



Genetic fidelity and variability of micropropagated cassava plants (*Manihot esculenta* Crantz) evaluated using ISSR markers

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ABSTRACT. Molecular markers are efficient for assessing the genetic fidelity of various species of plants after *in vitro* culture. In this study, we evaluated the genetic fidelity and variability of micropropagated cassava plants (*Manihot esculenta* Crantz) using inter-simple sequence repeat markers. Twenty-two cassava accessions from the Embrapa Cassava & Fruits Germplasm Bank were used. For each accession, DNA was extracted from a plant maintained in the field and from 3 plants grown *in vitro*. For DNA amplification, 27 inter-simple sequence repeat primers were used, of which 24 generated 175 bands; 100 of those bands were polymorphic and were used to study genetic variability among accessions of cassava plants maintained in the field. Based on the genetic distance matrix calculated using the arithmetic

complement of the Jaccard's index, genotypes were clustered using the unweighted pair group method using arithmetic averages. The number of bands per primer was 2-13, with an average of 7.3. For most micropropagated accessions, the fidelity study showed no genetic variation between plants of the same accessions maintained in the field and those maintained *in vitro*, confirming the high genetic fidelity of the micropropagated plants. However, genetic variability was observed among different accessions grown in the field, and clustering based on the dissimilarity matrix revealed 7 groups. Inter-simple sequence repeat markers were efficient for detecting the genetic homogeneity of cassava plants derived from meristem culture, demonstrating the reliability of this propagation system.

Key words: Germplasm; Meristem culture; Molecular marker; Somaclonal variation

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important food source for millions of people worldwide. In Brazil, cassava is found throughout the country, from the State of Amazonas in the north to the State of Rio Grande do Sul in the south (Fukuda et al., 2003). Cassava is one of the most important food crops in tropical and subtropical regions in the world and occupies fifth position in terms of global calory consumption, just behind wheat, rice, corn, and sorghum (Adeyemo, 2009).

Cassava is drought-tolerant and adapts well to different climate and soil conditions. The most important part of the plant is the tuberous starchy root, which is used in food and animal feed. Additionally, this plant is very important in industry and is used for starch production and the manufacture of cosmetics and pharmaceuticals, among other products (Fukuda et al., 2002; Moro, 2009).

The propagation of cassava is performed vegetatively using cuttings; low production is caused by constant multiplication in addition to infestation by diseases transmitted through successive generations (Silva et al., 2002).

Because of the slow and low propagation rate of cassava, the development of alternative techniques that allow for rapid multiplication and provision of planting materials that are free of pests and pathogens is very important. Among these alternatives, micropropagation (Souza et al., 2009) allows for the clonal production of high-quality plants in a short period of time and in reduced physical space as well as ensures phytosanitary quality (Grattapaglia and Machado, 1998).

In cassava, the establishment of *in vitro* cultures to initiate micropropagation begins by growing meristems, which allows for the production of healthy plantlets for forming stock plants that are able to support new plantings with high-quality propagating material. However, clonal fidelity is one of the most important prerequisites in the micropropagation of any crop species (Ribeiro, 2012).

A major limitation often encountered with *in vitro* culture is the presence of somaclonal variation among subclones of a parental line; somaclonal variation arises as a direct consequence of the *in vitro* culture of plant cells, tissues, or organs (Venkatachalam et al., 2007).

According to Alves et al. (2004), understanding the mechanisms leading to soma-

clonal variation and the appropriate procedures for preventing its occurrence and the development of early detection methods are important factors for ensuring quality and safety in the production of micropropagated plantlets.

One strategy for detecting somaclonal variants is the use of molecular markers; these markers are part of the genome, and thus can be used to avoid environmental effects and, consequently, misidentifications (Borba et al., 2005).

Inter-simple sequence repeat (ISSR) markers have been successfully used to detect genetic differences or similarities in several micropropagated plants, including gerbera (Bhatia et al., 2009), anthurium (Gantait and Sinniah, 2011), and grape (Nookaraju and Agrawal, 2012). The high reproducibility, simplicity, and low cost of the experimental procedures of ISSR should be considered when processing a large number of samples (Goulão and Oliveira, 2001).

Therefore, the objective of this study was to evaluate the genetic diversity of accessions of cassava plants (*Manihot esculenta* Crantz) maintained in the field and the genetic fidelity among different accessions of micropropagated plants using ISSR markers.

MATERIAL AND METHODS

Plant material and DNA extraction

As the source of plant material, the young leaves of 22 cassava accessions from the Embrapa Cassava & Fruits Germplasm Bank, Cruz das Almas, State of Bahia (Table 1) were used.

Table 1. Identification, common name, origin, and suitability of 22 cassava accessions maintained in the field for the assessment of *in vitro* genetic fidelity and diversity using ISSR markers.

No.	Accessions	Common name	Origin	Suitability
1	BGM 212	Eucalipto	Boa Vista do Tupim-BA	Human consumption
2	BGM 264	Cigana	Amargosa-BA	Flour
3	BGM 561	Cambará	Itambé-PE	Flour
4	BGM 563	Pi90	Muritiba-BA	Flour
5	BGM 337*	CM375/1	Cali-Colômbia	-
6	BGM 316*	CM367/2	Cali-Colômbia	-
7	BGM 340*	CM305/5	Cali-Colômbia	-
8	BGM 640	Sacai I	Paraipaba-CE	Flour
9	BGM 1723	Jussara CNPMF	Cruz das Almas-BA	Flour
10	BGM 1840	São Pedro	Belém-PA	Flour
11	BGM 638	Guarani	Pentecoste-CE	Flour
12	BGM 1123	Aipim valenca	Piritiba-BA	Human consumption
13	BGM 668	Nacionalista	Viçosa-MG	Ornamental
14	BGM 1037	Casco de burro	Aurora do Norte-TO	Human consumption
15	BGM 1811**	Formosa	Cruz das Almas-BA	Flour/starch
16	BGM 1245	MNag-1	Nigéria-África	-
17	BGM 1324	Mandioca folha virada	Afogados da Engazeira-PE	Flour
18	BGM 1345	Macaxeira preta	Congo-PB	Human consumption
19	BGM 1348	Caideira	Sumé-PB	Flour
20	BGM 1371	Mandioca alagoa	Soledade-PB	Flour
21	BGM 116	Cigana preta	Castro Alves-BA	Flour/starch
22	BGM 1282	Macaxeira-cará	Ouricuri-PE	Human consumption

Source: Cassava Germplasm Bank of Embrapa Cassava & Fruits. *Fitness not informed; **Resistant to bacterial infection.

Four plants from each accession were used: 1 plant was maintained in the field (c), and 3 plants were cultured *in vitro* (1i, 2i, 3i) at the Tissue Culture Laboratory of Embrapa Cassava

& Fruits for 7 subcultures.

DNA extraction was performed at the Molecular Biology Laboratory of Embrapa Cassava & Fruits using the protocol as described by Doyle and Doyle (1987), with modifications. DNA in the samples was quantified on 0.8% agarose gels and visualized under ultraviolet light. The DNA was diluted to 5 ng/μL for amplification reactions.

Amplification

DNA amplification was performed in a thermocycler (96-Well Thermal Cycler, Applied Biosystems, Foster City, CA, USA) using 27 ISSR primers (Table 2). The final volume of the reactions was 15 μL and contained 0.2 mM dNTPs, 1X buffer, 2.5 mM MgCl₂, 1 U Taq DNA polymerase, 0.4 mM primer, and 10 ng genomic DNA.

Table 2. List of ISSR primers used and their sequence (5'-3').

Primer	Sequence (5'-3')
ISSR 12	GAGAGAGAGAGAGARC
ISSR 24	AGTGTGTGTGTGTGT
ISSR 25	CGTGTGTGTGTGTGT
ISSR 31	CRCACCACCACCACCAC
ISSR 32*	CYCACCACCACCACCAC
ISSR 33	CAGCAGCAGCAGCAG
ISSR 34	CAGCAGCAGCAGCAGRC
ISSR 35*	CAGCAGCAGCAGCAGYC
ISSR 36	CRCAGCAGCAGCAGCAG
ISSR 37	CYCAGCAGCAGCAGCAG
ISSR 39	GTGGTGGTGGTGGTGRC
ISSR 40	GTGGTGGTGGTGGTGYC
ISSR 41	CRGTGGTGGTGGTGGTG
ISSR 42	CYGTGGTGGTGGTGGTG
ISSR 47	CYTGTGGTGGTGGTGGTGT
ISSR 58	ACGACGACGACGACGRC
ISSR 59	AGAAGAAGAAGAAGARC
ISSR 69	TTGTTGTTGTTGTTGRC
ISSR 83	CTGCTGCTGCTGCTGRC
ISSR 86	CGACGACGACGACGARC
ISSR 90	GAAGAAGAAGAAGAARC
ISSR 91	GATGATGATGATGATRC
ISSR 92	GACGACGACGACGACRC
ISSR 93	GAGGAGGAGGAGGAGRC
ISSR 94	GTAGTAGTAGTAGTARC
ISSR 97*	GCAGCAGCAGCAGCARC
ISSR 101	GGAGGAGGAGGAGGARC

*Primers not used for molecular analysis because they were monomorphic.

The DNA amplification program consisted of initial denaturation at 95°C for 2 min, followed by 39 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, with a final extension for 10 min at 72°C and hold at 14°C. The amplified products were subjected to electrophoresis on a 2.5% agarose gel in 0.5X TBE buffer. The gels were stained with ethidium bromide and photographed under ultraviolet light.

Data analysis

Amplified DNA fragments were classified as having a presence or absence of bands, and a matrix was built, with 1 representing the presence and 0 representing the absence of the

band on the gel. Genetic distances between accessions were evaluated using the dissimilarity matrix generated by the arithmetic complement of the Jaccard's index, and the clustering of genotypes was generated using the unweighted pair-group method using arithmetic averages and the Gene software (Cruz et al., 2003). A dendrogram was constructed using the STATISTICA (Statistica, 2002) software.

RESULTS

Study of genetic diversity among cassava accessions maintained in the field

Of the 27 ISSR primers tested, only 24 showed polymorphisms. These 24 primers generated a total of 175 bands, of which 100 were polymorphic (57.1%). The number of bands from each primer ranged from 2-13, with an average of 7.3 bands per primer (Table 3).

Table 3. List of ISSR primers, number of amplified products, number of monomorphic and polymorphic bands, and percent polymorphism in 22 cassava accessions.

Primer	Bands			% Polymorphism
	Total	Monomorphic	Polymorphic	
ISSR 12	13	2	11	84.6
ISSR 24	9	4	5	55.6
ISSR 25	6	2	4	66.7
ISSR 31	7	5	2	28.6
ISSR 33	11	8	3	27.3
ISSR 34	10	3	7	70.0
ISSR 36	10	6	4	40.0
ISSR 37	4	3	1	25.0
ISSR 39	11	6	5	45.5
ISSR 40	8	1	7	87.5
ISSR 41	10	1	9	90.0
ISSR 42	9	4	5	55.6
ISSR 47	9	4	5	55.6
ISSR 58	7	5	2	28.6
ISSR 59	2	1	1	50.0
ISSR 69	7	3	4	57.1
ISSR 83	7	5	2	28.6
ISSR 86	3	2	1	33.3
ISSR 90	9	1	8	88.9
ISSR 91	6	1	5	83.3
ISSR 92	2	1	1	50.0
ISSR 93	9	6	3	33.3
ISSR 94	3	0	3	100.0
ISSR 101	3	1	2	66.7
Total	175	75	100	57.1
Average	7.3	3.1	4.2	56.3

The minimum number of polymorphic bands (1 band) was obtained for ISSR primers 37, 59, 86, and 92. The maximum number of polymorphic bands was 11 bands and was obtained from the ISSR 12 primer, indicating 84.6% polymorphism (Table 3).

The electrophoretic profile of 22 accessions from the field using the ISSR 90 primer is shown in Figure 1.

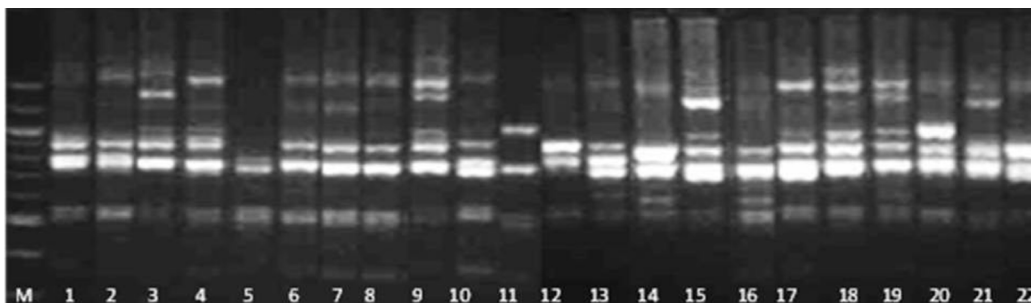


Figure 1. Electrophoretic profile of the ISSR 90 primer on a 2.5% agarose gel for 22 cassava accessions (lane 1 - BGM 212; lane 2 - BGM 264; lane 3 - BGM 561; lane 4 - BGM 563; lane 5 - BGM 337; lane 6 - BGM 316; lane 7 - BGM 340; lane 8 - BGM 640; lane 9 - BGM 1723; lane 10 - BGM 1840; lane 11 - BGM 638; lane 12 - BGM 1123; lane 13 - BGM 668; lane 14 - BGM 1037; lane 15 - 1811; lane 16 - BGM 1245; lane 17 - BGM 1324; lane 18 - BGM 1345; lane 19 - BGM 1348; lane 20 - BGM 1371; lane 21 - BGM 116; lane 22 - BGM 1282) maintained in the field and present in the Cassava Active Germplasm Bank (Banco Ativo de Germoplasma - BAG - Mandioca) of Embrapa Cassava & Fruits, Cruz das Almas, State of Bahia.

In the present study, the greatest genetic dissimilarity among cassava accessions was between BGM 668 and 1840 (0.32), and the lowest genetically dissimilar accessions were BGM 1037 and 1324 (0.07) (Table 4). This behavior reflects the different origins of these materials.

Silva et al. (2011) evaluated the intra- and inter-specific genetic diversity of *Manihot* accessions using ISSR markers and observed the greatest dissimilarity (0.69) between *M. esculenta* var. *mandiocaba* and *M. dichotoma* and the lowest dissimilarity (0.49) between *M. dichotoma* var. *undulata* and *M. caerulescens*. In this case, the authors observed greater dissimilarity among individuals of the same species compared to among different species of the same genus.

Zayed et al. (2013) used ISSR markers to measure the genetic distance between 4 cassava genotypes imported from Indonesia, Brazil, and Thailand (2 genotypes). The authors found greater dissimilarity (0.25) between genotypes from Brazil and Indonesia and the lowest dissimilarity between genotypes from Thailand and Brazil.

These observations and the results of the present study clearly demonstrate the efficiency of ISSR markers for revealing genetic diversity in cassava, both among genotypes of the same species and among species of the same genus.

A dendrogram was constructed from 100 polymorphic bands, and the arithmetic complement of the Jaccard's index was used to construct the genetic similarity matrix (Figure 2). The 22 cassava accessions were separated into 7 groups according to the unweighted pair-group method using arithmetic averages grouping method, and the cutoff was chosen according to the criteria of Mingoti (2005).

The 1st group (G1) was formed by 2 accessions, BGM 212 and BGM 1282. The 2nd group (G2) was formed by the accessions BGM 264, BGM 340, BGM 337, BGM 640, BGM 316, BGM 561, BGM 563, and BGM 1723. The 3rd group (G3) was formed only by BGM 638. The 4th group (G4) contained the accessions BGM 1123, BGM 1348, BGM 1037, BGM 1324, BGM 1345, BGM 1245, BGM 1371, and BGM 116. The 5th (G5), 6th (G6), and 7th groups (G7) were formed by accessions BGM 1811, BGM 668, and BGM 1840, respectively.

Table 4. Genetic dissimilarity matrix estimated based on Jaccard's coefficient for 22 cassava accessions.

	BGM 212	BGM 264	BGM 561	BGM 563	BGM 337	BGM 316	BGM 340	BGM 640	BGM 1723	BGM 1840	BGM 638	BGM 1123	BGM 668	BGM 1037	BGM 1811	BGM 1245	BGM 1324	BGM 1345	BGM 1348	BGM 1371	BGM 1116	BGM 1282	
BGM 212	0.00																						
BGM 264	0.15	0.00																					
BGM 561	0.17	0.17	0.00																				
BGM 563	0.17	0.20	0.10	0.00																			
BGM 337	0.16	0.17	0.17	0.18	0.00																		
BGM 316	0.17	0.16	0.18	0.20	0.14	0.00																	
BGM 340	0.17	0.13	0.16	0.17	0.14	0.17	0.00																
BGM 640	0.19	0.14	0.17	0.18	0.12	0.15	0.12	0.00															
BGM 1723	0.19	0.18	0.17	0.16	0.17	0.19	0.18	0.12	0.00														
BGM 1840	0.23	0.20	0.23	0.24	0.22	0.24	0.23	0.21	0.22	0.00													
BGM 638	0.20	0.22	0.22	0.22	0.19	0.21	0.19	0.14	0.19	0.28	0.00												
BGM 1123	0.20	0.21	0.25	0.24	0.21	0.23	0.18	0.21	0.23	0.28	0.16	0.00											
BGM 668	0.24	0.21	0.23	0.28	0.27	0.23	0.24	0.23	0.26	0.32	0.25	0.23	0.00										
BGM 1037	0.17	0.18	0.21	0.17	0.19	0.18	0.18	0.19	0.19	0.26	0.14	0.12	0.18	0.00									
BGM 1811	0.23	0.22	0.24	0.25	0.19	0.18	0.19	0.21	0.20	0.29	0.19	0.19	0.20	0.12	0.00								
BGM 1245	0.23	0.22	0.23	0.23	0.23	0.26	0.18	0.19	0.22	0.28	0.21	0.18	0.22	0.10	0.22	0.00							
BGM 1324	0.22	0.20	0.23	0.23	0.22	0.20	0.20	0.23	0.22	0.21	0.20	0.17	0.25	0.07	0.17	0.15	0.00						
BGM 1345	0.23	0.23	0.23	0.26	0.24	0.25	0.18	0.23	0.23	0.28	0.21	0.20	0.23	0.12	0.21	0.13	0.08	0.00					
BGM 1348	0.26	0.22	0.23	0.24	0.22	0.22	0.19	0.19	0.23	0.25	0.23	0.15	0.24	0.14	0.19	0.16	0.18	0.15	0.00				
BGM 1371	0.23	0.15	0.17	0.19	0.21	0.21	0.17	0.17	0.19	0.26	0.21	0.21	0.22	0.16	0.23	0.18	0.17	0.16	0.16	0.00			
BGM 1116	0.23	0.16	0.19	0.22	0.19	0.18	0.14	0.20	0.20	0.28	0.23	0.18	0.23	0.13	0.18	0.20	0.19	0.17	0.15	0.12	0.00		
BGM 1282	0.16	0.17	0.17	0.20	0.20	0.18	0.15	0.22	0.21	0.26	0.21	0.23	0.24	0.17	0.20	0.21	0.20	0.19	0.18	0.17	0.18	0.00	

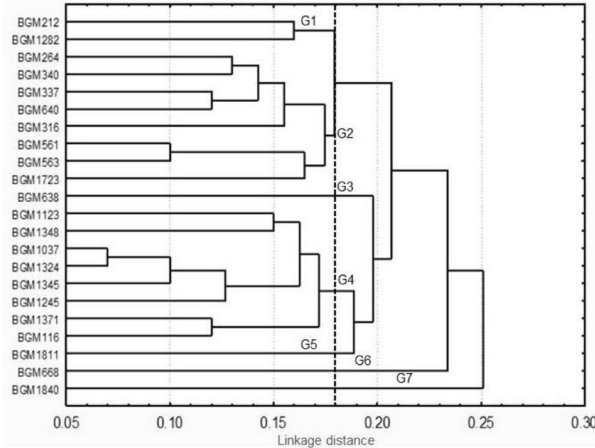


Figure 2. Dendrogram generated by unweighted pair group method using arithmetic averages using 100 polymorphic bands from 24 ISSR markers showing the genetic relationships between 22 cassava accessions found in the Cassava Active Germplasm Bank of Embrapa Cassava & Fruit.

Among the groups formed, only the 2nd group (G2) contained accessions with similar suitability. Although they originated from different regions of Brazil, these accessions are suitable for flour production. The other groups (G1, G3, G4, G5, and G7) consisted of accessions suitable for human consumption and/or flour production, except for the 6th group (G6), which showed no relation to suitability to other accessions.

This preliminary study of genetic diversity among accessions maintained in the field revealed variability that can be used in the breeding program of this species.

Genetic fidelity between accessions from the field and cultured *in vitro*

For most of the electrophoretic profiles tested in our study with 24 ISSR markers, the fidelity between accessions in the field and those multiplied *in vitro* for 7 subcultures was assessed. Figure 3 shows the electrophoretic profiles of plants maintained in the field (c) and matched plants from 7 subcultures *in vitro* (i) using the ISSR 39 primer.

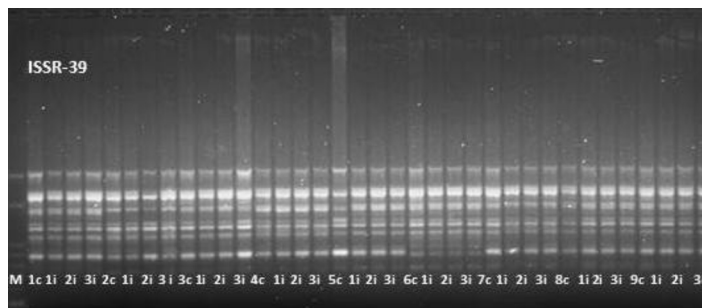


Figure 3. Electrophoretic profile on a 2.5% agarose gel of the ISSR 39 primer in plants maintained in the field (c) and matched clones derived from meristems (1i, 2i, 3i) of nine cassava accessions (1-9). Lane 1 - BGM 212; lane 2 - BGM 264; lane 3 - BGM 561; lane 4 - BGM 563; lane 5 - BGM 337; lane 6 - BGM 316; lane 7 - BGM 340; lane 8 - BGM 640; lane 9 - BGM 1723. Lane M = 1-kb DNA ladder (Invitrogen).

DISCUSSION

Several authors have used tissue culture to regenerate cassava plants using either organogenesis with meristem cultures (Oliveira et al., 2000) or axillary buds and nodal explants (Konan et al., 1997; Lima et al., 2002), or through somatic embryogenesis (Hankoua et al., 2005; Saelim et al., 2006). However, there have been no reports of studies examining somaclonal variation in cassava plants regenerated by one of the systems mentioned above, except for the study by Raemakers et al. (2001), who observed variations in the shape, color, and size of leaves, the presence of branches, and reduced size and vigor in transgenic plants regenerated from the culture of friable embryogenic callus in 6 of the 10 cassava genotypes tested. These observations were performed at the morphological level, with no other records using molecular markers. Molecular markers are widely used for studies of genetic similarity, divergence, variability, and molecular characterization of the cassava crop (Costa et al., 2003; Vieira et al., 2008, 2010).

Several authors have also used ISSR markers to determine the genetic fidelity of micropropagated plants. Pathak and Dhawan (2012) confirmed the clonal stability of 22 subcultures of apple rootstock Merton 793 plants regenerated from axillary buds using 15 ISSR primers. These authors observed a homogeneous amplification profile for all micropropagated plants compared with the donor plant.

Similarly, Lu et al. (2011) used 45 ISSR primers to assess genetic identity and monitor somaclonal variation of 30 banana cultivars during micropropagation using meristems as the source of explants. The authors also observed a homogeneous amplification profile in all micropropagated plants compared with their parent plants.

The results of the present study are consistent with those of previous reports describing the applicability of ISSR markers for studying the fidelity of meristem-derived plants. However, interesting observations may be made regarding the likelihood of somaclonal variation with the use of the ISSR 37 (Figure 4) and ISSR 25 (Figure 5) primers, which show examples of genetic differences between accessions multiplied *in vitro* and their corresponding accessions maintained in the field.

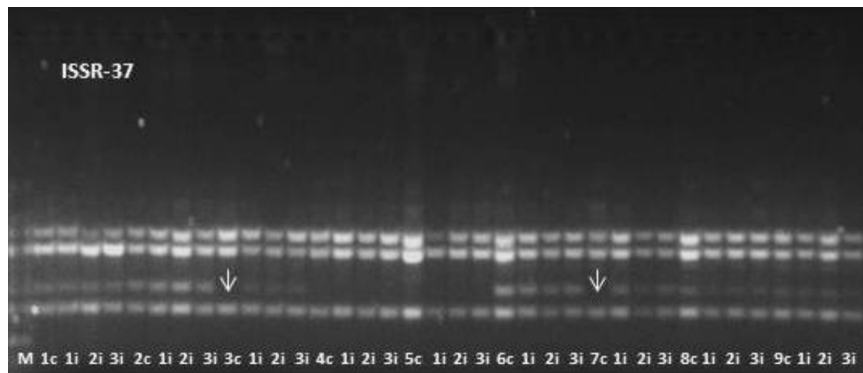


Figure 4. Electrophoretic profile on a 2.5% agarose gel of the ISSR 37 primer in plants in the field (c) and matched clones derived from meristems (1i, 2i, 3i) of nine cassava accessions. Lane 1 - BGM 212; lane 2 - BGM 264; lane 3 - BGM 561; lane 4 - BGM 563; lane 5 - BGM 337; lane 6 - BGM 316; lane 7 - BGM 340; lane 8 - BGM 640; lane 9 - BGM 1723. Lane M = 1-kb DNA ladder (Invitrogen). White arrows indicate accessions from the field with genetic divergences compared with matched material multiplied *in vitro*.

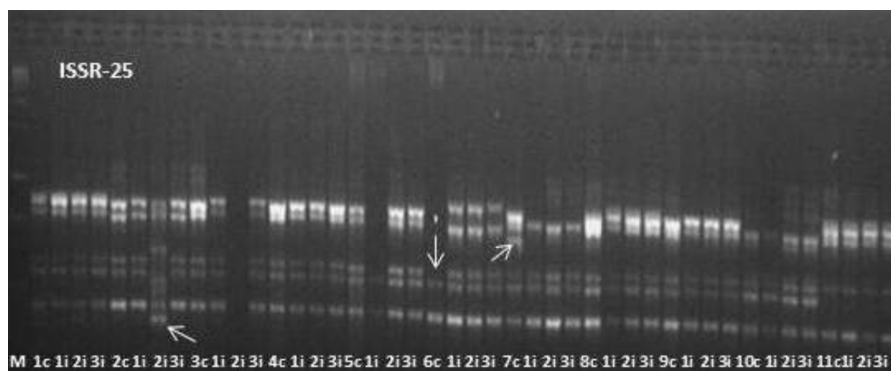


Figure 5. Electrophoretic profile on a 2.5% agarose gel of the ISSR 25 primer in plants maintained in the field (c) and matched clones derived from meristems (1i, 2i, 3i) of 11 cassava accessions. Lane 1 - BGM 212; lane 2 - BGM 264; lane 3 - BGM 561; lane 4 - BGM 563; lane 5 - BGM 337; lane 6 - BGM 316; lane 7 - BGM 340; lane 8 - BGM 640; lane 9 - BGM 1723, lane 10 - BGM 1840, lane 11 - BGM 638. Lane M = 1-kb DNA ladder (Invitrogen). White arrows represent genetic divergences between materials.

Figure 4 shows the difference between the accessions maintained in the field and those propagated *in vitro* when using the ISSR 37 primer, e.g., for the BGM 561 and BGM 340 accessions, for which the *in vitro* accessions exhibited a band while their matched field accessions did not. For the BGM 561 accession, this difference appeared only when the ISSR 37 primer was used. The genetic difference between the BGM 340 accession maintained in the field and its corresponding *in vitro* accession was evident for primers ISSR 37, 25, 90, 47, and 36. These results indicate that there may have been a problem with the collection or that somaclonal variation was present, which may be exploited in future cassava breeding programs.

The primer that generated the largest number of genetic differences between the material from the field and the accessions multiplied *in vitro* was ISSR 25. Figure 5 shows that this primer revealed genetic differences between field accessions and matched subcultured representatives of the BGM 316 and BGM 340 accessions. The BGM 316 accession exhibited a missing band in the field accession compared with the accession subcultured *in vitro*, and the BGM 340 accession showed a missing band compared with its matched *in vitro* materials.

However, for the BGM 316 accession, this missing band was only observed for this specific primer, and there were insufficient replicates to demonstrate the presence of somaclonal variation or any other occurrence, such as collection error. Further studies of the BGM 430 accession are necessary to determine whether there was somaclonal variation or whether there were problems in the collection.

The cassava germplasm bank maintained in the field is always changing locations, which may lead to problems in identifying and swapping of accessions, which can affect genetic analysis at the DNA level.

Based on the results of this study, the micropropagation/subculture method adopted by the Plant Tissue Culture Laboratory of Embrapa Cassava & Fruit clearly maintained the fidelity of accessions grown in the field and multiplied *in vitro*.

To better identify somaclonal variation, a greater number of *in vitro* replicates of the material being multiplied and the use of other techniques, such as cytogenetics, flow cytometry, sequencing, cloning, and sequence alignment of fragments, are required in addition to field tests to determine the behavior of possible somaclonal variation.

Molecular analysis revealed the genetic uniformity among most plants cultured *in vitro* and matched plants maintained in the field for the assessed accessions.

The ISSR markers were efficient for determining the genetic homogeneity of cassava plants derived from meristem culture, thus demonstrating the reliability of the propagation system.

There may be somaclonal variation of the BGM 340 accession, but further studies are necessary to support this hypothesis.

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