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Genetic Diversity of Cassava (*Manihot esculenta* Crantz) in Ecuador by Using SSR Markers

Nelly Paredes¹, Valeria Alulema², Luis Lima¹, Marten Sørensen^{3*}, and Álvaro Monteros-Altamirano⁴

¹ INIAP, Estación Experimental Central de la Amazonía, Vía Sacha San Carlos km 3 de la entrada a la Parker, Orellana, Ecuador; nelly.paredes@iniap.gob.ec (N.P.); luis.lima@iniap.gob.ec (L.L.)

² UCE (Universidad Central del Ecuador) - INBIOMED (Instituto de Investigación en Biomedicina), Hospital Docente de Calderón, Edificio CEGEMED, Quito, Ecuador; vealulema@uce.edu.ec / (V.A)

³ Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Sobi/Plen-KU, Thorvaldsensvej 40, 3., DK-1871 Frederiksberg C, Denmark

* Correspondence: ms@plen.ku.dk

⁴ INIAP, Estación Experimental Santa Catalina, Panamericana sur km 1, Quito, Ecuador; alvaro.monteros@iniap.gob.ec (Á.M.-A.)

Abstract: Cassava (*Manihot esculenta* Crantz), domesticated in the Amazonian region of South America, presents an important diversity in Ecuador, where it is a main staple food; however, only few Ecuadorian cassava accessions have been included in international molecular assessments. The purpose of this study was to apply suitable cassava microsatellites to characterize the genetic variability of the Ecuadorian cassava collection composed mainly of local landraces from the Coast, Andes and Amazonia regions. The use of microsatellite markers allowed the determination of the genetic diversity of the collection. Seven selected SSR primers, permitted to identify homozygous and heterozygous materials within the cassava collection of 133 accessions. The loci presented an average genetic diversity value of 0.7 and an average PIC value of 0.67, which is considered high. Low number of duplicates (8.8%) were identified in the Ecuadorian collection which is not fully duplicated at CIAT. Currently, a wide range of cassava diversity is still cultivated in multi-crop agro-ecosystem, mainly in the Coast and Amazonian regions. Especially in the Amazonian region, due to important cultural uses of cassava by local ethnic communities, more in depth studies in the region could unveil the genetic diversity present *in situ* today.

Keywords: SSR; variability; ethnobotany

1. Introduction

In the Euphorbiaceae family, the genus *Manihot* contains 110 species ranging in habit from herbs to small trees [1,2]. Within this genus, cassava (*Manihot esculenta* Crantz) is the sixth major crop globally [1,3]. In the XI century, cassava was taken from Brazil to the Caribbean and Central America. In the XVI century, Portuguese took cassava to the west coast of Africa in the XVIII century, in the XIX century to the African east coast and from there onwards to India; the Spanish took cassava to the Pacific [4]. Nowadays, cassava is an important energy source in the diet of millions of people in tropical and subtropical areas of America, Asia and Africa [5,6]. As cassava grows under marginal conditions, it presents a great potential to increase food security in developing countries [1,7]. This species can be exploited as a root food crop, vegetable, feed, or industrial uses such as starch, ethanol, or bioplastics [6–8].

Cassava was domesticated somewhere between 3000 to 12000 B.C. in the Amazonian region of South America [2,9,10]. Archaeological evidence showed domestication in the Amazonia (5000 to 7000 B.C.) and use of cassava in the Peruvian coast before 4,000 BC; and Colombia and Venezuela from 3,000 to 7,000 B.C. [4,11]. According to Nassar [12] and

Schaal et al. [1], there are two centers of diversity for the genus: one in Brazil and another in Mexico; however, domestication occurred from wild *M. esculenta* subsp. *flabellifolia* populations in the Brazilian Amazonia [1,13–17]. Cassava's primary gene pool (GP-1) comprises three subspecies, the cultivated *M. esculenta* subsp. *esculenta* and two wild progenitors *M. esculenta* subsp. *flabellifolia* and *M. esculenta* subsp. *peruviana* [17–19]. The closest wild relative is *M. pruinosa* [11]. It has also been determined that cassava is a functional diploid ($2n=2x=36$) but probably a segmental allotetraploid [2,7,20].

Genetic diversity of several national cassava collections has been studied by using different molecular techniques such as Isozymes for Costa Rica [21]; RAPDS for Ghana [22] and Venezuela [23]; SSR markers for Brazil [24–27] and Colombia [28]; SNP in Sierra Leone [29] and AFLP in Guyana [30]. In addition, evaluation of intercontinental collections has also been assessed, e.g. from America and several countries of Africa [31,32], America and Asia [33,34,35] and South America, Africa and Asia [36,37]. In spite of the important diversity of cassava present in Ecuador, only few Ecuadorian cassava accessions have been included in studies such as in Roa et al. [33,34]; Chavarriaga-Aguirre et al. [38], Kawuki et al. [31] and Raji et al. [36].

The purpose of this study was to apply suitable cassava microsatellites to characterize the genetic variability of the Ecuadorian cassava collection composed mainly of local landraces from the Coast, Andes and Amazonia regions, which have long been neglected in international studies.

2. Materials and Methods

Plant materials of Cassava

The cassava collection evaluated in this study is a subset of the landraces collected along with continental Ecuador, as explained in Monteros-Altamirano et al. [39]. This collection encompasses 136 accessions collected in the Coast, Andes and Amazonia regions of Ecuador. The Ecuadorian collection was planted at the Central Amazonian Experimental Station of INIAP, located at Via Sacha-San Carlos at 250 m a.s.l., with an average temperature of 24 °C and average precipitation of 3100 mm. Then, apical leaves samples, smaller than 1.5 cm in size, were taken for molecular characterization. Approximately 50 leaves were collected per accession in Ziploc bags with silica gel to avoid deterioration and allow the plant tissue to dry, allowing the sample to be preserved. The silica gel was changed every 24 hours for two days or until the leaf tissue was dehydrated.

Extraction and quantification of DNA

the sample in an Eppendorf tube and 1000µl of prewarmed extraction buffer (CTAB 2%, PVP 1%, NaCl sodium chloride 1.4M, Tris-HCL pH8 0.1M, EDTA Ethylenediamine Tetraacetate Acid Disodium Salt pH8 0) were added, 2M and 2µl of β-mercaptoethanol). The samples were incubated at 65°C in the water bath for one h with shaking every 30 minutes. Subsequently, they were centrifuged at 14000 rpm for 10 minutes; then the supernatant was recovered and 750µl of CIA (Chloroform-Isoamyl Alcohol in proportion 4:1). After shaking, it was centrifuged at 4000 rpm for 15 minutes. The supernatant was recovered in a new tube, and again 750 µl of CIA was added, vortexed and centrifuged at 10,000 rpm for 5 minutes. The supernatant was transferred to a new tube, 200 µl of ice-cold ethanol was added and incubated at -20 °C for 30 minutes. It was centrifuged at 5000 rpm for one minute, after which the extracted DNA was captured. The obtained DNA was washed with 70% ethanol, then the ethanol was removed, and the DNA was dried at temperature. The DNA pellet was re-suspended in 200 µl of T.E. buffer with 2 µl of RNase enzyme (10 µg / ml) for every 100 µl of DNA obtained. The samples were conserved until their quantification at -20°C. After DNA extraction, the concentration and quality of the DNA samples were determined. Samples were analyzed by using the BioTek Epoch™ microplate spectrophotometer. The Take3 microplate of the spectrophotometer consists of 16 wells, where 2µLblank (ultrapure water) were placed in the first two wells and 2µL

DNA in each of the following wells. The reading was performed using the equipment software, and the data obtained were imported into an Excel table. Subsequently, the DNA was diluted at a concentration of 5ng / μ L with ultrapure water and tartrazine and stored at -20 °C for later use.

Validation and amplification of DNA

The extracted DNA samples were validated to determine their amplification capacity using microsatellite primer (SSRY5). The cocktail of the PCR reaction was: Buffer PCR (5X), MgCl₂ (25mM), dNTP's (5mM), Primer (10uM) F, Primer (10uM) R, Taq polymerase (5U/uL), DNA (5ng/ μ L). After the amplification of the samples, 18 μ L of mineral oil was placed in each sample and amplified in the Basic Gradient thermocycler from Biometra. The amplification program included an initial cycle of denaturation at 94°C per 5 min, 30 cycles of cyclical denaturation at 94°C per 45 seconds, 1 minute of binding at 55°C, 2 min cyclic elongation at 72°C, and a final cycle of elongation at 72°C per 10 min, to finally stabilizing at 10 °C for 5 min [41]. Seven primers (Table 1) taken from those proposed by Chavarriaga-Aguirre et al. [42] and Mba et al. [43] were applied. Primers were previously tested to determine different combinations: duplex 1 (SSRY40 and SSRY 153), duplex 2 (SSRY68 and SSRY31), duplex 3 (SSRY3 and SSRY151) and monoplex (SSRY100).

Table 1. Microsatellite primers used in the molecular analysis of the cassava collection.

Locus	Motif	Size (pb)	Sequence	MT (°C)
SSRY3	(CA)17	250	5' TTAGCCAGGCCACTGTTCTT 3' 3' GCGAGGTTCAAATATGCGAT 5'	55
SSRY31	(GA)21	190	5'CTTCATCACGTGTTAATACCAATC 3' 3' ATTGTTGTGGTTGCAGGACA 5'	55
SSRY40	(GA)16	231	5' TGCATCATGGTCCACTCACT 3' 3' CATTCTTTTCGGCATTCCAT 5'	55
SSRY151	(GA)126	182	5' AGTGGAATAAGCCATGTGATG 3' 3' CCCATAATTGATGCCAGGTT 5'	45
SSRY68	(CT)12 (CCCT)17	290	5' GCTGCAGAATTTGAAAGATGG 3' 3' CAGCTGGAGGACCAAAAATG 5' 3' CAGCTGGAGGACCAAAAATG 5'	55

Subsequently, to visualize the amplified DNA bands, 2 μ L of the amplified DNA samples previously mixed with the loading buffer (blue juice) were loaded into the wells of a 2% agarose gel with a 100 bp molecular DNA Ladder (10488-058 INVITROGEN). The samples were run in a horizontal electrophoresis chamber at 110v for 30 minutes. The gels were visualized in the Dolphin View Wealtec photo-reducer.

Genotyping of cassava DNA samples

In the SAGA-GT Microsatellite software, a project was created for cassava, detailing the information of each primer such as size, the channel in which they amplify (700–800 nm), the range of band size, the duplexes formed and the position of the cassava samples was located in advance in each position of the gel. First, the acrylamide gel was prepared with 20 ml of K.B. Gel Matrix Plus 6.5%, 150 µl of APS (Ammonium Persulfate) at 10% and 15 µl of Temmed (Tetramethylethylene-diamine at 99%), the mixture was placed between the glass plates of the LI-COR 4300 and the comb was placed, after 1 hour of polymerization of the gel, it was placed in the LI-COR 4300 with the 1X TBE (Tris-Borate-EDTA) K.B. Plus LI-COR buffer. Subsequently, a pre-run of 25 min at 1500 V was performed to focus the laser at 700 and 800 nm. Then, 0.8 µL of the amplified products were loaded, previously diluted with Blue Stop Solution LI-COR in a 1:1 ratio and denaturated at 94 ° C for 5 min, and the run was started at 1500 V for 1 hour and a half. The molecular weight marker IRDye 30-350 bp were used.

Statistical analysis

The molecular characterization data were obtained using the SAGA GT-SSR version 3.3 software, a reading assistant for the images provided by the LI-COR. The data matrix obtained from SAGA was imported into Excel, where it was purged and saved to continue with the analysis.

Analysis of genetic diversity

The Power Marker V3.0 program was used for the genetic diversity analysis [44]. The following parameters were obtained: Number of observations, sample size, allele frequency, number of generated genotypes, and number of alleles per locus, total or observed heterozygosity (Ho), genetic diversity or expected heterozygosity (He) and Polymorphism Information Content (PIC).

Cluster Analysis

For Cluster Analysis, binary data matrix (1 and 0) was elaborated and analyzed in the Power Marker V3.0 software; a UPGMA tree representation was made to represent the individual relationships between accessions.

Identification of duplicates

The identification of duplicates was carried out through multilocus microsatellites genotypes and using similarity percentages obtained in the Excel Microsatellites add-on software, which has a value of 100% for similar individuals.

Variance analysis

To determine the number of groups with the most significant variability between them, a molecular analysis of variance tested from two to ten groups was used with the Arlequin 2.0 program.

3. Results

Genotyping of SSR markers in LI-CORD

Homozygous and heterozygous individuals were identified within the cassava collection of 133 accessions. The primer SSRY40 allowed to identify 51 homozygous and 77 heterozygous individuals; with SSRY153 40 homozygous and 93 heterozygous individuals; with SSRY3 72 homozygous and 57 heterozygous individuals; with SSRY151, 19 homozygous and 144 heterozygous; with SSRY68, 64 homozygous and 63 heterozygous; with SSRY31 36 homozygous and 90 heterozygous and with the SSRY100, 69 homozygous and 58 heterozygous individuals were identified.

Table 2. Genetic diversity analysis of 7 cassava SSR markers

Locus	Allele frequency	Observations <i>n</i>	Alleles <i>n</i>	Genetic Diversity	Ho	PIC
SSRY40	0.64	128	6	0.55	0.6	0.52
SSRY153	0.53	133	6	0.65	0.7	0.61
SSRY3	0.63	129	6	0.56	0.44	0.52
SSRY151	0.21	133	10	0.87	0.86	0.85
SSRY68	0.4	127	10	0.78	0.5	0.75
SSRY31	0.43	126	8	0.72	0.71	0.69
SSRY100	0.42	127	10	0.76	0.46	0.74
Average	0.47	129	8	0.7	0.61	0.67

Table 2 shows the SSRY151 locus, which presented the highest genetic diversity (0.87) for the other loci. The loci presented an average genetic diversity value of 0.7 for the seven primers. It is important to note that the primers presented values greater than 0.5 for the PIC. This parameter is essential to determine the index of information provided by these markers within the population. The SSRY151 locus was the most polymorphic with a PIC of 0.85, while the SSRY40 and SSRY3 loci were the least polymorphic with a PIC of 0.52. In addition, the SSRY3 locus presented the lowest heterozygosity observed with a value of 0.44, while on average, for the loci, there was a Ho of 0.61. Average PIC value was of 0.67.

Allelic frequency

The allelic frequency for each primer was determined to analyze the variability of the loci in detail. Allele frequencies and sizes at each locus are detailed in Appendix 1.

Multivariate analysis: Clusters description

The cluster analysis grouped accessions according to their genotype, allowing a better analysis of the population's genetic diversity. This analysis was performed using the UPGMA method on the main coordinates of the individuals. The dendrogram shown in Figure 1 represents the genetic relationships of the germplasm, where two main groups were identified (G1 and G2).

Figure 1. Dendrogram of 133 accessions of *Manihot esculenta*, indicating two groups based on SSR genotyping. UPGMA and Simple Matching similarity coefficient was used.

Group 1

This group is formed by subgroup A and contain mostly accessions from Manabí, few accessions from Santo Domingo de Los Tsáchilas, and a minimum number of accessions from Esmeraldas, all these provinces are from the Coast of Ecuador (Appendix 3).

Group 2.

This group has subgroups B and C and contains all the accessions collected in the Amazonian region, few accessions from Manabí, Santo Domingo de Los Tsáchilas, one accession from Esmeraldas. Subgroup B is made up of accessions from the north-central part of the Amazonian region (Pastaza, Napo, Sucumbíos and Francisco de Orellana) and from the coastal region the accessions of Manabí and Santo Domingo de Los Tsáchilas. In contrast, subgroup C comprises all the accessions collected in Morona Santiago and Zamora Chinchipe; it also contains accessions from Pastaza, Sucumbíos, Napo and Francisco de Orellana, all provinces from the Amazonia. In this group, there are also accessions from the coastal region (Esmeraldas, Manabí, Santo Domingo de los Tsáchilas) and accessions from Tungurahua. The latter province is known as an Andean province; however, the region of the Tungurahua province named “Baños”, located in the foothills of the Andes, is known as the door of the Ecuadorian Amazonia toward the province of Pastaza (Appendix 3).

Analysis of variance

The Molecular Analysis of Variance (AMOVA) was carried out, grouping the accessions by regions of Ecuador (Coast, Sierra and Amazonia).

The analysis of this grouping is detailed in Table 3.

Table 3. Molecular analysis of variance of 133 accessions from the Ecuadorian cassava collection.

Source	df	SS	MS	Est. Var.	%
Among Populations (Regions)	2	10.605	5.302	0.049	2%
Within Populations	263	665.684	2.531	2.531	98%
Total	265	676.289		2.580	100%

Table 3 shows a low percentage of variation among populations or regions (2%), but most of the variation (98%) is due to differentiation among individuals.

Nei Genetic Distance

The genetic distance of Nei [45] was determined as another parameter to analyze the population genetic structure. For details of the groups' distance, see *Appendix 2*, where a Nei distance of 0.401 was observed between the G1 and G2 groups.

Appendix 2 shows the genetic distances among the provinces, the greater differentiation are between the provinces of Esmeraldas (Coast) and Tungurahua (Sierra) with a genetic distance of 0.525; Esmeraldas and Morona Santiago (Amazonia) with a genetic distance of 0.518; Santo Domingo de los Tsáchilas (Coast) and Morona Santiago (Amazonia) 0.473; Esmeraldas and Francisco de Orellana (Amazonia) 0.443; Santo Domingo de Tsáchilas and Fco de Orellana 0.414. This data indicates clear differentiation between accessions from the Coast and Sierra and between Amazonia and the Coast of Ecuador.

Identification of duplicates

The accessions that meet 100% genetic similarity are three pairs of duplicates (ECU18505, ECU18524); (ECU19070, ECU19071); (ECU19107, ECU19135) and two triplicates (ECU19134, ECU19148, ECU19149); (ECU19115, ECU19125, ECU19118). The duplicates found in the Ecuadorian collection correspond only to 8.8% of the analyzed accessions.

4. Discussion

Genetic diversity of the collection

The use of microsatellite markers allowed the determination of the genetic diversity of the collection. Furthermore, these markers generated a critical amount of genetic information due to their ability to cover the entire genome, in addition to not presenting intergenic interactions and having simple inheritance [23,46–48].

Cassava is considered a diploid species, although it is sometimes considered polyploid and possibly an allopolyploid (tetra or hexaploid) [49]. However, only homozygous and heterozygous diploid individuals were found in this study, similarly to Pincay [50]. Our results are consistent with Domínguez et al. [51]; De Carvalho et al. [20]; Ceballos et al. [3]; Ceballos et al. [7] who indicate that cassava is a diploid species.

In the 133 national cassava accessions, 56 alleles were identified using seven primers. The SSRY151, SSRY68, and SSRY100 loci presented the highest polymorphism index. However, the seven primers presented between 6 and 11 alleles with an average of 8 alleles/loci. Casalla [52], mentioned that a marker is highly polymorphic when identifying more than two alleles per locus. Primer SSRY100 presented the highest number of alleles, similar to Arguello [53], indicating that this primer can be used in future characterizations due to its great discriminating power. Other studies with a similar number of alleles per locus are Beovides et al. [54] from 2 to 10; Arguello [53] range from 3 to 9 or Pincay [50] from 8 to 13.

The observed heterozygosity (H_o) presented an average value of 0.61 with values that vary between 0.5 and 0.86, similar to those found by Pincay [50]. These high values confirm that cassava is heterozygous in nature, as in Arguello [53], who also mentions that the asexual reproduction mode of cassava influences the levels of inherited heterozygosity. On the other hand, according to Morillo et al. [55], this high heterozygosity may be related to the allogamous nature of the species. Furthermore, the heterozygosity of cassava was confirmed by other studies such as Alzate et al. [28]. Sosa et al. [56] stated that heterozygosity is one of the critical diversity quantification indices.

Representativeness of the Ecuadorian collection of cassava

High genetic diversity and a low number of duplicates (8,8%) were identified in the Ecuadorian collection. The 100% duplicates are based mainly on collection-sites closeness, e.g. identical ECU19070 and ECU19071 accessions were collected in Tungurahua in the same place called Río Verde; ECU18505 and ECU18524 were collected in close parishes of the neighboring Coastal provinces of Manabí and Esmeraldas. The triplicate (ECU19134, ECU19148, ECU19149) were collected all in the same province of Pastaza in the Amazonia; and (ECU19115, ECU19125, ECU19118) were collected in the Amazonian province of Sucumbíos. Only the identical ECU19107 and ECU19135 were collected in distant parishes, although from two bordering provinces of Napo and Pastaza in the Amazonia. It is essential to mention that our collection is not wholly duplicated at CIAT, as stated by Tay [4], who indicates that 116 Ecuadorian accessions are conserved at CIAT (CGIAR); however, 134 accessions are considered as only *in situ* (missing from CGIARs) and not duplicated elsewhere. Cross passport reference between CIAT and INIAP's collections by region determined that CIAT holds 74% of the Ecuadorian Coastal accessions and only 25% of our Amazonian accessions; however, INIAP holds only 14% of the accessions collected in the Sierra from CIAT. Therefore, this Ecuadorian collection filled the gap of missing accessions, especially from the Amazonian region of Ecuador.

Cassava in Ecuadorian farmers' fields

In the Coastal region, Manabí province is a traditional commercial production area. Mainly smallholders intercrop cassava and other cash crops such as coffee, peanut and maize, among others [57,58]. In communities closer to urban centers, cassava is essentially a cash crop, but in more isolated communities, the crop is used mainly for family consumption and animal feed [58]. Industrial cassava byproducts have been produced and exported from Manabí [59], e.g. family farmers in this province have extracted cassava starch for over a hundred years [59,60]. Producer-processor associations occurred years ago [60] and continue today. Our SSR data indicate that accessions from the Manabí province are grouped except for a few materials (Figure 1, Appendix 3). This may be because this province has been supported by several international projects collaborating with INIAP and CIAT [60] which probably increased cassava genetic resources to help the commercial production in the area. Additionally, the genetic distance of Nei (Appendix 2) indicates that there is a difference with other materials from the Coast: Esmeraldas (0.350)

and Santo Domingo de Los Tsáchilas (0.297), which are provinces that have not had commercial exploitation of yuca like Manabí and consequently less gene flow.

Unlike the Coastal region, where cassava mestizo producers are more inclined to commerce, the cropping shift to self-consumption added to the high cultural importance within indigenous communities in the Amazonia region. Although our collection did not show high genetic diversity within the Amazonian region (Appendix 2), we believed several local landraces are still underrepresented. Currently, they are in the hands of local farmers, especially in indigenous communities of the Amazonian region, e.g. at least five landraces of cassava (*lumu*) have been reported growing together in one *chakra* or *chagra* (swidden garden) at Kichwa communities of Napo [61]. In the *chakra* Kichwa, similar to other indigenous communities, cassava grows along with other species such as maize, rice, plantain, and beans [62] up to 25 species [63]. Between four and 13 varieties of cassava (*kene*) are managed by one Waorani family in the *chagra de yuca* or *kewenkore* [64]. The Jívaroan indigenous group in Pastaza province, intercrop cassava that occupies most of the cropland of the households [65] and in the same province where Quichua, Shiwiari, and Zapatero indigenous people live reported up to 16 varieties of cassava per household [63]. In this region, cassava presents cultural importance, e.g. Cassava-cropping knowledge is transmitted among women's generations of Kichwas through the delivery of good seeds and practices in the *chakra*, accompanied by advice [66]. Kichwa mainly uses cassava for self-consumption as food or *chicha* (fermented cassava beverage); surplus might be directed to the market, especially by mestizos [67]. Then, *in situ* studies must be conducted to understand the genetic diversity among Amazonian indigenous communities and strengthen the conservation and traditional use of local cassava.

Supplementary Materials: N/A

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Appendix 1. Alleles found with their frequencies and sizes in 133 accessions from the cassava collection.

Locus	Al- lele	Frecuency G1	Frecuency G2	Locus	Al- lele	Frecuency G1	Frecuency G2
SSRY40	220	0.021	0.000	SSRY68	248	0.063	0.110
	224	0.688	0.583		250	0.042	0.440
	228	0.000	0.161		254	0.000	0.110
	230	0.083	0.078		256	0.000	0.041
	234	0.000	0.018		258	0.063	0.014
	236	0.125	0.106		260	0.000	0.018
SSRY153	96	0.500	0.518		262	0.021	0.005
	102	0.167	0.018		264	0.104	0.055
	104	0.083	0.005		266	0.458	0.096
	106	0.000	0.087		288	0.167	0.046
	114	0.063	0.188		160	0.500	0.381
	116	0.104	0.174		162	0.000	0.009
SSRY3	236	0.438	0.014	SSRY31	166	0.042	0.037
	240	0.229	0.032		172	0.000	0.014
	242	0.146	0.697		176	0	0.055
	244	0.042	0.000		180	0.125	0.037
	248	0.021	0.005		188	0.021	0.183
	250	0.000	0.216		192	0.313	0.193
SSRY151	174	0.000	0.009	SSRY100	192	0.042	0.041
	178	0.000	0.028		196	0.021	0.018
	180	0.000	0.156		200	0.000	0.188
	182	0.042	0.138		206	0.000	0.037
	184	0.313	0.041		210	0.063	0.037
	186	0.021	0.073		212	0.646	0.339
	188	0.021	0.092		214	0.083	0.156
	192	0.396	0.023		220	0.00	0.014
	210	0.083	0.234		240	0.042	0.060
	216	0.08333	0.188		242	0.021	0.046

Note: alleles with less frequency are indicated in blue and alleles with greater frequency in red.

Appendix 2. Nei genetic distance matrix between 10 provinces (Coast, Sierra and Amazonia) and 2 countries of origin of the cassava collection accessions.

	COAST			AMAZONIA						SIERRA
	ESM	MAN	SD	SUC	FO	NA	PA	MS	ZC	TU
ESM	0.000									
MAN	0.350	0.000								
SD	0.342	0.297	0.000							
SUC	0.314	0.083	0.222	0.000						
FO	0.443	0.119	0.414	0.132	0.000					
NA	0.379	0.123	0.362	0.101	0.168	0.000				
PA	0.333	0.103	0.295	0.116	0.143	0.114	0.000			
MS	0.518	0.213	0.473	0.192	0.265	0.166	0.161	0.000		
ZC	0.415	0.183	0.320	0.157	0.251	0.159	0.184	0.311	0.000	
TU	0.525	0.175	0.384	0.126	0.245	0.151	0.221	0.241	0.165	0.000

ESM: Esmeraldas, MAN: Manabí, SD: Santo Domingo de los Tsáchilas, SUC: Sucumbíos, FO: Francisco de Orellana, NA: Napo, PA: Pastaza, MS: Morona Santiago, ZC: Zamora Chinchipe, TU: Tungurahua.

Appendix 3. Grouping of 133 Ecuadorian accessions of cassava by using SSR markers.

GROUP 1		GROUP 2							
ACC	PROVINCE	ACC	PROVINCE	ACC	PROVINCE	ACC	PROVINCE	ACC	PROVINCE
ECU-18423	Manabí	ECU-17603	Pastaza	ECU-18554	Manabí	ECU-19117	Sucumbíos	ECU-19150	Francisco de Orellana
ECU-18497	Manabí	ECU-17604	Pastaza	ECU-18559	Manabí	ECU-19118	Sucumbíos	ECU-19151	Francisco de Orellana
ECU-18505	Manabí	ECU-17605	Pastaza	ECU-18560	Manabí	ECU-19119	Sucumbíos	ECU-19152	Francisco de Orellana
ECU-18506	Manabí	ECU-17606	Pastaza	ECU-18562	Manabí	ECU-19120	Sucumbíos	ECU-19153	Francisco de Orellana

ECU-18511	Santo Domingo de los Tsáchilas	ECU-17607	Pastaza	ECU-19070	Tunguragua	ECU-19121	Sucumbíos	ECU-19154	Francisco de Orellana
ECU-18513	Santo Domingo de los Tsáchilas	ECU-17608	Sucumbíos	ECU-19071	Tunguragua	ECU-19122	Sucumbíos	ECU-19155	Francisco de Orellana
ECU-18516	Santo Domingo de los Tsáchilas	ECU-17611	Sucumbíos	ECU-19089	Napo	ECU-19123	Sucumbíos	ECU-19156	Francisco de Orellana
ECU-18523	Santo Domingo de los Tsáchilas	ECU-17612	Zamora Chinchipe	ECU-19090	Napo	ECU-19124	Sucumbíos	ECU-19157	Francisco de Orellana
ECU-18524	Esmeraldas	ECU-17614	Zamora Chinchipe	ECU-19091	Napo	ECU-19125	Sucumbíos	ECU-19158	Francisco de Orellana
ECU-18536	Santo Domingo de los Tsáchilas	ECU-17615	Zamora Chinchipe	ECU-19092	Napo	ECU-19126	Sucumbíos	ECU-19159	Francisco de Orellana
ECU-18539	Esmeraldas	ECU-17617	Zamora Chinchipe	ECU-19093	Napo	ECU-19127	Sucumbíos	ECU-19160	Francisco de Orellana
ECU-18540	Manabí	ECU-17618	Zamora Chinchipe	ECU-19094	Napo	ECU-19128	Sucumbíos	ECU-19161	Francisco de Orellana
ECU-18541	Manabí	ECU-17619	Zamora Chinchipe	ECU-19095	Napo	ECU-19129	Sucumbíos	ECU-19162	Francisco de Orellana
ECU-18543	Manabí	ECU-17620	Zamora Chinchipe	ECU-19096	Napo	ECU-19130	Sucumbíos		
ECU-18545	Manabí	ECU-17621	Morona Santiago	ECU-19097	Napo	ECU-19131	Sucumbíos		
ECU-18546	Manabí	ECU-17622	Morona Santiago	ECU-19098	Napo	ECU-19133	Sucumbíos		
ECU-18547	Manabí	ECU-17623	Morona Santiago	ECU-19100	Napo	ECU-19134	Pastaza		
ECU-18548	Manabí	ECU-17624	Morona Santiago	ECU-19101	Napo	ECU-19135	Pastaza		
ECU-18555	Manabí	ECU-17625	Morona Santiago	ECU-19102	Napo	ECU-19136	Pastaza		
ECU-18556	Manabí	ECU-17627	Morona Santiago	ECU-19103	Napo	ECU-19137	Pastaza		
ECU-18561	Manabí	ECU-17628	Morona Santiago	ECU-19104	Napo	ECU-19138	Pastaza		
ECU-18564	Manabí	ECU-17640	Esmeraldas	ECU-19105	Napo	ECU-19139	Pastaza		
ECU-18566	Manabí	ECU-17730	Zamora Chinchipe	ECU-19106	Napo	ECU-19140	Pastaza		
ECU-18567	Manabí	ECU-18407	Manabí	ECU-19107	Napo	ECU-19141	Pastaza		
		ECU-18495	Manabí	ECU-19108	Napo	ECU-19142	Pastaza		
		ECU-18510	Santo Domingo de los Tsáchilas	ECU-19109	Francisco de Orellana	ECU-19143	Pastaza		
		ECU-18517	Santo Domingo de los Tsáchilas	ECU-19110	Francisco de Orellana	ECU-19144	Pastaza		
		ECU-18522	Santo Domingo de los Tsáchilas	ECU-19111	Francisco de Orellana	ECU-19145	Pastaza		
		ECU-18529	Manabí	ECU-19113	Francisco de Orellana	ECU-19146	Pastaza		
		ECU-18533	Santo Domingo de los Tsáchilas	ECU-19114	Sucumbíos	ECU-19147	Pastaza		
		ECU-18535	Santo Domingo de los Tsáchilas	ECU-19115	Sucumbíos	ECU-19148	Pastaza		
		ECU-18542	Manabí	ECU-19116	Sucumbíos	ECU-19149	Pastaza		