



## GENETIC DIVERSITY OF CASSAVA (*Manihot esculenta* Crantz), (EUPHORBIACEAE) GROWN IN THREE AGROCLIMATIC ZONES OF CENTRAL AFRICAN REPUBLIC

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### ABSTRACT

Cassava (*Manihot esculenta* Krantz) introduced in the Central African Republic (CAR) in the 1850s, is now the staple food of the population. It does not know the genetic diversity of cultivated accessions in peasant communities. To assess this diversity, microsatellite technique was used on the 179 accessions identified. 137 alleles were amplified with an average of 5.95 alleles at the 23 loci. Analysis of genetic diversity within varieties across five villages showed that 46 accessions of 49 have a genotypic homogeneity is 93.87%. Only accessions "Tokonenanga" (Ndanga) "Touguenlag" (Soungbe) and "Sereka" (Karama) are each represented by two different genotypes.

**Keywords:** *Manihot esculenta*; genetic diversity; CAR.

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## INTRODUCTION

Introduced into Africa by Portuguese sailors in the 16th, the first cuttings arrived in the Central African Republic (CAR) in the 1850s by following Tisserant. Views are important in the diet of the population, cassava is cultivated today in all agricultural regions of the country. The tuber yield among producers declined for a number of years, the impact was noticed by the rising price of chips on the market. This low production was mainly attributed to the African cassava mosaic. To overcome this problem, improved IITA accessions were introduced in 1995 distributed on-farm to increase performance.

Of these processes for the introduction of cassava in Central Africa, we have no knowledge of the genetic diversity even if the maintenance ecotypes is provided by peasant producers themselves through their traditional practices based on morphological traits, anecdotes and other. No study to date has been done on the diversity of cassava grown in the CAR, we do not know the real diversity after various processes of introduction of plant material in the country. If you want to choose a policy of crop improvement and conservation strategy for populations of cassava-farm, the evaluation of the genetic diversity of available resources would be a valuable asset. It can lead to the discovery of new ecotypes through traditional farming practices and identifying new combinations with maximum genetic variability, which can then be used for further breeding and introgression of desirable genes in diverse germplasm genetic base available [1,2].

Varietal selection peasant CAR leads introductions and withdrawals continual accessions, some authors for their part have shown in similar research [3, 4, 5]. Introductions and drop accessions contribute to the structuring of varietal diversity and adaptation of crops in relation to the physical environment and socio-economic development. They can also lead to an increase or decrease of genetic diversity in CAR. Fukuda and his team [6] et al. (1996) showed that genetic erosion is mainly due to the expansion of the agricultural frontier, the advancement of urban space, the magnitude of biotic, a biotic and finally replacing traditional accessions from accessions improved. The management of diversity of cassava RCA has not escaped this process. For this, knowledge of the extent, structure and conservation of germplasm of cassava is therefore essential to reduce genetic erosion and help improvement programs. Easy identification and selection of genotypes is then an essential step [7] for any improvement program.

In addition, accessions farmers, the basis of ex situ collections are recognized as reservoirs of useful genes for crop improvement [8]. Around the world, breeding programs generally aim primarily at increasing yields, development of resistance to disease and drought, as well as improving the nutritional properties of edible organs. There is however a lack of knowledge of the genetic diversity among several accessions available for the improvement of the crop.

For this study, the technique of microsatellites is retained. The SSR microsatellite repeats are a very effective way to assess the genetic diversity because they can easily be adapted for classification and identification of many organisms and are particularly useful in study of the variation in allele frequency of unlinked loci. Using this technique, several studies have shown a high level of heterozygosis in various populations of cassava [9, 10, 11]. The purpose of this study was to evaluate the genetic variability among accessions introduced traditional and improved cassava grown in the Central African Republic using microsatellite markers. We could get an idea of the extent and structure. This study took into account the study of diversity within and between varieties.

## MATERIALS AND METHODS

### Study area and collection of materials

The Central African Republic is a landlocked country located in the heart of Africa (Figure 1). Its economy is mainly based on agriculture. It employs nearly 68% of the workforce and accounts for 56% of Gross Domestic Product (GDP) between 2005-2006. Areas of arable land in the country represent a potential of 15 million hectares, less than 2 million are cultured

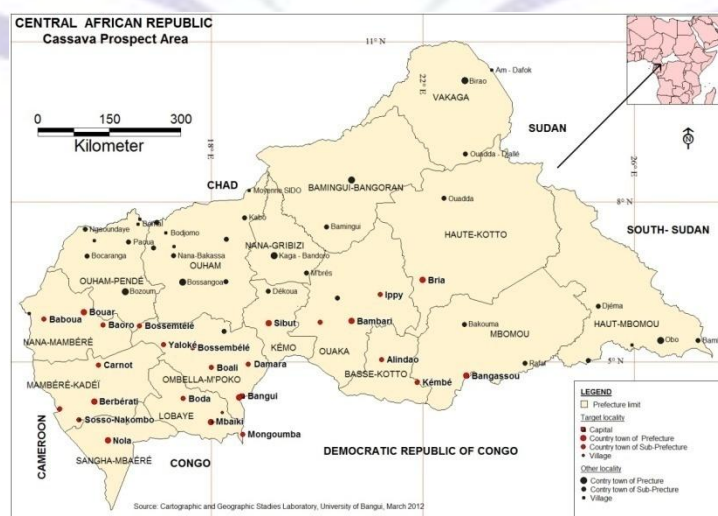


Fig 1: The study sites



The first fact-finding mission targeted roads serving the country. The technique is to order every time you see a field along these roads. Total of 130 accessions were reported and the leaves were collected. The second sample is however achieved in five villages were selected according to their ability in cassava production and after three agroclimatic zones selected for this study. 49 accessions were identified and three leaves were sampled accessions for a total of 147 samples for molecular analysis for the study of genetic diversity within varieties. Total of 179 accessions were reported for the study of genetic diversity.

### Selection of Molecular Markers

The choice of markers is focused on some work that has developed microsatellite markers specific to cassava [9, 12] For complete bibliographic data, other studies have led to a selection of markers that have been used in the study of the genetic diversity of cassava [10, 13, 14, 15] . The research is directed towards those who are able to reveal a larger number of alleles. Types of repeats are also part of the selection criteria taking simple patterns as targets (Table 1).

**Table 1 : List of selected markers**

Amorces	Real size	Minimal size	Maximal size	alleles number	Motifs
SSRY4	307	288	308	5	(GA) <sub>16</sub> TA(GA) <sub>3</sub>
SSR8	308	300	318	4	(CA) <sub>14</sub> CT(CA) <sub>2</sub>
SSRY9	298	276	294	5	(GT) <sub>15</sub>
SSRY12	286	274	288	4	(CA) <sub>19</sub>
SSRY19	234	216	234	6	(CT) <sub>8</sub> (CA) <sub>18</sub>
SSRY20	163	154	180	6	(GT) <sub>14</sub>
SSRY25	316	280	310	5	(GA) <sub>27</sub>
SSRY26	141	140	164	5	(GA) <sub>18</sub>
SSRY52	286	264	286	4	(GT) <sub>19</sub>
SSRY63	310	302	312	3	(GA) <sub>16</sub>
SSRY64	214	232	260	4	(CT) <sub>13</sub> CG(CT) <sub>6</sub>
SSRY68	307	268	308	6	(CT) <sub>12</sub> CC(CT) <sub>17</sub>
SSRY82	231	200	228	6	(GA) <sub>24</sub>
SSRY100	230	210	260	5	(CT) <sub>17</sub> TT(CT) <sub>7</sub>
SSRY101	233	230	257	5	(GCT) <sub>13</sub>
SSRY103	292	292	304	5	(GA) <sub>22</sub>
SSRY105	265	240	266	4	(GT) <sub>6</sub> GC(GT) <sub>2</sub> (GA) <sub>16</sub>
SSRY110	267	272	286	5	(GT) <sub>12</sub>
SSRY135	273	260	276	4	(CT) <sub>16</sub>
SSRY164	207	160	204	4	(GA) <sub>29</sub>
SSRY169	120	110	122	5	(GA) <sub>19</sub> AAA(GAA) <sub>2</sub>
SSRY175	156	104	154	8	(GA) <sub>38</sub>
SSRY179	246	204	246	6	(GA) <sub>28</sub>
GA126	197-243	202	236	6	
GA127a	234-259	234	256	4	



## DNA extraction

DNA was extracted from 0.15 g of sample. The grinding of the samples was performed in a mortar filled with liquid nitrogen until a powder. The homogenate was transferred to a 15 mL tube containing 5 mL of extraction buffer prior to incubation at 74 ° C. Then, the 15 mL tube containing the crushed cassava leaves is incubated for 20 minutes. After cooling to room temperature, 5 mL of CIAA are added into the tube and mixed by inversion of the tube several. The tube was centrifuged at 6000 g for 15 minutes at room temperature. The supernatant was transferred to a 13 mL tube to which 5 mL of isopropanol. After mixing by centrifugation, one can visualize a ball cap of the DNA formed and transferred to a microtube containing 400 ul of ultrapure water. After recovery of the DNA extract was stored at -20 ° C.

## PCR amplification and Migration on the Li horn

The PCR amplifications were performed in a Mastercycler Gradient, Mastercycler or Eppendorf , 25 ng of DNA in a final volume of 10µl of buffer (10 mM Tris-HCl (pH 8), 100 mM KCl, 0.05% w / v gelatin and 2.0 mM MgCl<sub>2</sub>) containing 0.1 mM of the primer extended by a tail universal M13, 0.1 mM of the other primer, 160 mM of dNTPs, 1 U of Taq DNA polymerase (Life Technologies, USA), and 0.1 mM of M13 tail marked with a fluorescent dye IR700 IR800 or (MWG, Germany). The PCR program used was as follows: initial denaturation at 95 ° C for 1 min, 10 cycles of touchdown (94 ° C for 30 s, T<sub>m</sub> (+5 ° C, -0.5 ° C / cycle) for 1 min, 72 ° C for 1 min), 25 additional cycles of amplification: 94 ° C for 30 sec, 1 min and T<sub>m</sub> 72 ° C for 1 min, and a final elongation step at 72 ° C for 8 min. PCR products were diluted 10 times and then subjected to gel electrophoresis in 6.5% polyacrylamide system analyzer fragments 4300 Li-Cor (LI-COR, USA). Allele sizes were determined using the analysis software SAGA-GT Version 3.2 (LI-COR, USA).

## Statistical Analysis

Dissimilarities between pairs of individuals are calculated with the Darwin software version 5.0 [16] by Simple Matching index according to:

$$d_{ij} = 1 - \frac{1}{L} \sum_{l=1}^L \frac{m_l}{\pi}$$

where  $d_{ij}$  is the dissimilarity between  $i$  and  $j$ , the number of loci  $L$ ,  $\pi$  ploidy and the number of common alleles between  $i$  and  $j$  for locus  $l$ . The dissimilarity matrix obtained is shown as a dendrogram constructed by the neighbor-joining method [17]. This representation was used to test the identity of three samples for each accession. In case dissimilarity intravarietal was observed, the samples were compared by locus locus. Eliminated the redundant to repeat dissimilarity analysis which helped build a dendrogram genotypes CAR. The calculation of genetic parameters is done on all samples CAR. Diversity is described using the software GENETIX 4.02 [18] by calculating the frequency of alleles, observed and expected heterozygosities the (respectively), fixation indices  $F_{st}$  and  $F_{is}$  heterogeneity that characterizes the frequency allelic between subdivisions of a population based on Wright, under the null hypothesis of Hardy-Weinberg.

## RESULTS

### Heterozygosis and fixation index CAR

Total of 137 alleles were amplified using 23 microsatellite markers. The number of alleles per locus obtained in this study (Table 2) is between 4 (SSRY 105) and 9 (SSRY20) for an average of 5.95. Heterozygosity value is between 0.4149 (SSRY8) and 0.8958 (SSRY175 and SSRY179) with a mean heterozygosity of 0.6914 (mutilocus). This value is greater than the expected heterozygosity (0.6666).  $F_{is}$  values are between -0.02821 (SSRY8) and 0.6370 (SSRY9) for an average of -0.03198. The negative value of  $F_{is}$  confirms heterozygosity excess (Table II). The value of  $F_{is}$  negative (-0.03198) -0.02821 varies (SSRY8) to 0.41035 (SSRY4). Cassava CAR has an excess of heterozygous individuals.

**Table 2: heterozygosity and fixation index CAR (96 genotypes).**

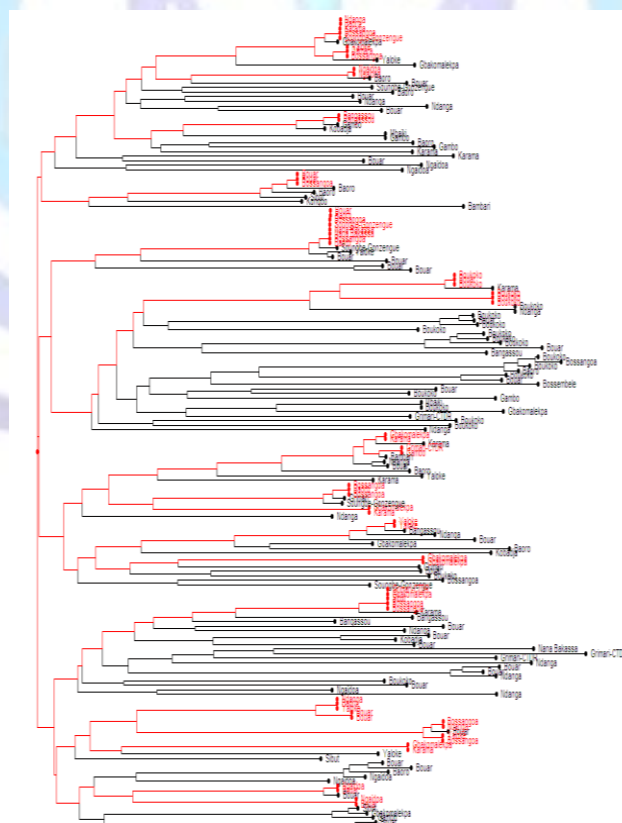
Locus	Alleles number	Hexp.	Hn.b.	Hobs	Fis
GA126	8	0.8066	0.8108	0.8646	-0.06669
GA127a	5	0.5667	0.5697	0.5625	0.01270
SSRY100	6	0.7781	0.7822	0.8316	-0.06357
SSRY101	6	0.7081	0.7118	0.8646	-0.21598
SSRY105	4	0.5444	0.5473	0.5938	-0.08539
SSRY110	6	0.4864	0.4890	0.5263	-0.07675
SSRY12	4	0.6665	0.6700	0.6563	0.02062
SSRY103	6	0.7579	0.7619	0.8646	-0.13559



SSRY135	4	0.7151	0.7189	0.7684	-0.06926
SSRY164	6	0.5569	0.5598	0.5313	0.05130
SSRY169	5	0.5429	0.5449	0.6146	-0.12867
SSRY175	8	0.8320	0.8364	0.8958	-0.07148
SSRY179	8	0.7794	0.7835	0.8958	-0.14426
SSRY20	9	0.6669	0.6704	0.7083	-0.05685
SSRY25	5	0.5349	0.5377	0.5938	-0.10488
SSRY26	5	0.6957	0.6993	0.7917	-0.13282
SSRY4	7	0.7014	0.7051	0.4167	0.41035
SSRY63	4	0.6149	0.6181	0.5938	0.03955
SSRY64	5	0.7327	0.7365	0.8021	-0.08951
SSRY68	8	0.8337	0.8381	0.6146	0.26770
SSRY8	5	0.4014	0.4036	0.4149	-0.02821
SSRY82	8	0.7164	0.7201	0.8438	-0.17275
SSRY9	5	0.6931	0.6968	0.6526	0.06370
<b>Multilocus</b>	<b>5.95652</b>	<b>0.6666</b>	<b>0.6701</b>	<b>0.6914</b>	<b>-0.03198</b>

### Inter varietal genetic diversity

The NJ tree is presented on molecular data cassava accessions that were collected during the fact-finding missions in CAR. Thus, 179 accessions are included in the molecular analyzes. Figure 3 shows that some accessions that differ in their names and backgrounds have the same genotype, it is the case of "Beniga", "Laka-Bossongoa", "Laka", "Zimongbona2", "Ngbokeré", "Gbanambana" "Bessanwe", "sanwen" (Table 3). This can be justified by way of diversity management by farmers.



**Fig 2: dissimilarities between molecular data obtained on 179 accessions. In red, these are accessions signalled under different names and collected in different zones that present genotypes in the same way.**











## DISCUSSION

The study of genetic diversity has focused on the 179 accessions revealed 137 alleles from 23 loci with an average of 5.95 alleles and 96 genotypes representing diversity. Similar results were obtained by other authors who worked on cassava [19, 20, 10, 21, 22, 23]. Peroni and his team [24] evaluated 137 specimens of 58 cassava accessions from Brazil. Using 9 loci, they were able to show an average of 4.56 alleles ranging from 2 to 7. Fregene and his team [10] (2003) analyzing 283 accessions of several countries using 67 loci found an average 5.02 loci. Marcos [25] (2009) studying 42 accessions collected in five regions of Brazil found an average of 5.0 alleles per locus. These data show the degree of variability of cassava grown according to certain production areas.

Heterozygosity value obtained (0.6914) is greater than the expected heterozygosity (0.6666) with a  $F_{is}$  negative (-0.03198) explains differentiation of individuals within the collection of cassava CAR. Similar work carried out by Marcos [25], Lokko and his team [23] (2006), Fregene and his team [10] have given respectively averages of heterozygosity of the order of 0.570, 0.447, 0.535, 0.637. The values obtained are lower than our cons. In French Guiana, Pujol and his team [26] showed that there is a positive correlation between the diversity of plants and their level of heterozygosity. For weeding, farmers tend to eliminate less vigorous plants which would justify the high level of heterozygosity. These high values of  $H_O$  can be supported by the process of allo fertilization in crop systems cassava. Nature protogynous flowers cassava, where male flowers open 7-8 days after the female flowers, plays a role in the proportion of cross-pollination in cassava [27]. Heterozygosity may well be attributed to the traditional management of diversity of cassava in Central Africa, as cultural practices vary among peasant producers. For confirmation, Fregene and his team [10] (2003), in a study of 283 cassava accessions from different countries, attributed the high genetic diversity found in agricultural practices "burned" by the Indian farmers. Due to the nature of preferential crossing in cassava, a large number seeds that survive (slash and burn) can germinate and give new plants the natural and artificial selection uses these plants, leading to new accessions of cassava fields. The incidence of new accessions in agricultural systems have long been described in cassava [28, 29, 26, 11]. Another agricultural practice that has been documented to increase the genetic diversity of plant material exchange between farmers [28, 26, 15]. A combination of these agricultural practices may play an important role in the diversification of cassava in Central African Republic.

Several groups of accessions from different regions have the same genotype (Table 31). This is the case of "Beninga" (Mbaïki), "Laka-Bossongoa" (Bossongoa), "Laka" (Soungbe-Gozenque), "Zinongbona2" (Bossongoa), "Ngbokeré" (Nana Bakassa), "Gbanambana" (Nana Bakassa), "Bessanwe" (Baoro), "sanwen" (Bouar). Faraldo and al. (2000), studying cassava accessions of three distinct groups (Indigenous Park of the Xingu Ribeira Valley is in São Paulo and the Amazon region), found a great similarity among accessions of São Paulo and the Amazon. Peroni and his team [24] also showed a high genetic similarity among accessions of the Ribeira Valley and those of the Rio Negro (Amazon). The identity between the genotypes of accessions reported in different villages, show the possible exchange of materials between communities. However, some accessions have been reported in different villages under the same name, have the same phenotypic characteristics as well as the same genotype, it is the case of "Sawesse" Nana Bakassa collected and Soungbe Gozenque who have the same characters morphological and genotypes. This is proof that peasant producers have the ability to maintain accessions in a wider area. The criteria for appointment are also involved in the variation of diversity. They can contribute to maintaining, increasing and decreasing diversity from the peasant mode of management. Another example is "Six months" and "Zagbang" collected and Baoro Bangassou respectively, have the same genotype but different name and sampling areas. His explains why some peasant producers rename both accessions once arrived in their fields. [30] Kizito (2006) in similar work on cassava grown in Uganda got the same result. In addition, two plants of the same genotype can have different phenotypes they grow in different ecological conditions. Phenotypic diversity, which is found on the ground by general names for the different accessions, is therefore an indicator of genetic variability. The interpretation of the named range must be integrated into an understanding of the local modes of identification and classification of plants. The humanities approach proposes to add another level, that of knowledge, skills and practices: cultural diversity. Of the 49 accessions, three showed genotypic variability within varieties. This change is significant if we consider that cassava is propagated vegetatively. "Tokonenanga" two heterozygous genotypes for the locus with an allele SSR135 common for all people. This is consistent with a hypothesis of inbreeding. This confirms the advantage, if tests can be done on other loci. The presence of a common allele genotypes of "Tounguenlag" demonstrates the existence of a relationship. Two new alleles of SSR103 (285) and SSR4 (307) explain the effect of sex, knowing that the cassava plant is cross-pollinated. The accumulation of somatic mutations may be one of the causes of this variation if one considers that some exogenous (environmental) measures can influence accessions in their midst.

"Sereka" presents a considerable genetic distance between its genotypes (11 loci on 23). SSR8 genotypes (303/316 and 308/308) have no common allele is not proof of relationship between genotypes "Sereka." Mode of management of accessions could also have been a cause of this variation by simple confusion. Convergence phenotypic another accession can also lead to a mixture in which the appointment can not be revealed by a simple phenotypic observation. If we consider that the cultivation of cassava based are by vegetative propagation, crop development by absence of sexual reproduction is subject to the accumulation of somatic mutations. The frequency of somatic mutation depends on the mutation rate and the extent of the selection [31]. For example, the color diversity within accessions (cassava flower) is also a product of somatic mutation: this diversity is due to a partial fixation of a mutation that occurs in the process of chlorophyll synthesis. The contribution of somatic mutation in the evolution of some vegetatively propagated crops, in the case of grapes [32, 33, 34, 34, ], and yam [35], has already been demonstrated. Despite its recent introduction in the country, cassava also appears to have undergone this type of event. During the investigation, interviews with farmers helped us to know they were able to observe in their fields of seedlings grown from seeds (sexual reproduction), the question is whether there was an introduction-conscious or unconscious cassava accessions from seeds in their field?



Molecular data combined with interviews with farmers and observations in the field, show that there was incorporation of plants grown from seeds in the field, combined with the process of vegetative propagation. We can say that the sexual process and contributed to the clonal evolution of cassava CAR. This trend may well be supported by about Balloux and Halkett and their teams [36, 37] as what, in a strictly clonal population, the heterozygote excess is due to the accumulation of mutations fixed independently on each individual. Populations of crops produced by farmers are not stable, especially among pollinators. In fact, they undergo a change in each generation of allelic and genotypic frequencies under the influence of evolutionary forces: mutation, natural selection, and human selection, migration of individuals, populations and genetic drift. Among these factors, only the mutation is not directly influenced by peasant farming practices and environmental factors [38, 39]. This variation may be a result of selection of peasant farmers through a spontaneous insertion plants that would come from sexual recombination. Furthermore, they have shown the multilocus genotypes genetically different in a same accession ("Tokonenanga", "Tounguenlag" and "Sereka"). Similar work showed high genetic variation in a small rural community in Guyana [29]. Different levels of variation within our collection can be a variety of sources: the mode of management of accessions by peasant producers, the possible exchanges of plant material between different communities, as well as programs aimed at the introduction of accessions improved narrow genetic base. Despite the variations in the genotype of each of the three accessions ("Tokonenanga", "Tounguenlag", "Sereka"), 93.87% of the accessions are homogeneous. This demonstrates the ability of producers to maintain and differentiate accessions in their fields.

5 villages surveyed, the average accessions collected is 9.8. Sardos [15] studying the genetic diversity of cassava grown in Vanuatu received an average of 10.4 out of 10 villages. By comparing our results with those obtained in Ghana where cassava was introduced to the 16th century, the average is 3.5 per village accessions [40]. This number is high for a country like CAR where cassava is a recent introduction from Ghana. Manu Aduening and team [41] al. (2005) therefore suggested that the evolution of cassava in Ghana is limited by environmental constraints. Unlike as in Central Vanuatu, cassava is subjected to environmental factors associated with adverse weather conditions, cassava mosaic disease, pests, etc.. Another hypothesis is that the appointment of genetic resources differs between countries. Ghanaian farmers adopt a system composed of polyclonal multiplication of plants from seeds [40] (Manu-Aduening and al., 2005). By CAR as against Vanuatu [15] (Sardos, 2008): Farmers have a long experience of making observations in their diversity especially after vegetative propagation and naming and renaming of accessions based on observations at the scale of a country where there are many ethnic groups and languages with inking cultural vernacular. These data expose the similarity of the situation between the RCA and Vanuatu. The study of genetic diversity is reminiscent of the relationship that could have the number of clones that had been initially introduced [41] (Tisserant, 1953) on the Central African country and the large number of genotypes available today, confirmed by SSR markers which revealed a number of allele between 4 and 9. This situation is similar to Vanuatu [15] (Sardos, 2008) compared to the number of genotypes available today with a number of alleles between 2 and 7, can not be attributed only clones initially introduced. It Would there have been other sources of diversification, due to some means which led to an increase in the gene pool across the respective countries as well as the management mode of peasant producers. Environmental factors, sexuality due to outcrossing nature of cassava, as well as the accumulation of somatic mutations have all contributed both to the diversification of cassava accessions. Fis negative value (table) shows that there is excess heterozygosity between the two populations (CAR and Vanuatu). If we consider that cassava is vegetatively propagated across the two countries, the effect of heterozygosity is expected from the independent accumulation of mutations at fixed genotypes [36, 37] (Balloux and al., 2003; Halkett and al., 2005). The effect of the same sex can increase diversification. Globally, many studies on local diversity of cassava showed an excess of heterozygous individuals [26, 13, 11] (Pujol and al., 2005; Balyejusa Kizito and al., 2007; Rocha and al., 2008). Amazon, the selection favoring heterozygous individuals has been demonstrated [22, 43, 26], and the excess of the heterozygote is generally attributed to selection among individuals recombinant. The hypothesis of a mixed system (clone-sex) justify heterozygosity excess in both populations. A similar result has already been demonstrated by [15] Sardos (2008), showing heterozygosity excess (Fis: - 0.1050) compared to the diversity of cassava in Vanuatu.

## CONCLUSION

This study shows that 96 genotypes were found on les137 alleles were amplified with an average of 5.95 alleles on 23 locus. L bulk of accessions introduced improved in CAR have a common genetic background. Regarding the study of intra-varietal genetic diversity, 93.87% of the 49 accessions collected at village level have shown genotypic homogeneity. Only "Tokonenanga" (Ndanga), "Tounguenlag" (Soungbe) and "Sereka" (karama) are each represented by two different genotypes. This study also shows that he does not have a y-structuring of cassava accessions grown bitter after CAR (Sweet and Bitter).

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