydrate in tropical regions, cassava (*Manihot esculenta* Crantz) is used in various ways as food and in the processing industry. Brazil is the largest producer in Latin America and the second world's producer (FAO, 1999).

A predominantly outcrossing species, presenting male and female flowers in the same inflorescence, male flowers are smaller and formed in the upper section of the inflorescence, while a smaller number of female flowers are located in the lower section. Flowering in cassava is protogynic, where the female flowers are functional one week before the male flowers (Fukuda, 1999). Even so, inbreeding may exist if crosses should occur among related individuals or clones, which is favored by mass interaction.

Recently, simple sequence repeats (SSRs) or microsatellite molecular markers have been used to estimate outcrossing rates and genetic variability in several plant species, including cassava (Chavarriga et al., 2000), considering they are codominant, polymorphic, multiallelic markers and amplified by the polymerase chain reaction (PCR).



Figure 1. Protected open-pollinated fruits in the field (A, B) and resulting progenies (C).

## Objectives

This study was to estimate the multilocus and unilocus outcrossing rates and the genetic variability among 10 cassava varieties originating from different regions in Brazil and their progenies with microsatellite markers, providing more information regarding the mating system of this species.

## Materials and Methods

Nine cassava ethnovarieties originating from different regions in Brazil and a cultivated variety (cv. Mantiqueira) (Table 1) were planted in a randomized block design with 10 replications at the Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo, Piracicaba, SP. Progenies were obtained with open-pollinated seeds from the 10 varieties (Figure 1). However, due to low germination rates only eight progenies were evaluated.

Recently expanded leaves from 10 mother plants and their progenies were collected for DNA extraction. DNA quantification was performed in 4% polyacrylamide gel and revealed with silver staining. DNA amplification was conducted with nine pre-selected primers (Chavarriga-Aguirre et al., 2000). The annealing temperature was 55°C for primers GAGG-5, GA-12, GA-126, GA-127, GA-131, GA-134, GA-136 and GA-140 and 56°C for primer GA-21. Amplification products were separated in 6% polyacrylamide gels, stained with silver nitrate.

Data was analyzed with POPGENE version 3.11 software (Yeh, 1999) for estimates of genetic diversity parameters. The MLTR software (Ritland, 1997) was used for the mating system analysis.

Table 1. List of the cassava (*Manihot esculenta*) varieties studied, including identification, folk name, usage and origin (settlement and geographic origin).

Nº	Identification	Folk name	Usage	Origin
1	DG 61	Macaxeira	Flour	Carvoeiro – Rio Negro/AM
2	DG 118	Macaxeira	Flour	São João/Guarabira – RioSolimões/AM
3	DG 44	Antonio	Flour	D. Pedro II / Barcelos / Anati - Rio Negro/AM
4	DG 47	Mamaroca	Flour	Carvoeiro - Rio Negro/AM
5	DG 131	Manteiguinha	Food	Cananéia – Vale do Ribeira/SP
6	DG 2	Branca	Flour	Ih Comprida - Vale do Ribeira/SP
7	DG 137	Mantiqueira	Food	cv. Mantiqueira – IAC/SP
8	DG 59	Samuama	Flour	Carvoeiro - Rio Negro/AM
9	DG 73	Socó	Flour	Panacarina – Rio Branco/RR
10	DG 43	Prezinha	Flour	Barcelos - Rio Negro/AM

## References

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## Results and Discussion

A total of 240 individuals were evaluated, including 10 mother plants and eight progenies, varying from 8 to 20 plants per progeny. Thirty-two alleles were observed, with 3 to 5 per primer, in a total of nine loci (Table 2, Figure 2). The highest observed heterozygosity was 0.591±0.286 (progeny n° 4) and the highest expected heterozygosity was 0.512 ± 0.136 (progeny n° 5). The polymorphic loci varied from 77.8% to 100% among the eight progenies.

A 0.923±0.047 multilocus outcrossing rate was estimated, indicating that 92.3% of the seeds were originated by outcrossing events and that the ethnovarieties studied are preferentially allogamous. Silva et al. (2003) found a similar multilocus outcrossing rate in cassava ethnovarieties using allozyme markers. The single locus outcrossing rate was 0.785±0.066, and the difference in the multilocus and single locus rates was 0.139±0.049, indicating low crossing rates among related individuals (13.9%). The proportion of selfing events within progenies was estimated as 12.4%, while the crossing rate among full-sib individuals was estimated in 52.7%, a relatively high percentage, corresponding to the experimental design used in the field, which contained 10 replications (clones) of each variety distributed at random.

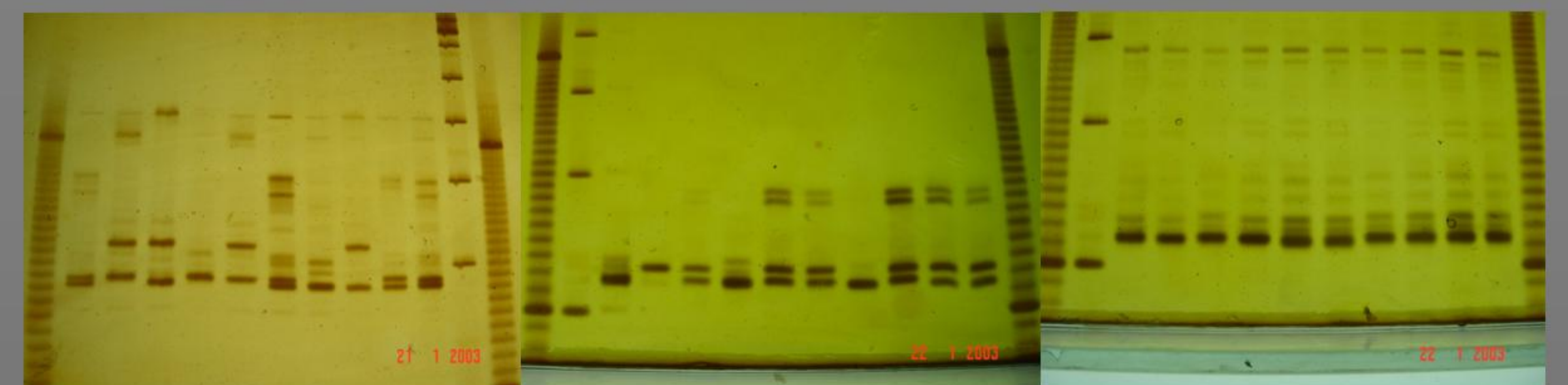


Figure 2. Zymograms of primers GA-126, GA-127 and GA-12, used in the progeny analysis of *Manihot esculenta*.

Table 2. Individual multilocus outcrossing rate by progeny ( $\hat{i}$ ) and mating system parameters for *Manihot esculenta* ethnovarieties.

Progenies	n	$\hat{i}$	(se) <sup>1</sup>
1	20	1.00	(0.00)
2	10	0.66	(0.19)
3	15	0.99	(0.09)
4	20	0.84	(0.10)
5	20	1.00	(0.00)
6	13	0.97	(0.06)
7	8	1.00	(0.00)
8	9	1.00	(0.00)
Fixation index for maternal genotypes ( $\hat{F}^m$ )		0.00	(0.00)
Multilocus outcrossing rate ( $\hat{i}^m$ )		0.923	(0.047)
Single locus outcrossing rate ( $\hat{i}^s$ )		0.785	(0.066)
Bi-parental inbreeding ( $\hat{i}^m - \hat{i}^s$ )		0.139	(0.049)
Correlation of inbreeding within progeny ( $\hat{r}^i$ )		0.124	(0.068)
Correlation of outcross paternity within progeny ( $\hat{r}^p$ )		0.527	(0.089)
Probable number of pollinators ( $1/\hat{r}^p$ )		1.90	

## Conclusions

The results allow us to conclude that the Brazilian cassava ethnovarieties studied are highly polymorphic with a preferentially allogamous breeding system, and a multilocus outcrossing rate estimated as 92.3%.