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Genetic diversity of cassava (*Manihot esculenta* Crantz) varieties grown in Daloa district in Central-Western Côte d'Ivoire

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Cassava is an important staple food in Côte d'Ivoire produced in several areas including the Daloa district in Central-Western Region. However, this plant experiences biotic and abiotic constraints that strongly limit its productivity. Proper knowledge of genetic diversity is important to mitigate these constraints and select resistant and well adapted genotypes to increase the productivity. This study assesses the genetic diversity of four varieties of cassava (Bocou 1, Bonoua, Yace and Yavo) cultivated in Daloa. A total of 266 samples of leaves were collected and genomic DNA was genotyped with 5 simple sequence repeats (SSR) microsatellite markers. In all, 28 alleles were recorded from all the loci with an average number of alleles ranging from 2.6 to 2.9. The average heterozygosity obtained for all loci was higher than expected (p = 0.008) and significant genetic diversity was observed within all the varieties (Fis = -0.43, p=0.02) for Bocou1, (Fis = -0.59, p = 6.10^{-4}) for Bonoua, (Fis = -0.32, p = 0.05) for Yace and (Fis = -0.38, p=0.02) for Yavo. A strong genetic differentiation were also observed between varieties, except between varieties Bocou1 and Bonoua where differentiation was moderate (Fst = 0.13). Genetic structure of the population exhibited two or three clusters depending of the variety which might be due to the continuous exchange of plant materials among farmers, selection-base varieties, and use of several varieties in the same fields. This study provides improved understanding of the genetic basis of the varieties which can be exploited to fight against biotic and abiotic stresses in this area.

Key words: Cassava, Côte d'Ivoire, Daloa, genetic diversity, *Manihot esculenta,* microsatellite, population structure.

INTRODUCTION

Cassava is a Euphorbiaceous native to Central America. This plant is the staple food for more than 500 million people in the tropics and subtropics (EL-Sharkawy, 2004). According to FAO (2013), cassava is the world's fourth largest vegetable food behind maize, rice and wheat. In Côte d'Ivoire, cassava is produced mainly in the

south, west and center regions. Average annual production is about 2.41 MT, with an average yield of 6.5 t/h (N'zué et al., 2004, 2013); though of strategic importance for food security and poverty reduction, this crop experiences many abiotic and biotic constraints that limit its production in areas such as the district of Daloa located in Central-Western part of Côte d'Ivoire. In order to mitigate these constraints, cassava breeding programs are being considered (N'Zué et al., 2001, 2013; Kabeya et al., 2012). They consist of responding to the emergence of pests, adapting to abiotic conditions, increasing yields and reducing the level of cyanogenic compounds (Piero et al., 2015). To better understand the genes involved in traits of agronomic interest against pests and for selecting better genotypes that are resistant to pathogens, knowledge on the genetic diversity of cassava become crucial. This study aims to evaluate the genetic potential of four varieties of cassava; Bocou1, Bonoua, Yacce and Yavo, the most profitable and the most cultivated in the district of Daloa.

MATERIALS AND METHODS

Study site and sample collection

The study was carried out in the Daloa district located in the forest zone in Central-West region of Côte d'Ivoire (6° 53' N, 6° 27' W). This area is covered with dense semi-deciduous forest, has ferralitic soil, a tropical climate with two rainy seasons and two dry seasons (Koffie-bikpo and Kra, 2013). Sampling was conducted throughout January 2018 and consisted of harvesting apparently healthy young cassava leaves belonging to four varieties at each selected site in the four cardinal points of the city area, taking into account the availability of the desired varieties. Samples were conserved in coded plastic bags and sent to URGEM laboratory for molecular analyzes. A total of 266 fresh leaf samples were collected, including 58 for Bocou1, 74 for the Bonoua, 70 for Yace and 64 for Yavo varieties as illustrated in Table 1.

DNA extraction

DNA was extracted using standard procedures according to Risterucci et al. (2000) with slight modifications as follow; 800 µL of MATAB were substitute by 400 µL of PBS and 400 µL of ASL. The concentration and DNA quality were check on 3% agarose gel in 0.5X of TBE buffer stained with ethidium bromide and visualized with a gel viewer before polymerase chain reaction (PCR) implementation.

Polymerase chain reaction analysis

PCR was carried out in thermal Cycler BIO-RAD T100^{TM} using microsatellite primers listed in Table 2. These primers were chosen according to their good distribution on the cassava genome (Sraphet et al., 2011; Whankaew et al., 2011) and their high polymorphism (Kawuki et al., 2013). The PCR amplification mixture (mix) was carried out in a final volume of 25 µL comprising 2.5 µL of DNA solution, 2.5 µL of 10X buffer, 1.5 µL of $MgCl_2$ (0.5 mM), 1.6 µL of dNTP (200 µM), 1.3 µL of each of forward and reverse primers (10 µM), 0.3 µL of Taq polymerase (5U/µL) and 15.4 µL of ultra-pure water. PCR conditions included an initial denaturation of 45 sec at 94°C, followed by hybridization of 1 min at the annealing temperature defined for each primer used as shown in Table 2. The final extension included 1 min at 72°C completed in 35 cycles and final hold at 4°C. The PCR products were checked for amplification product on 3% agarose gel stained with ethidium bromide in 0.5X of TBE buffer at 100 V for 45 min and visualized under UV gel viewer.

Microsatellite markers reliability test

Quality and neutrality of markers used to evaluate the genetic diversity of different cassava varieties were calculated per locus (De Meeûs et al., 2007).

Genetic analysis

The data generated were analyzed for genetic diversity parameters. These parameters are estimated for each locus and the average on all the loci. The CREATE software made it possible to format the database for FSTAT and GENETIX software. The average number of alleles per locus reflects the richness of alleles in the studied population *(*A = Σ(a /L), where *a* is the number of alleles at a given locus and L number of loci studied). The polymorphism (P) is the percentage of polymorphic loci in the study population. A locus is considered polymorphic when the frequency of the most common allele is less than 0.95. The heterozygosity observed (Ho) and expected (He) defined by Nei (1978) and the fixation index F_{IS} (F_{IS} = (He-Ho)/He) made it possible to evaluate within-population diversity. The allelic frequency and the number of rare alleles defined as those having a frequency less than 0.05 were also calculated for each locus. Genetic differentiation was examined with F_{ST} based on the formula: $F_{ST} = (H_T-H_S)/H_T$, where H_S is the expected heterozygosity of an individual within subpopulation and H_T the expected heterozygosity of an individual in the total population (Wright, 1978).

Genetic structure of cassava cultivars

Population structure of cassava cultivars were visualized based on dendrogram using Nei's method (Nei, 1978). This method is based on the dissimilarity matrix between individuals using Darwin software version 6.0. Similarity index is calculated between two individuals according to the following formula: $Dij = \left(1 - \frac{1}{L}\right)\sum_{l=1}^{L}\left(\frac{ml}{\pi}\right)$, where D_{ij}, the dissimilarity between the individuals i and j; L, the number of loci; π = ploidy and ml the number of matched alleles per locus. In addition, a model-based approach was also applied to confirm the genetic structure. The number of groups (K) in each cassava cultivar was determined with the STRUCTURE software using the admixture model (Pritchard and Donnelly, 2001). The statistic ΔK, which is based on the rate of change in the log probability of the data between successive K-values, was then used to detect the true number of K populations in the dataset (Evanno et al., 2005). This test was performed using the correlated model of

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Table 1. Sites where cassava samples were collected.

Site	Variety				
	Bocou1	Bonoua	Yace	Yavo	Total
Site 1	14	18	16	17	65
Site 2	14	19	19	15	67
Site 3	11	17	16	16	60
Site 4	19	20	19	16	74
Total	58	74	70	64	266

Table 2. Microsatellite markers used to assess genetic diversity.

Name	Type of repeat	Primers (forward and reverse)	Annealing temperature (ºC)	Reference
EME20	(ATG)9	5'-CAG-CAC-CAG-TCA-ACA-TTC-CTG-3' 5'-CCT-TCT-GGC-AAT-GAG-CTC-ATG-3'	58	Sraphet et al. (2011)
EME303	(CT)11	5'-ATT-GGG-AAG-CAT-TGG-TGT-AGA-A-3' 5'-CAC-AAA-CAA-AAC-CCT-GTG-ACC-T-3'	58	Whankaew et al. 2011
EME222	(GAT)6	5'-CCC-ACT-CTC-TGT-CCA-CTT-C-3' 5'-CTT-CGA-CTC-TTC-TTT-ACG-GG-3'	58	Sraphet et al. (2011)
SSRY100	(CT)17TT(CT)7	5'-ATC-CTT-GCC-TGA-CAT-TTT-GC-3' 5'-TTC-GCA-GAG-TCC-AAT-TGT-TG-3'	58	Kawuki et al. (2013)
SSRY61	(CA)12	5'-GGC-TGC-TTT-ACC-TTC-TAC-TCA-A-3' 5'-CAA-GAA-CGC-CAA-TAT-GCT-GA-3'	58	Whankaew et al. (2011)

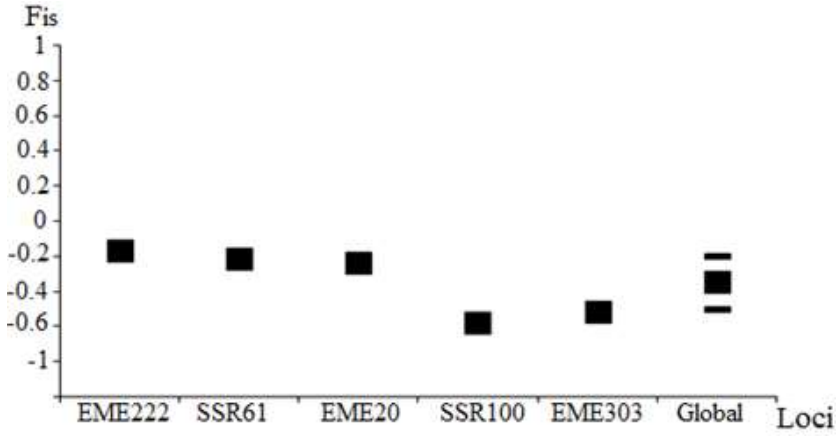

Figure 1. F_{IS} estimation per microsatellite locus.

allelic frequencies based on the clustering of individuals with similar allelic frequencies.

RESULTS

Microsatellite markers reliability test

The 5 microsatellite markers have good congruence and variances quite homogeneous regarding F_{IS} calculated per locus as illustrated in Figure 1. Therefore, all these markers were used for subsequent analysis.

Genetic diversity parameters

Genetic diversity parameters were assessed with 5 microsatellites markers, and the results are presented in Table 3. All these markers were polymorphic for the four varieties. A total of 28 alleles were observed. The number of alleles varied from four to seven throughout loci with an average number of 5.6 alleles. The rate of rare alleles

Table 3. Mean of genetic diversity parameters across all loci.

Locus	No. of allele	Rare allele	Ho	He	F_{IS}	F_{ST}	P-value
EME222	7	2	0.752	0.645	-0.166	0.15	0,05
SSRy61	5	1	0.688	0.574	- 0.199	0.10	0.05
EME20	5	1	0.721	0.597	- 0.208	0.13	0.04
SSRy100	7	0	0.985	0.625	- 0.576	0.19	0.004
EME303	4	0	0.909	0.599	- 0.517	0.19	0.007
Mean	5.600	0.800	0.811	0.608	- 0.333	0.15	0,008

He = expected heterozygosity, Ho = observed heterozygosity, F_{IS} = fixation index, F_{ST} = genetic differentiation index.

Table 4. Mean of genetic diversity parameters for each variety across all loci.

Population	No. of allele	Rare allele	H_0	H_e	F_{IS}	P-value
Bocou1	2.6	1	0.76	0.53	-0.43	0.02
Bonoua	2.75	1	0.92	0.58	-0.59	0.0006
Yacé	2.75	0	0.74	0.56	-0.32	0.05
Yavo	2.9	2	0.80	0.58	-0.38	0.02

Table 5. Estimated F_{ST} between pairs of the four varieties of cassava.

Population	Bocou1	Bonoua	Yace	Yavo
Bocou1	-	0.13	0.24	0.24
Bonoua	0.13	-	0.25	0.23
Yace	0.24	0.25	-	0.25
Yavo	0.24	0.23	0.25	-

in the overall population is 14.28%. The EME222 marker recorded the highest number of rare alleles as shown in Table 3. The mean number of alleles across all loci obtained for varieties Bocou1, Bonoua, Yace and Yavo were 2.6, 2.75, 2.75 and 2.9 respectively. Of the 28 alleles revealed by these 5 markers across loci and varieties, only one rare allele occurred in variety Bocou1 and Bonoua, and 2 occurred in variety Yavo. The average expected heterozygosity (He) across all the varieties and loci ranged from 0.574 in SSRy61 to 0.645 in EME222 with an average of 0.608, while observed heterozygosity ranged from 0.688 to 0.985 in SSRy61 and SSRy100, respectively, with an average of 0.811. The average heterozygosity obtained for all loci was higher than expected (p=0.008). A significant genetic diversity was observed within all the varieties (F_{IS}=-0.43, p=0.02) for Bocou1, (F_{IS}=-0.59, p=6.10^{-4}) for Bonoua, (F_{IS}=-0.32, p=0.05) for Yace and (F_{IS}=-0.38, p=0.02) for Yavo as illustrated in Table 4.

Genetic differentiation between varieties

F_{ST} values indicate good genetic differentiation between varieties except between varieties Bocou1 and Bonoua where there is moderate genetic differentiation with a F_{ST} value equal to 0.13 as described in Table 5.

Population structure of cassava cultivars

Genetic structure within cassava varieties is described by radial style dendrograms based on dissimilarity matrices between individuals. These graphs shapes testify the existence of a spatial structuring of the diversity within these varieties. Thus, samples from different sites are grouped together to form distinct genetic groups within the four varieties as shown in Figure 2.

According to admixture model, varieties Bocou1, Bonoua and Yavo comprised of 2 clusters (K = 2) each, with a maximum likelihood for value ΔK = 696, 106 and 98 for these varieties respectively. Variety Yacé comprised 3 clusters (K = 3) with a maximum likelihood for a value of ΔK = 16. For variety Bocou1, there are 37 individuals (64%) in cluster 1 and 21 individuals (36%) in cluster 2. Variety Bonoua has 39 individuals (53%) in cluster 1 and 35 individuals (47%) in cluster 2. Concerning variety Yavo, individuals are distributed evenly over 2

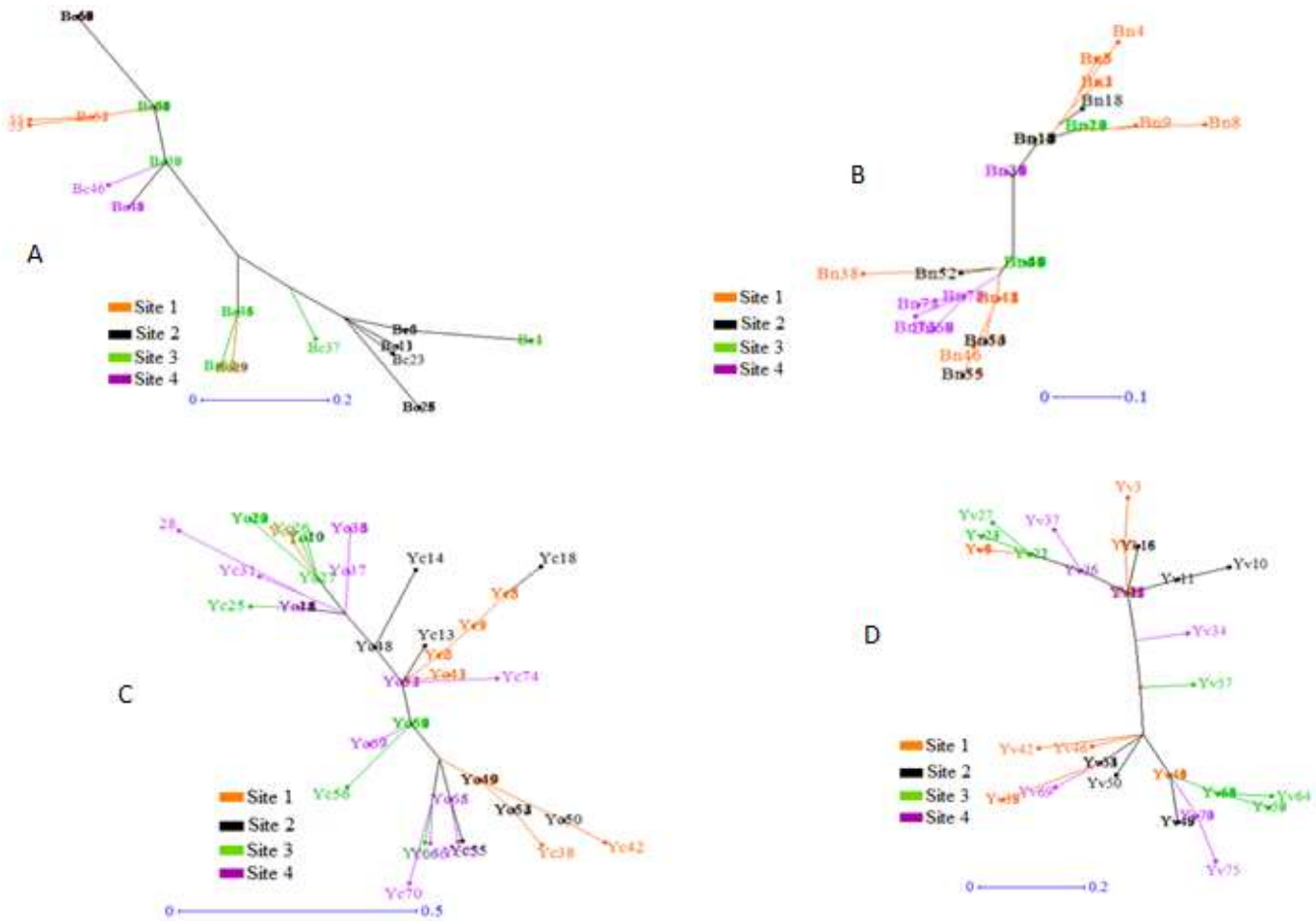

Figure 2. Dendrograms describing the genetic structure of cassava populations according to dissimilarity between individuals (A, B, C and D represent varieties Bocou1, Bonoua, Yacé and Yavo respectively).

clusters (50% each). Variety Yacé has 21 individuals (30%) in cluster 1, 35 individuals (46%) in cluster 2 and 14 individuals (24%) in cluster 3 as shown in Figure 3.

DISCUSSION

Genetic diversity and structure within and between 4 cassavas varieties cultivated in Daloa region were assessed with five microsatellite markers considered adequate to give reliable results on genetic parameters of cassava (Moyib et al., 2007). These markers were all polymorphic. Low levels of rare alleles were observed in these varieties, indicating the good quality of the markers (Meye, 2013).

Genetic diversity paramerters

The average numbers of allele obtained in the different varieties analyzed are greater than 2 and reveal a good allelic richness within these varieties but less than an average number of alleles of 4.76 observed by Kizito et al. (2005) in Uganda. The highest average number of allele from these authors may be due to the fact that their study was carried out on unselected traditional varieties with more diversity than selected variety used in our study. This mean that the varietal selection lost alleles and therefore genetic diversity contained in wild collections is higher than selected variety observed (Nassar, 1978). Low genetic variability among the cassava varieties was also observed by other authors in Nigeria using the same molecular markers (Kabeya et al., 2012; Afonso et al., 2019). This narrow variability is a drawback from the point of view of breeders, because they need high genetic variability to improve agronomic traits and the genotypes are selected only based on very few agronomic traits such as maturation time, height and yield. Increasing genetic variability is crucial for breeding programs (Kabeya et al., 2012). This shows the need to always maintain an ever more diverse wild core that is referred to when the varietal selection has led to the loss of some interesting trait in the wildlife species.

The average values of FIS are negative for all loci in the

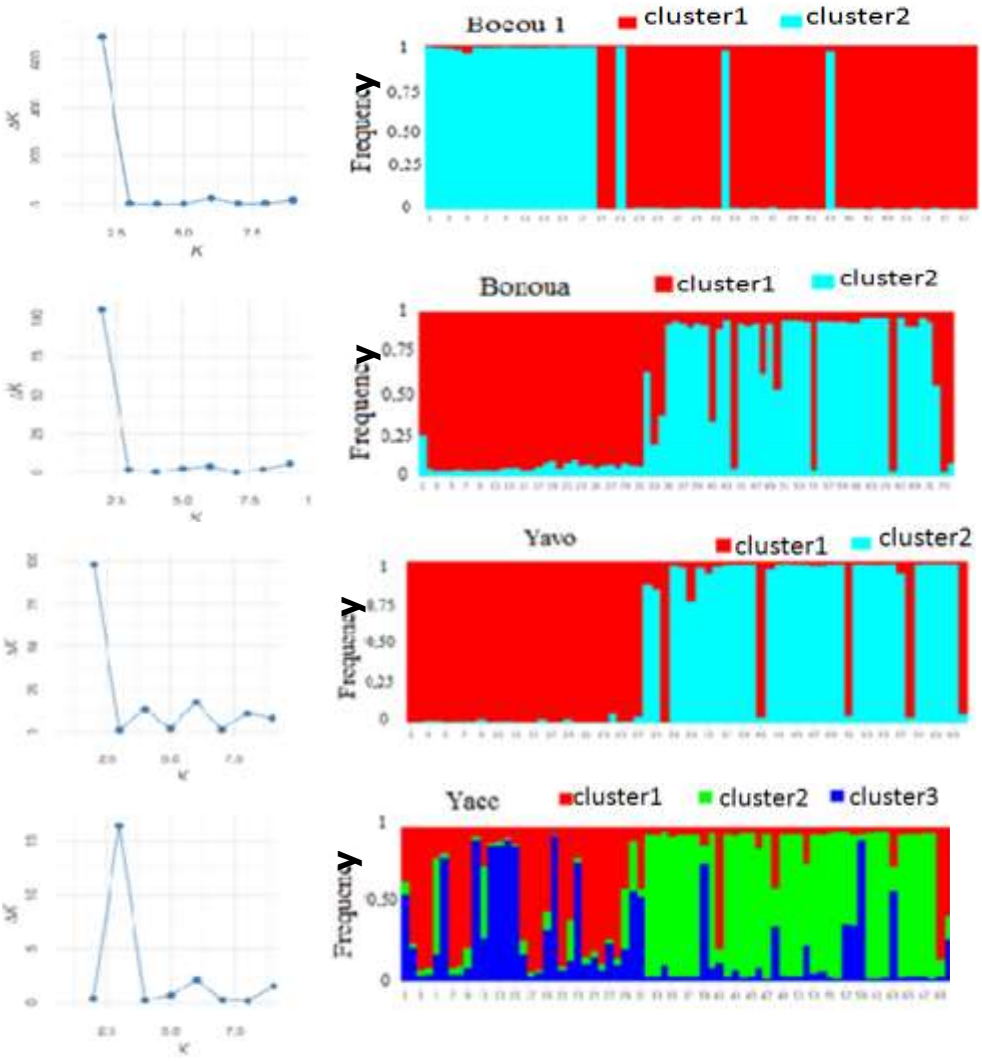

Figure 3. Clusters within cassava varieties using the admixture model (each color is representative of a cluster).

different varieties of cassava. There is therefore an excess of heterozygotes within the varieties that could be explained accordingly. One could think of the clonal reproduction of cassava which results in identical individuals to the original one. Otherwise, the excess of heterozygotes could be due to the natural sex-based reproduction systems of cassava. This argument is supported by Silva et al. (2003) who showed a pollen rate of cassava greater than 0.6 which reveals high levels of pollination with varying levels of selfing in this plant. The average number of alleles greater than 2 and the strong heterozygosity reflect the existence of genetic diversity within the varieties of cassava considered.

Let us note that the varieties studied (Yacé, Yavo, Bocou1 and Bonoua) are already improved (selected) varieties from the National Center for Agricultural Research (CNRA) and therefore from several crosses between landraces (N'Zué et al., 2013). This could explain the high number of heterozygosity and therefore the no-equilibrium situation of Hardy-Weinberg (Beninga, 1992).

Chromosomal mutations due to environmental pressures might arise and thus contribute to genetic diversity. One of the factors behind this diversity is the continuous exchange of plant material with interesting agronomic traits among farmers in different localities (Missihoun et al., 2012). It could also be due to cultural practices based on the use of several varieties in fields that by gene exchange which creates genetic variability (Meye, 2013). In fact, the genetic diversity of cassava in a locality increases with varietal diversity (Adriano et al., 2013). In addition, when several varieties are grown by many households over large areas, they are minimally threatened and may simply be subject to in situ conservation of genetic diversity (Jarvis et al., 2000). Furthermore, it is noted that the clonal spread of cassava leads to an accumulation of pests, reducing yields. Thus, the higher the genetic diversity, the higher is the

resistance to pathogens (McKey et al., 2012).

However, many varieties are abandoned because of the adoption of higher-yielding varieties and only few are maintained in cultivation which could thus favor genetic erosion (Kombo et al*.,* 2012). Indeed, genetic diversity is higher in Amerindian villages (Elias et al., 2000) because, farmers of these areas exploit seedlings from seeds to enhance the diversity of their plant material (Peroni et al., 2007). Seed multiplication is therefore source of new genotypes (Rival and Mckey, 2008) but is unfortunately unknown in Côte d'Ivoire.

Genetic differentiation between varieties

Moderate genetic differentiation between cassava varieties observed (F_{ST} = 0.19) could be explained by a low gene flow between varieties because of few gene exchange between the improved varieties (Lokko et al., 2006). However, when the varieties are considered two by two, the genetic differentiation is strong except varieties Bocou1 and Bonoua which present a moderate genetic differentiation. We can say that gene flow is higher between these two varieties than between the others.

Population structure of cassava cultivars

The analysis carried out with STRUCTURE presented two main clusters for Bocou1, Bonoua and Yavo varieties and three genetic clusters for the Yacé variety. There is thus more diversity within these varieties which confirms their instability which could offer improvement possibilities for these varieties (Trochet et al., 2014).

Conclusion

Cassava is one of the income-generating crops grown in Côte d'Ivoire. This study evaluated the genetic diversity of four varieties of cassava cultivated in Daloa district. It comes up the existence of a moderate genetic diversity and differentiation within and between these varieties. Population structure defined two or three clusters depending on variety. This study is the first step in understanding the range of genetic diversity of cassava varieties grown in Daloa. The information generated is of paramount importance to justify and guide conservation strategies for cassava genetic resources in the area, to serve as a reference not only to the region but also to the country and a parental selection guide in the sense of varieties improvement.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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