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Esterase isozymes for the characterization of "unnamed" cassava cultivars (*Manihot esculenta* Crantz)

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ABSTRACT. Esterase isozymes were used as molecular markers to discriminate and cluster seven "unnamed" cultivars (accesses A-G) of *M. esculenta*. The "unnamed" cassava cultivars were compared to 25 different *M. esculenta* cultivars (cultivars BG), which have been maintained in the germplasm collection of the Agronomy Department, State University of Maringá. 4-Methylumbelliferyl acetate, 4-methylumbelliferyl propionate and α–naphthyl acetate were utilized as substrates for isoesterase detection and comparative analysis. Similarity between plants, using Jaccard's coefficient, ranged from 47.6% to 100%. A dendrogram produced by cluster analysis showed identity between cultivar BG 23 and plants of accession D. Plants of accesses B and G were also clustered with cultivar BG 23 showing 95% and 89% similarity, respectively. Plants of accesses A and E were similar to BG 1, showing 95% and 90% similarity, respectively. Plants of accession F were clustered with cultivar BG 9, showing 94% similarity. The dendrogram also showed that most of the cultivars were clustered together with 85-90% similarity. Thus, we conclude that esterase isozymes may be used as molecular markers of cassava genotypes for the characterization of "unnamed" cultivars of *M. esculenta*.

Key words: esterase, isoesterase, isozymes, cassava, *Manihot esculenta*, polymorphism, genetic similarity.

RESUMO. Izoenzimas esterases para discriminar cultivares "sem nome" de mandioca (*Manihot esculenta*). Isoenzimas esterases foram usadas como marcadores moleculares para discriminar e agrupar sete cultivares "sem nomes" (acessos A-G) de *Manihot esculenta*. Os cultivares "sem nomes" de mandioca foram comparados com 25 diferentes cultivares (BG) que vêm sendo mantidos na coleção de germoplasma do Departamento de Agronomia, da Universidade Estadual de Maringá. Acetato e propionato de 4-metilumbeliferona e acetato de α–naftil, foram os substratos utilizados para a detecção e análise comparativa das isoesterases. A similaridade entre as plantas, usando o coeficiente de Jaccard, variou de 47,6% até 100%. O dendrograma produzido pela análise de agrupamento mostrou identidade entre as plantas do cultivar BG23 e as plantas do acesso D. As plantas dos acessos B e G também foram agrupadas com o cultivar BG 23, mostrando similaridade de 95% e 89%, respectivamente. As plantas dos acessos A e E foram similares às plantas BG 1, mostrando 95% e 90% de similaridade, respectivamente. As plantas do acesso F foram agrupadas com as plantas do cultivar BG 9, mostrando 94% de similaridade. O dendrograma mostrou também que a maioria dos cultivares foram agrupados com 85-90% de similaridade. Assim, concluímos que as isozimas esterases podem ser utilizadas como marcadores moleculares de genótipos de mandioca, para a caracterização dos cultivares sem nomes de *M. esculenta*.

Palavras-chave: esterase, isoesterase, isoenzimas, cassava, *Manihot esculenta*, polimorfismo, similiridade genética.

Although cassava (*Manihot esculenta*) reproduces from seeds, most plants are disseminated by vegetative propagation (Nassar, 1992). Vegetative multiplication should affect genetic variability and facilitate its random distribution. As a consequence, the cultivars from the Brazilian territory present also a casual and non-uniform nomenclature (Leitão Filho, 1971). The same variety or cultivar may

present different denominations, and on the other hand, different cultivars present the same denomination in different places (Silva, 1979). This is a frequent occurrence in the State of Paraná where the origin of the cassava cultivars is ignored and they are not characterized as improved genetic material.

The problem of nomenclature is more marked for cassava cultivars that are planted in small accession (domestic land plots and gardens) which commonly are useful for consumption in natura and are referred to as "table cassava". Most of the plants are "unnamed" cultivars of *M. esculenta*. These cultivars without names are easily available to the population, and can be "mixed" with the varieties cultivated for commercial purposes since their distribution is random.

Botanical and agronomic characteristics might be used for the characterization of the different cassava cultivars, but there is some disagreement about such parameters as floral structures (Cours, 1951; Valeriano, 1955; Rogers, 1963; Leon *et al.*, 1967), coloration of adult stems, number of foliate lobes, leave shape and coloring (Sarmiento, 1969; Leitão Filho, 1971), and root anatomy (Viégas, 1976) when applied to discriminate close varieties of *M. esculenta*.

Thus, in the present study esterase isozymes were used as molecular markers to discriminate and cluster "unnamed" cultivars of *M. esculenta*. Esterase isozymes are proteins capable of hydrolyzing esters that are present in biological material of all kinds of organisms, and are usual markers in genetic studies because they are easy to detect and appear to be highly polymorphic (Davis, 1964). In the present study, the "unnamed" cassava cultivars were compared to different *M. esculenta* cultivars which have been maintained in the germplasm collection of the Agronomy Department, State University of Maringá.

Material and methods

The "unnamed" cultivars of *M. esculenta* were collected at seven different sites (accesses A-G; domestic lands) in the municipality of Maringá (State of Paraná). Samples of 10-20 plants of each accession were collected and compared to the cassava cultivars maintained in a germplasm collection (collection BG). The *M. esculenta* collection BG consists of cultivars originating from traditional cultivars collected in the southwestern and northwestern regions of the State of Paraná, in the South of Brazil, and cultivars produced at the Agronomy Institute of Campinas (IAC), State of São Paulo, Southeast Brazil (Table 1). These cultivars have been maintained by vegetative propagation for five years and are useful in production programs (Gonçalves-Vidigal *et al.*, 1997); they are cultivated yearly at the Iguatemi Experimental Farm of the State University of Maringá.

Table 1. Geographic origin of the *Manihot esculenta* BG cultivars maintained at the germplasm bank of the State University of Maringá, Maringá (PR)

BG	Cultivar	Region	City
01	Fibra	NW – PR	Paranavai
02	Branca de Santa Catarina	NE – SP	Campinas
03	IAC 12-829	NE – SP	Campinas
04	IAC 13	NE – SP	Campinas
05	Fécula Branca	SW – PR	Santa Helena
06	Alegretti	SW – PR	Santa Helena
07	Amarela	SW – PR	Santa Helena
08	IAC 12-829	NE – SP	Campinas
09	Espeto	NW – PR	Araruna
10	Vermelha	NW – PR	Mandaguaçu
11	Verdinha	SW – PR	Santa Helena
12	Verdinha	SW – PR	Santa Helena
13	Amarela	SW – PR	Santa Helena
14	Branca	NW – PR	Mandaguaçu
15	Amarela	NW – PR	Mandaguaçu
16	IAC 14	NE – SP	Campinas
17	IAC 576-70	NE – SP	Campinas
18	Renascença	SW – PR	Renascença
19	Stalina	SW – PR	Santa Helena
20	Araruna	NW – PR	Araruna
21	José Mendes	NW – PR	Mandaguaçu
22	Fécula Branca	SW – PR	Santa Helena
23	Amarela	NW – PR	Mandaguaçu
24	Mico	NW – PR	Araruna
25	Pão-do-Chile	NW – PR	Mandaguaçu

NW – PR: Northwestern region of the State of Parana; SW – PR: Southwestern region of the State of Parana; NE – SP: Northeastern region of the State of São Paulo

The electrophoretic evaluations were carried out using samples of young unexpanded leaves (10-20 mm length) of each *M. esculenta* plant. The leaves were individually homogenized with a glass rod in an Eppendorf microcentrifuge tube using 80 µl of 1.0 M phosphate buffer, pH 7.0 containing 5% PVP-40, 0.01 M DTT (dithiothreitol), 10 mM sodium metabisulfite, 50 mM ascorbic acid, 1.0 mM EDTA, and 0.5% β-mercaptoethanol solution (Resende, 1999). After homogenization, the samples were centrifuged at 14,000 rpm for 30 minutes at 4 °C in a Beckman GS-15R centrifuge.

The esterase isozymes were analyzed by procedures originally described by Resende (1999). The supernatants were absorbed with Watman n°. 3 paper strips (5 x 6 mm) which were inserted vertically into a 14% starch gel (penetrose-30) prepared in 0.01 M Tris and 0.0028 M citric acid buffer pH 7.5. In the electrode chambers we used 0.1 M Tris and 0.028 M citric acid pH 7.5. Electrophoresis was carried out at 4 °C for approximately 5-6 hours, at 35 mA (8.5 V/cm of gel).

4-methylumbelliferyl esters (acetate and propionate) and α–naphthyl acetate were utilized as substrates (Tashian, 1969; modified by Resende, 1999)

for isoesterase detection and comparative analysis between plants from the accesses and plants from BG cultivars. 4-methylumbelliferyl acetate substrate (4 mg) was dissolved in 500 µl acetone and the volume was completed to 10 ml using twice-distilled water. After staining with 4-methylumbelliferyl acetate the gel was washed with tap water and incubated for 30-60 min in a solution containing 50 ml 0.05 M sodium acetate, pH 6.5, 40 mg fast blue RR salt and 4 mL 1% α-naphthyl acetate (Resende, 1999).

Data were analyzed by comparing the esterase patterns on the basis of presence or absence of each esterase isozyme. The similarity between the plants of access A-G and the plants of BG cultivars was calculated using Jaccard's coefficient, and UPGMA cluster analysis was performed using the NTSYS-pc sofware (Rohlf, 1989).

Results

Five different electrophoretic phenotypes for esterase isozymes were observed in plants of accession A (A1-A5). Plants of accesses B, D, and G showed three different phenotypes (B1-B3; D1-D3; and G1-G3, respectively). Four and two electrophoretic phenotypes were observed in plants of accesses F (F1-F4) and E (E1-E2) while only one electrophoretic phenotype was observed in 10 plants of accession C. Figure 1 shows the five electrophoretic patterns for esterase isozymes stained with 4-methylumbelliferyl acetate and the three electrophoretic patterns for esterase isozymes stained with α-naphthyl acetate observed in plants of accesses A and G. At least five plants of each of the 25 BG cassava cultivars were used with the three substrates to check the consistency of isozyme banding patterns.

The esterase isozymes patterns observed with 4-methylumbelliferyl acetate, 4-methylumbelliferyl propionate, and α-naphthyl acetate in plants of seven accesses and of cultivars BG of *M. esculenta* produced a total of 24 esterase isozymes, 9 of which were consistent in all cultivars (Est-1/Est-2/Est-3/Est-4/Est-5/Est-7/Est-16/Est-7/Est-18), while 15 were polymorphic (Table 2). Substrate preference for 4-methylumbelliferyl esters and α-naphthyl acetate was observed; Est-5 and Est-7 were observed with 4-methylumbelliferyl propionate and isozymes from Est-16 to Est-24 were detected with α-naphthyl acetate.

The relationship between plants of the accesses and plants of cultivars BG was estimated using Jaccard's coefficient of similarity (Table 3). Similarity between all plants ranged from 47.6% (BG 16 and F3; BG 16 and BG 25) to 100% (BG 3 and BG 8; BG 23 and D3). A dendrogram produced by cluster analysis (Figure 2) showed the identity of BG 23 ("Amarela") and plants with the D3 electrophoretic phenotype of accession D. Plants of accesses B (B3 electrophoretic phenotype) and G (G1 electrophoretic phenotype) were also clustered with cultivars BG 23, showing 95% and 89% similarity, respectively. Plants of accesses A (A5 electrophoretic phenotype) and E (E2 electrophoretic phenotype) were similar to BG 1 ("Fibra"), showing 95% and 90% similarity, respectively. Plants of accession F (F2 electrophoretic phenotype) were clustered with cultivar BG 9 ("Espeto"), showing 94% similarity. The dendrogram also showed that most cultivars clustered together at 85-90% similarity.

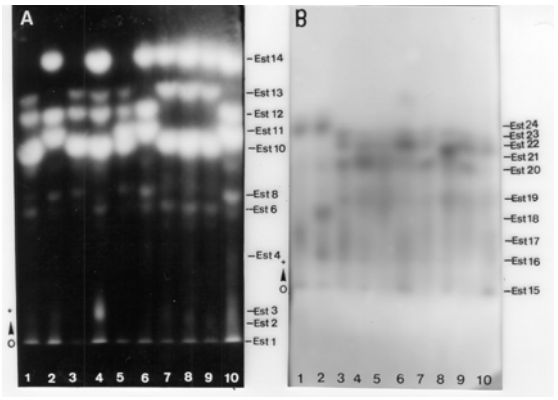

Figure 1. Electrophoretic esterase phenotypes of *Manihot esculenta* cultivars detected with 4-methylumbelliferyl acetate (A) and α -naphthyl acetate (B): samples 1-5 correspond to accession A showing Est-1/Est-2/Est-3/Est-4/Est-6/Est-10/Est-12/Est-13/Est-15/Est-16/Est-17/Est-24 (sample 1), Est-1/Est-2/Est-3/Est-4/Est-8/Est-11/Est-12/Est-14/Est-15/Est-16/Est-17/Est-18/Est-20/Est-24 (sample 2), Est-1/Est-2/Est-3/Est-4/Est-8/Est-10/Est-12/Est-13/Est-15/Est-16/Est-17/Est-19/Est-20/Est-22/Est-23 (sample 3), Est-1/Est-2/Est-3/Est-4/Est-6/Est-10/Est-12/Est-13/Est-14/Est-15/Est-16/Est-17/Est-18/Est-19/Est-20/Est-22 (sample 4), Est-1/Est-2/Est-3/Est-4/Est-8/Est-11/Est-12/Est-13/Est-15/Est-16/Est-17/Est-19/Est-22/Est-23 (sample 5) electrophoretic phenotypes; samples 6-10 correspond to plants of accession G showing Est-1/Est-2/Est-3/Est-4/Est-6/Est-8/Est-11/Est-12/Est-14/Est-15/Est-16/Est-17/Est-19/Est-20/Est-22/Est-23 (samples 6 and 10), Est-1/Est-2/Est-3/Est-4/Est-6/Est-8/Est-10/Est-13/Est-14/Est-15/Est-16/Est-17/Est-19/Est-20/Est-21/Est-23 (samples 7, 8, and 9)

Discussion

Althrough vegetative propagation is a prominent feature of cassava plants, the variation in the isoesterase patterns observed in cultivars BG and accesses A-G indicates genomic variability among cassava cultivars. The genetic variability observed in the present study agrees with the results observed in the analysis of other enzymatic systems in cassava (Resende, 1999). High levels of polymorphism were also found for the *M. esculenta* germplasm collection from African varieties (Lefévre and Charrier, 1993a, b).

Table 2. Isoesterase phenotypes in the seven *Manihot esculenta* accesses (A-G) for 4-methylumbelliferyl acetate and 4-methylumbelliferyl propionate (from Est-1 to Est-14), and α-naphthyl acetate (from Est-15 to Est-24)

	Est-1	Est-2	Est-3	Est-4	Est-5*	Est-6	Est-7*	Est-8	Est-9	Est-10	Est-11	Est-12	Est-13	Est-14	Est-15	Est-16	Est-17	Est-18	Est-19	Est-20	Est-21	Est-22	Est-23	Est-24
A1	+	+	+	+	+	+	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-	-	-	+
A2	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	+	+	_	+	-	-	-	+
A3	+	+	+	+	+	-	+	+	-	+	-	+	+	-	+	+	+	-	+	+	-	+	+	-
A4	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	-	+	+	-	+	-	-
A5	+	+	+	+	+	-	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	-	-	-
B1	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	-	+
B2	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+
B3	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	-	-
C1	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	-
D1	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-	-
D2	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	-	+	-	-	-	-	+
D3	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+
E1	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-
E2	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	-	-	+	-	-
F1	+	+	+	+	+	-	+	+	-	-	+	-	-	+	+	+	+	+	+	+	-	+	+	-
F2	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	+	-	+
F3	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	-	-
F4	+	+	+	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+	+	-	-	-	-	+
G1	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	-	+	+	-	+	+	-
G2	+	+	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	+	-	+	-	-
G3	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	-	+	-	+	-

* isoesterases substrate-specific for 4-methylumbelliferyl propionate

Table 3. Esterase polymorphism in the *Manihot esculenta* cultivars detected with the use of 4-methylumbelliferyl acetate, 4-methylumbelliferyl propionate, and α- naphthyl acetate

Substrates	Polymorphic Isoesterases	Total Isoesterases
4-Methylumbelliferyl acetate and propionate	8	14
α-Naphthyl acetate	7	10
Total	15	24

Domestication of cassava using indigenous agronomic strategies which valued heterogeneity rather than homogeneity (Chernela, 1963), and the apomixis process in cassava (Nassar, 1992; Nassar *et al.*, 1993) should be important factors promoting the greater variability present in the *M. esculenta* species. Indeed, apomixis provides fixation of heterosis (Nassar, 1994; Nassar *et al.*, 1998).

In the present study, the majority of the unnamed cultivars clustered together, while cultivar BG showed different clustering. However, the identity between the "Amarela" cultivar (BG 23) and plants of accession D, and the high similarity between plants of accesses A, B, C, E, F, G and cultivars BG were clear and permitted genome assignment of the A-G access plants. We suspect that the identity and genomic similarity between plants of accesses D, B, G, and cultivar "Amarela" (BG 23) reflect a popular preference for cassava with yellow root coloration used for consumption in nature. The unnamed cultivars of *M. esculenta* contain genetic information for esterase isozymes usually found in cultivars "Fibra" and "Espeto" which are traditional cultivars from the northwestern region of Paraná cultivated by regional producers and useful for flour and starch production.

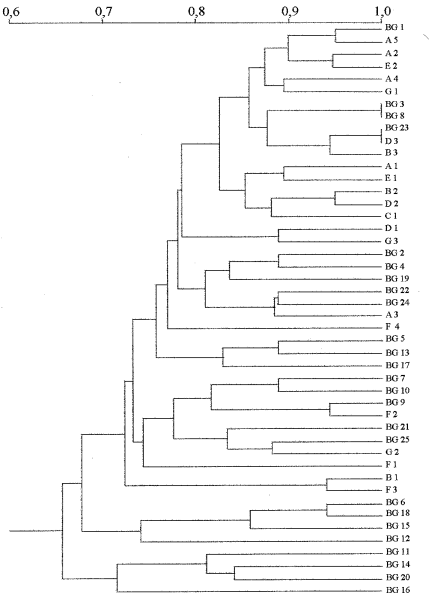

Figure 2. Dendrogram representing the relationship between the 25 *Manihot esculenta* cultivars (BG 1: Fibra, BG 2: Branca de Santa Catarina, BG 3: IAC 12-829, BG 4: IAC 13, BG 5: Fécula Branca, BG 6: Alegretti, BG 7: Amarela, BG 8: IAC 12-829, BG 9: Espeto, BG 10: Vermelha, BG 11: Verdinha, BG 12: Verdinha, BG 13: Amarela do Mato Grosso, BG 14: Branca, BG 15: Amarela, BG 16: IAC 14, BG 17: IAC 576-70, BG 18: Renascença, BG 19: Stalina, BG 20: Araruna, BG 21: José Mendes, BG 22: Fécula Branca, BG 23: Amarela, BG 24: Mico, BG 25: Pão do Chile) and accesses A-G of the cassava cultivars based on UPMGA cluster analysis of the esterase isozymes detected with 4-methylumbelliferyl acetate and propionate, and α-naphthyl acetate using Jaccard´s similarity coefficient

Table 4. Jaccard´s coefficient of similarity of the *Manihot esculenta* cultivars BG (BG 1: Fibra, BG 2: Branca de Santa Catarina, BG 3: IAC 12-829, BG 4: IAC 13, BG 5: Fécula Branca, BG 6: Alegretti, BG 7: Amarela, BG 8: IAC 12-829, BG 9: Espeto, BG 10: Vermelha, BG 11: Verdinha, BG 12: Verdinha, BG 13: Amarela do Mato Grosso, BG 14: Branca, BG 15: Amarela, BG 16: IAC 14, BG 17: IAC 576-70, BG 18: Renascença, BG 19: Stalina, BG 20: Araruna, BG 21: José Mendes, BG 22: Fécula Branca, BG 23: Amarela, BG 24: Mico, BG 25: Pão do Chile) and cassava accesses A-G. The coefficients were calculated from isozyme esterase data for 15 polymorphic bands

BG 1	1.000																																												
BG 2	0.750	1.000																																											
BG 3	0.850	0.789	1.000																																										
BG 4	0.762	0.889	0.800	1.000																																									
BG 5	0.895	0.737	0.842	0.750	1.000																																							
BG 6	0.800	0.737	0.667	0.667	0.700	1.000																																						
BG 7	0.800	0.650	0.750	0.667	0.789	0.619	1.000																																					
BG 8	0.850	0.789	1.000	0.800	0.842	0.667	0.750	1.000																																				
BG 9	0.850	0.789	0.800	0.800	0.750	0.667	0.750	0.800	1.000																																			
BG 10	0.895	0.650	0.750	0.667	0.789	0.700	0.889	0.750	0.842	1.000																																		
BG 11	0.667	0.684	0.619	0.619	0.571	0.650	0 571	0.619	0.700	0.650	1.000																																	
BG 12	0.700	0.722	0.650	0.650	0.684	0.778	0.524	0.650	0.650	0.600	0.550	1.000																																
BG 13	0.895	0.737	0.750	0.667	0.889	0.789	0.700	0.750	0.750	0.789	0.650	0.778	1.000																															
BG 14	0.762	0.789	0.714	0.800	0.667	0.667	0.591	0.714	0.800	0.667	0.789	0.650	0.667	1.000																														
BG 15	0.667	0.684	0.619	0.619	0.737	0.833	0.650	0.619	0.545	0.571	0.524	0.722	0.737	0.545	1.000																													
BG 16	0.619	0.632	0.500	0.650	0.600	0.600	0.524	0.500	0.571	0.600	0.722	0.667	0.684	0.737	0.550	1.000																												
BG 17	0.789	0.722	0.650	0.650	0.778	0.684	0.684	0.650	0.737	0.778	0.722	0.765	0.882	0.650	0.632	0.765	1.000																											
BG 18	0.750	0.778	0.700	0.700	0.737	0.941	0.650	0.700	0.619	0.650	0.600	0.722	0.737	0.619	0.882	0.550	0.632	1.000																										
BG 19	0.750	0.882	0.789	0.789	0.833	0.650	0.650	0.789	0.789	0.650	0.684	0.722	0.833	0.700	0.684	0.632	0.824	0.684	1.000																									
BG 20	0.714	0.833	0.750	0.750	0.700	0.619	0.619	0.750	0.750	0.619	0.833	0.600	0.700	0.842	0.571	0.684	0.684	0.650	0.833	1.000																								
BG 21	0.714	0.737	0.750	0.750	0.700	0.700	0.789	0.750	0.750	0.789	0.571	0.600	0.619	0.667	0.650	0.524	0.600	0.737	0.650	0.619	1.000																							
BG 22	0.750	0.882	0.789	0.789	0.737	0.650	0.737	0.789	0.789	0.737	0.778	0.632	0.737	0.700	0.600	0.632	0.824	0.684	0.882	0.833	0.737	1.000																						
BG 23	0.850	0.789	0.895	0.800	0.842	0.750	0.750	0.895	0.800	0.750	0.700	0.571	0.750	0.714	0.700	0.500	0.650	0.789	0.789	0.750	0.750	0.789	1.000																					
BG 24	0.762	0.789	0.800	0.714	0.750	0.750	0.750	0.800	0.714	0.750	0.700	0.650	0.750	0.636	0.700	0.571	0.737	0.789	0.789	0.750	0.842	0.889	0.800	1.000																				
BG 25	0.667	0.600	0.700	0.619	0.737	0.650	0.737	0.700	0.700	0.737	0.600	0.550	0.650	0.545	0.684	0.476	0.632	0.684	0.684	0.571	0.833	0.684	0.789	0.789	1.000																			
A 1	0.762	0.789	0.800	0.714	0.750	0.667	0.842	0.800	0.714	0.750	0.700	0.571	0.750	0.636	0.700	0.571	0.737	0.700	0.789	0.750	0.750	0.889	0.800	0.895	0.700	1.000																		
A 2	0.850	0.789	0.800	0.714	0.750	0.842	0.667	0.800	0.800	0.750	0.789	0.650	0.842	0.714	0.700	0.571	0.737	0.789	0.789	0.750	0.667	0.789	0.895	0.800	0.700	0.800	1.000																
A 3	0.750	0.882	0.789	0.789	0.737	0.737	0.737	0.789	0.700	0.737	0.684	0.632	0.737	0.700	0.684	0.632	0.722	0.778	0.778	0.737	0.833	0.882	0.789	0.889	0.684	0.889	0.789	1.000														
A 4	0.850	0.700	0.800	0.714	0.750	0.750	0.750	0.800	0.800	0.842	0.789	0.571	0.750	0.714	0.619	0.571	0.737	0.700	0.700	0.667	0.750	0.789	0.895	0.800	0.789	0.800	0.895	0.789	1.000													
A 5	0.950	0.800	0.900	0.810	0.850	0.762	0.762	0.900	0.900	0.850	0.714	0.667	0.850	0.810	0.636	0.591	0.500	0.714	0.800	0.762	0.762	0.800	0.900	0.810	0.714	0.810	0.900	0.800	0.900	1.000												
B 1	0.800	0.737	0.842	0.750	0.700	0.789	0.619	0.842	0.750	0.700	0.650	0.684	0.700	0.750	0.650	0.524	0.600	0.737	0.650	0.619	0.700	0.650	0.842	0.667	0.650	0.667	0.842	0.737	0.842	0.850	1.000											
B 2	0.857	0.800	0.900	0.810	0.850	0.682	0.850	0.900	0.810	0.762	0.636	0.591	0.762	0.727	0.714	0.522	0.667	0.714	0.800	0.762	0.762	0.800	0.900	0.810	0.714	0.900	0.810	0.800	0.810	0.905	0.762	1.000										
B 3	0.800	0.833	0.842	0.750	0.789	0.789	0.700	0.842	0.750	0.700	0.737	0.600	0.789	0.667	0.737	0.524	0.684	0.833	0.833	0.789	0.700	0.833	0.944	0.842	0.737	0.842	0.944	0.833	0.842	0.850	0.789	0.850	1.000									
C 1	0.857	0.800	0.810	0.810	0.762	0.682	0.762	0.810	0.900	0.762	0.714	0.667	0.762	0.810	0.636	0.591	0.750	0.636	0.800	0.762	0.682	0.800	0.810	0.727	0.636	0.810	0.810	0.714	0.810	0.905	0.762	0.905	0.762	1.000								
D 1	0.714	0.833	0.750	0.750	0.700	0.700	0.700	0.750	0.750	0.619	0.737	0.600	0.700	0.667	0.737	0.524	0.684	0.737	0.833	0.789	0.619	0.833	0.842	0.750	0.650	0.842	0.842	0.737	0.750	0.762	0.700	0.850	0.889	0.850	1.000							
D 2	0.810	0.750	0.850	0.762	0.895	0.636	0.800	0.850	0.762	0.714	0.591	0.619	0.800	0.682	0.750	0.545	0.700	0.667	0.842	0.714	0.714	0.750	0.850	0.762	0.750	0.850	0.762	0.750	0.762	0.857	0.714	0.950	0.800	0.857	0.800	1.000						
D 3	0.850	0.789	0.895	0.800	0.842	0.750	0.750	0.895	0.800	0.750	0.700	0.571	0.750	0.714	0.700	0.500	0.650	0.789	0.789	0.750	0.750	0.789	1.000	0.800	0.789	0.800	0.895	0.789	0.895	0.900	0.842	0.900	0.944	0.810	0.842	0.850	1.000					
E 1	0.762	0.700	0.800	0.714	0.750	0.667	0.842	0.800	0.714	0.750	0.700	0.500	0.667	0.636	0.700	0.500	0.650	0.700	0.700	0.667	0.750	0.789	0.895	0.800	0.789	0.895	0.800	0.789	0.895	0.810	0.750	0.900	0.842	0.810	0.842	0.850	0.895	1.000				
E 2	0.900	0.842	0.850	0.762	0.800	0.800	0.714	0.850	0.850	0.800	0.750	0.700	0.895	0.762	0.667	0.619	0.789	0.750	0.842	0.800	0.714	0.842	0.850	0.850	0.667	0.850	0.947	0.842	0.850	0.950	0.800	0.857	0.895	0.857	0.800	0.810	0.850	0.762	1.000			
F 1	0.619	0.722	0.650	0.737	0.600	0.600	0.778	0.650	0.737	0.684	0.632	0.500	0.524	0.650	0.632	0.500	0.579	0.632	0.632	0.600	0.778	0.722	0.737	0.650	0.722	0.737	0.650	0.722	0.737	0.667	0.684	0.750	0.684	0.750	0.778	0.700	0.737	0.833	0.619	1.000		
F 2	0.800	0.737	0.750	0.750	0.700	0.619	0.789	0.750	0.944	0.889	0.737	0.600	0.700	0.750	0.500	0.600	0.778	0.571	0.737	0.700	0.789	0.833	0.750	0.750	0.737	0.750	0.750	0.737	0.750	0.842	0.850	0.700	0.762	0.700	0.850	0.700	0.714	0.750	0.750	0.800	0.778	1.000		
F 3	0.750	0.684	0.789	0.700	0.650	0.737	0.650	0.789	0.789	0.737	0.600	0.632	0.650	0.700	0.600	0.476	0.550	0.684	0.600	0.571	0.737	0.600	0.789	0.619	0.684	0.619	0.789	0.684	0.789	0.800	0.941	0.714	0.737	0.714	0.650	0.667	0.789	0.700	0.750	0.722	0.737	1.000		
F 4	0.714	0.833	0.842	0.750	0.700	0.700	0.700	0.842	0.667	0.619	0.571	0.684	0.700	0.667	0.737	0.524	0.600	0.737	0.737	0.700	0.700	0.737	0.750	0.750	0.571	0.842	0.750	0.833	0.667	0.762	0.789	0.850	0.789	0.762	0.789	0.800	0.750	0.750	0.800	0.684	0.619	0.737	1.000		
G 1	0.850	0.700	0.800	0.714	0.842	0.750	0.667	0.800	0.800	0.750	0.700	0.650	0.842	0.714	0.700	0.571	0.737	0.700	0.789	0.667	0.667	0.700	0.895	0.714	0.789	0.714	0.895	0.700	0.895	0.900	0.842	0.810	0.842	0.810	0.750	0.850	0.895	0.800	0.850	0.650	0.750	0.789	0.667	1.000	
G 2	0.750	0.684	0.789	0.700	0.833	0.571	0.833	0.789	0.789	0.833	0.600	0.550	0.737	0.619	0.600	0.550	0.722	0.600	0.778	0.650	0.833	0.778	0.789	0.789	0.882	0.789	0.700	0.778	0.789	0.800	0.650	0.800	0.789	0.714	0.650	0.842	0.789	0.789	0.750	0.722	0.833	0.684	0.650	0.789	1.000	
G 3	0.714	0.737	0.667	0.667	0.700	0.700	0.619	0.667	0.750	0.619	0.737	0.684	0.789	0.667	0.737	0.600	0.778	0.650	0.833	0.700	0.545	0.737	0.750	0.667	0.650	0.750	0.842	0.650	0.750	0.762	0.700	0.762	0.789	0.850	0.889	0.800	0.750	0.750	0.800	0.684	0.700	0.650	0.700	0.842	0.650	1.000

Thus, the present study shows that esterase isozymes as molecular markers of cassava genotypes may provide valuable information for the characterization of "unnamed" cultivars of *M. esculenta*.

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