

Set up of simple sequence repeat markers and first investigation of the genetic diversity of West-African watermelon (*Citrullus lanatus* ssp. *vulgaris* oleaginous type)

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Abstract *Citrullus lanatus* ssp. *vulgaris* oleaginous type (West-African watermelon) is a crop cultivated in sub-Saharan Africa for its dried seeds reported to be rich in nutrients. In previous studies, little polymorphism was found in watermelon—cultivated for its flesh with the use of microsatellite (SSR) markers. Such study has never been applied to the oleaginous type until now. The objectives of the present study were firstly to apply the SSR markers

set up for watermelon to the West-African watermelon and secondly to study the genetic structure of this type in Ivory Coast. For the first objective, 37 markers were studied on eight plants pertaining to four accessions. For the second objective, the polymorphic markers were applied on three morphologically and geographically separated accessions with twenty plants per accession. Multiple correspondence analysis (MCA), unweighted pair-group method with arithmetic averaging (UPGMA), molecular analysis of variance (AMOVA) and assignments test structure were applied. The optimal annealing temperature varied from 49 to 59°C according to the markers. Thirty-two markers that proved to amplify their respective loci were selected, but only nine of them appeared to show polymorphism on the set of 8 plants studied. The application of these markers on the three accessions revealed several features. No structuration into sub-populations was observed inside a given accession. The genetic variance proved to be substantially higher between the different accessions than inside a given accession. Moreover this analysis is a first hint that the morphology classification does not match the genetic structure of *C. lanatus*. The results of this work provide the first quantitative information regarding the genetic variability of *Citrullus lanatus* oleaginous type. In order to sharpen our understanding of the mechanisms responsible for the genetic variance inter/intra accessions, further studies based on a larger sample of plants and accessions are required.

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Introduction

In sub-Saharan Africa, *Citrullus lanatus* (Thunb.) Mats. et Nakai (oleaginous type—West-African watermelon, also called egusi in West-Africa (Merrick 1998), formerly considered as *C. lanatus* ssp. *vulgaris* (Schrad. ex Eckl. et Zeyh.) *Furca mucosopermis* group or ssp. *mucosopermis* Fursa—is cultivated and praised for its seeds reported to be rich in nutrients—~60% lipids and ~30% proteins (Loukou et al. 2007). This secondary crop constitutes a valuable income for a part of the population: the average price for one kg is 2.3 euros—7 times higher than one kg of coffee beans (Zoro Bi et al. 2003). However the harvest is intricate—especially the extraction of the seeds—and the plant is more and more disregarded by the farmers. In Ivory Coast, only the central region keeps cultivating and selling oleaginous Cucurbitaceae. The continuous decrease of the oleaginous *C. lanatus* culture associated with the lack of selection strategies may be one major reason of a genetic erosion of this plant.

In Ivory Coast, the plants are classified into two morphotypes according to the morphology of the seeds. The first morphotype (wlèwlè) is characterized by glossy seeds with a tapered proximal extremity. This morphotype is subdivided into three cultivars based on the size of the seeds (about 40, 60 and

120 mm²) (Fig. 1). In the second morphotype (bebu), the seeds are heavier and have a flat ovoid shape with rugged and thick ends (Zoro Bi et al. 2003).

African watermelon is generally considered as allogamous, monoecious, entomophilous and protandrous (Gusmini and Wehner 2003). The occurrence of autoincompatibility has not been demonstrated. However it is fair to say that the reproductive mechanisms of *C. lanatus* are far from being completely understood.

The genetic structure of *C. lanatus* has been investigated for several years. A study based on isozymes (Navot and Zamir 1987) revealed that *C. lanatus* var. *lanatus* showed a low rate of allozymic variability compared to *C. lanatus* var. *citroides* (L.H. Bailey) Mansf ex Greb. The works from Jarret et al. (1997) based on SSR markers, from Levi et al. (2001) on RAPDs, from Dane and Liu (2007) on SSRs led to types, and from Kwon et al. (2007) on SSRs led to the same conclusions. However, from Dane and Liu's analysis (2007), these studies did not take into account the oleaginous type.

Whether bebu and wlèwlè morphotypes correspond to different genetic groups of the species is not known. Recently, an analysis relying on the use of ISSR markers (Djè et al. 2006) showed the existence of distinct genetic groups inside a unique accession (Tahi 2006). Although this result has still to be confirmed, it suggests that a review of our knowledge of the reproduction mechanisms of *C. lanatus* oleaginous type is needed. In particular, a possible occurrence of geitonopollinisation is not excluded, as the male and female flowering periods overlap with each other (Ferreira et al. 2000; Loehrlein and Ray 2007). This also shows that studies on diversity

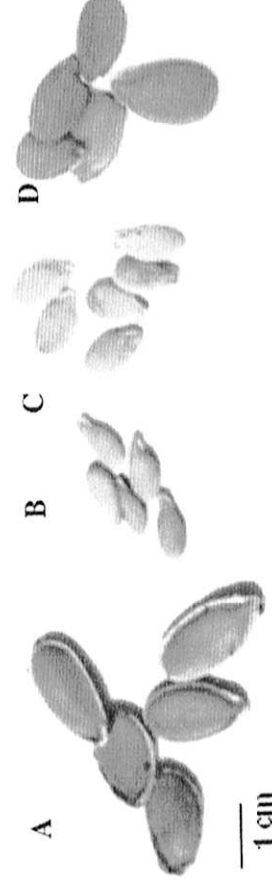


Fig. 1 Seeds of *C. lanatus* oleaginous type from the four recognized morphotypes: bebu (a), et wlèwlè (b, c, d). Morphotype bebu (accession 223), the seeds have a flat

ovoid shape with rugged and thick ends. b, c, d Morphotype wlèwlè (accession 161, 117 and 211 respectively), characterized by glossy seeds with a tapered proximal extremity

and reproduction mechanisms cannot be separated from each other in order to establish conservation and selection strategies for *C. lanatus* oleaginous type species.

In this paper, we first describe the set up of the markers for the oleaginous type and the polymorphism of these markers. The genetic structure inside three different accessions, geographically and morphologically well separated from each other, was investigated with the use of SSR markers.

Materials and methods

Plant material

Seeds from four different accessions—no 117 (wîèwîè 60 mm²), no 161 (wîèwîè 40 mm²), no 223 (bebu) and no 402 (wîèwîè 120 mm²)—were obtained from the collection of the University of Abobo-Adjamé (Ivory Coast) and two plants per morphotype were used for the set up of SSR markers. Three accessions (20 plants each) of two different morphotypes were then used for the study of genetic diversity:

- 117, from Gohitafla, centre part of the country, wîèwîè morphotype, ca. 60 mm² size seeds (Fig. 1c)
- 211, from Alépé, Southern part, wîèwîè morphotype, ca. 120 mm² size seeds (Fig. 1d)—223, from Alépé, Southern part, bebu morphotype, ca. 120 mm² size seeds (Fig. 1a)

In each accession, the seeds were selected randomly among all the harvested seeds.

The seeds were germinated in germination chambers (Snijders Scientific, Netherlands) under controlled conditions (20–30°C; obscurity).

DNA extraction

A CTAB extraction procedure based on the works from Levi and Thomas (1999) and Gusmini (2005) was used as follows. DNA was isolated from the tissue of young roots stocked at –80°C, as the extraction product is cleaner than that from cotyledons and young leaves. Small pieces (70–80 mg) were ground with liquid nitrogen and sand. The resulting powder was immediately transferred to a

microfuge tube with 700 µl extraction buffer [0.1 M Tris-Base, 1.4 M NaCl, 2.5% cetyltrimethylammonium bromide (CTAB), 20 mM EDTA-dissodium, 0.2% sodium dodecyl sulfate (SDS), 0.5% Sarkosyl, 250 mg polyvinylpyrrolidone (MW 40 (PVP-40) and 250 mg polyvinylpyrrolidone (PVPP)] and mixed thoroughly. After 30 min incubation at 60°C, 500 µl of chloroform was added. After mixing vigorously, it was centrifuged at 12,500 rpm for 5 min at room temperature. The aqueous phase was recovered in a new Eppendorf tube. After the addition of 500 µl isopropanol at –4°C, the DNA was left to precipitate for 20 min at –20°C, centrifuged at 12,500 rpm for 15 min. Then the pellet was washed with 500 ml ethanol (70%) and was centrifuged at 12,500 rpm for 15 min. Finally, the DNA was dissolved in 100 µl TE [10 mM Tris-HCl, 1 mM EDTA (pH 8.0)] and was centrifuged for 5 min at 12,500 rpm. The supernatant was transferred to a new container for final storage. The DNA concentration was estimated with a spectrophotometer. The DNA was stored at –20°C until use.

This protocol differed from those of Levi and Thomas (1999) and Gusmini (2005) by addition of 0.2% SDS to disrupt cell membranes (Levi Ammon, personal communication). Neither b-mercaptoethanol nor isoamylalcohol were used, as these toxic products did not improve the DNA quality and quantity.

PCR conditions

The microsatellite markers had been set up for watermelon previously (Jarret et al. 1997; Guerra-Sanz 2002; Joobeur et al. 2006; Kwon et al. 2007). Among these markers, only the 37 most polymorphic ones were selected. The resolution of these markers was performed for *C. lanatus* oleaginous type with optimal annealing temperature varying from 49 to 59°C. The best conditions were retained according to the intensity and sharpness of the bands. Amplification reactions were performed in a 15 µl volume containing 10–15 ng genomic DNA, 2.5 mM MgCl₂ (Amersham Bioscience Corp., United Kingdom), 100 µM of each dNTP (Amersham Bioscience Corp., UK) 0.05 µM primer F (0.5 µM primer R (Sigma, USA), 5 µM M13-labelled primer (Sigma) (Ducarme

¹ Levi Ammon: USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC 29414.

et al. 2008), 0.6 U Taq polymerase (Amersham Bioscience Corp., United Kingdom), and $1 \times$ Taq DNA Poly buffer (Amersham Bioscience Corp., UK).

The PCR amplifications were performed using a PTC 100 Thermal Cycler (MJ Re-search, USA) programmed as follow: 4 min at 94°C; 40 cycles of 30 s at 94°C, 30 s at annealing temperature (varying from 49 to 56°C), and 30 s at 72°C; and a final elongation step of 20 min at 72°C.

Electrophoresis and data analysis

PCR products (7.5 μ l) mixed with bromophenol blue markers (1 μ l for each well) were analyzed by electrophoresis using 1.5% w/v agarose gel (100 ml) with 1.5% of ethidium bromide in $1 \times$ TBE Buffer ($10 \times$ TBE: 108 g l^{-1} Trisbase, 55 g l^{-1} boric acid, 8.3 g l^{-1} EDTA pH 8) carried out at a constant voltage of 90 V for 140 min. A DNA ladder (Smartladder, Eurogentec, Belgium) was used in each electrophoresis gel as a molecular mass marker. Gels were visualized under UV light and recorded with a video image analyzer (Biocapt, Vilbert-Lourmat, Marne-la-Vallée, France).

The sequencer ABI 3100 DNA (Applied Biosystems, USA) was used to analyse the size of the amplified fragments we used. The marker 400 HD was used as internal marker. The size of alleles was determined by the Gene Mapper software v.3.5 (Applied Biosystems, USA). Two homozygote alleles were entirely sequenced, in order to guarantee that the PCR amplification actually targeted the desired SSR.

Data analysis

The polymorphism information content (PIC) was calculated *via* the formula established by Powell et al. (1996).

$$PIC = 1 - \sum_{j=1}^n f_j^2 \quad (1)$$

in which f_j is the frequency of the j allele and the summation extends over n alleles (Kwon et al. 2007). Observed and expected heterozygosity, as well as Wright's F -statistics F_{is} (Weir and Cockerham 1984) were calculated with the software Genetix 4.03. The number of populations showing a significant

deviation of Hardy–Weinberg equilibrium for each locus was calculated with the software Genpop 4.0 (Raymond and Rousset 1995).

A multiple correspondence analysis (MCA) was performed using the software SPAD 7.0. The existence of groups of individuals was tested according to the Ward aggregation criterion (based on the variance).

The genetic absolute distance (AGD) (Bowcock et al. 1994) was calculated using the Microsat software, especially designed by Minch et al. (1995) for microsatellite data analysis.

$$AGD = 1 - \text{proportion of shared alleles} \quad (2)$$

The unweighted pair-group method with arithmetic averaging (UPGMA) clustering was then used to construct dendrograms (Philip 3.68). The analysis of molecular variance (AMOVA) was realized to determine the proportions of the molecular variance between and within groups of accessions (GenAlex 6 in Microsoft Excel).

Assignment test were also realized with the entire set of accessions, by means of the Structure software (Pritchard et al. 2000).

Results

Set up of the markers for the oleaginous type and analysis of polymorphism

Among the 37 markers tested, 32 generated one clear band on agarose gel and were therefore selected for further sequencing in order to distinguish the homozygote genotypes. However, the sequencing revealed that only 9 markers were polymorphic. The annealing temperature was 53°C for ADPGPS and EST00675 and 57°C for CI 2–23, CI 1–20, CI 2–61, CI 2–140, MCPI-12, MCPI-21, MCPI-26.

Diversity of three accessions (117, 211 and 223) of *C. lanatus* oleaginous type

The 9 SSR markers generated a total of 20 alleles leading to 41 different profiles (Table 1). Thus several individuals could not be separated from each other. Accession 117 was not at the Hardy–Weinberg equilibrium according to three markers, and five

Table 1 N-HWE: Number of populations showing a significant deviation of Hardy–Weinberg equilibrium for each locus ($P < 0.05$)

Primers	Repeat motifs	Reference	NA*	NA	Non-HWE	Hhb	Hobs	PIC*	PIC
ADPGPS	(CT)18	Guerra-Sanz (2002)	4	3 (2, 2, 2)	117	0.36, 0.48, 0.36	0.25, 0.25, 0.15	/	0.50
EST00675	(CT)13	Guerra-Sanz (2002)	3	2 (1, 2, 1)	223 NP, 117 NP	0.00, 0.30, 0.05	0, 0.25, 0.05	/	0.12
Cl. 2-23	(CT)15	Jarret et al. (1997)/ Kwon et al. (2007)	5	4 (3, 3, 3)	117	0.48, 0.40, 0.14	0.50, 0.30, 0.05	/0.58	0.56
Cl. 1-20	(CT)18	Jarret et al. (1997)/ Kwon et al. (2007)	3	3 (1, 3, 1)	223 NP, 117 NP	0.00, 0.19, 0.00	0.00, 0.20, 0.00	/0.59	0.06
Cl. 2-61	(CT)18	Jarret et al. (1997)	4	2 (1, 2, 1)	223 NP, 117 NP	0.00, 0.50, 0.00	0.00, 0.32, 0.00	/	0.30
Cl. 2-140	(GA)20	Jarret et al. (1997)	3	2 (2, 2, 2)	/	0.50, 0.22, 0.18	0.35, 0.15, 0.10	/	0.50
MCPI-12	(AAG)7N69 (AT)26	Joobeur et al. (2006)	4	2 (2, 2, 2)	117	0.14, 0.47, 0.41	0.15, 0.23, 0.15	0.66	0.41
MCPI-21	(AG)11	Joobeur et al. (2006)	4	2 (1, 1, 1)	223 NP, 211 NP, 117NP	0.00, 0.00, 0.00	0.00, 0.00, 0.00	0.67	0.44
MCPI-26	(AAT)12	Joobeur et al. (2006)	5	2 (2, 1, 1)	211NP, 117 NP	0.14, 0.00, 0.00	0.15, 0.00, 0.00	0.63	0.46

NP non polymorphic locus, NA number of alleles (total and for accessions 223, 211 and 117 respectively), Hhb non-biased expected heterozygosity (total and for accessions 223, 211 and 117 respectively) (Nei 1978), Hobs observed heterozygosity (total and for accessions 223, 211 and 117 respectively) and polymorphism information content (PIC) of 9 SSR markers content in 3 accessions of 20 samples of *C. lanatus* oleaginous type. NA* and PIC* is the NA and the PIC found in the literature (Jarret et al. 1997; Guerra-Sanz 2002; Joobeur et al. 2006; Kwon et al. 2007)

Table 2 Level of heterozygosity, Wright's statistic, number of alleles per locus and AGD of 3 accessions (117, 211 and 223) of *C. lanatus* oleaginous type from SSR markers analysis

	Hexp	Hhb	Hobs	Fis	Allele/locus	AGD
223	0.1762 ± 0.2066	0.1808 ± 0.2119	0.1556 ± 0.1810	0.143NS	1.67	0.16
211	0.2763 ± 0.1903	0.2842 ± 0.19600	0.1885 ± 0.1176	0.343***	2.00	0.25
117	0.1242 ± 0.1568	0.1274 ± 0.1608	0.0556 ± 0.0635	0.570***	1.67	0.13
Total	0.3734 ± 0.1736	0.3766 ± 0.1751	0.1320 ± 0.0912	0.328***	2.44	0.37

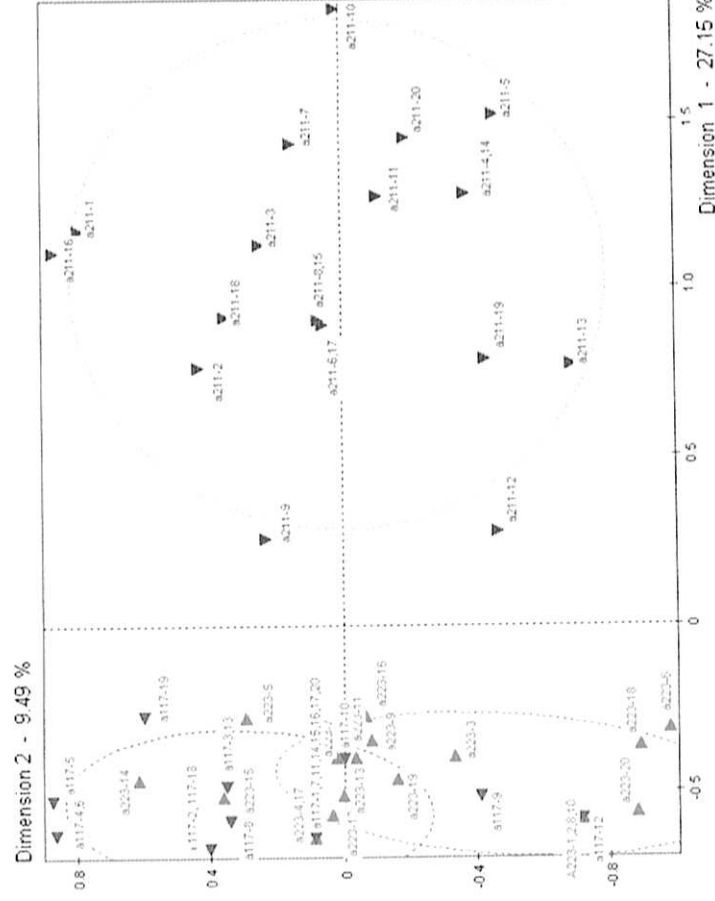
Hexp biased expected heterozygosity, Hhb non-biased expected heterozygosity (Nei 1978), Hobs observed heterozygosity, mean ± SD, Fis heterozygosity deficit (*** $P < 0.001$), AGD absolute genetic distance (Bowcock et al. 1994)

markers were not polymorphics for at least two accessions. The PIC varied from 0.06 (CI 1–20) to 0.56 (CI 2–23) (average 0.37). The number of alleles per locus was 1.67, 2.00 and 1.67 for the accessions 117, 211 and 223 respectively (Table 2). The genetic distances were 0.13, 0.25 and 0.16 for the accessions 117, 211 and 223 respectively. The genetic distance for all plants of three accessions was 0.37. The observed heterozygosity (Hobs) was 0.06, 0.12 and 0.16 for the accessions 117, 211 and 223 respectively, which was less than the expected (Hexp) one but not significantly. However the Fis value for the sample including all the fields was 0.328 showing an overall

lack of heterozygosity (the Fis of the accession 223 was not significant).

In order to represent graphically the genetic diversity, an MCA method was used to project the sample onto a two-dimensional space. The resulting plot contained 36% of the total information (27% dimension 1 and 9% dimension 2) (Fig. 2). The first axis separated the accession in two groups: the accession 211 (from Alépé, Southern part, wlewè morphotype) on one side and the accessions 117— from Gohitafla, centre, wlewè morphotype and 223—from Alépé, Southern part, bebu morphotype on the other side.

Fig. 2 Multiple component analysis plot of 3 accession type of *C. lanatus* oleaginous type from the SSR markers analysis. Accessions No.117 from Gohitafla (centre), wilewile morphotype; No. 211, from Alépé (southern part), wilewile morphotype; No. 223, from Alépé (southern part), bebu morphotype. The dotted ellipses refer to the clusters resulting from the Ward aggregation criterion (confidence interval 95%)



One cluster (at the right-hand side) resulting from the Ward aggregation criterion at the 95% confidence level only included individuals of the accession 211, and only 4 individuals of this accession were found outside the cluster, although at a very short distance from it. The second axis roughly separated the accessions 223 and 117. Each of the two clusters at the left-hand side resulting from the Ward aggregation criterion at the 95% confidence level contained 14 of the 20 individuals of one accession, either 117 in the upper cluster, or 223 in the lower one. The next dimensions did not bring any relevant information (data not shown).

Almost the same genetic relationship between accessions was observed with the UPGMA clustering method (Fig. 3) based on the absolute genetic distance. The cluster I contained individuals mainly from accession 223 as well as 2 individuals from accession 117 (A117-12 and A117-9). These two individuals also belong to the group including individuals from accession 223 according to the MCA based on the Ward aggregation criterion. The cluster II contains individuals mainly from accession 117, as well as from accession 223 (A223-4, 17, 11, 13, 15, 14). The individuals 11 and 13 from accession

113 belong to the two groups in the MCA that include most of individuals from 117 and 223, whereas the other individuals from accession 223 were grouped together in agreement with the MCA analysis. The cluster III was formed by three individuals labeled 117-19, 223-6 and 223-18.

These individuals lie outside any group in the MCA based on the Ward aggregation criteria. Finally, the cluster IV contains the individuals from accession 211.

The assignment tests applied on the entire set of accessions, by means of the Structure software (Pritchard et al. 2000) was performed with a number of sub-populations from $k = 1$ to $k = 8$. For $k = 2$, the accession 211 was well separated from the accessions 117 and 223. For $k = 3$ (Fig. 4), the accession 211 was still completely separated from the other ones. In contrast, plants from both accessions 223 and 117 were roughly separated into two groups with some plants from each accession falling into the group of the other one. Beyond $k = 3$, there was no detectable structure among the three accessions.

The three analyses MCA, UPGMA and Structure revealed a very similar decomposition into three groups, i.e.:

Fig. 3 Dendrogram (UPGMA method) of 3 oleaginous type from SSR markers analysis. The scale bar on the bottom of the figure represents the absolute distance (fraction of shared allele) between the sample. Accessions No. 117 from Gohitafla (*centre*), wîlèwè morphotype: No. 211, from Alépé (*southern part*), wîlèwè morphotype: No. 223 from Alépé (*southern part*), bebu morphotype

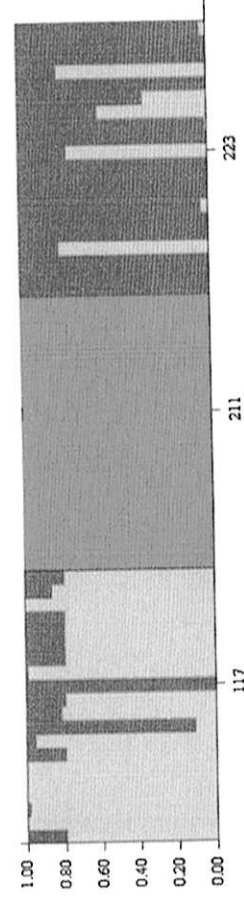
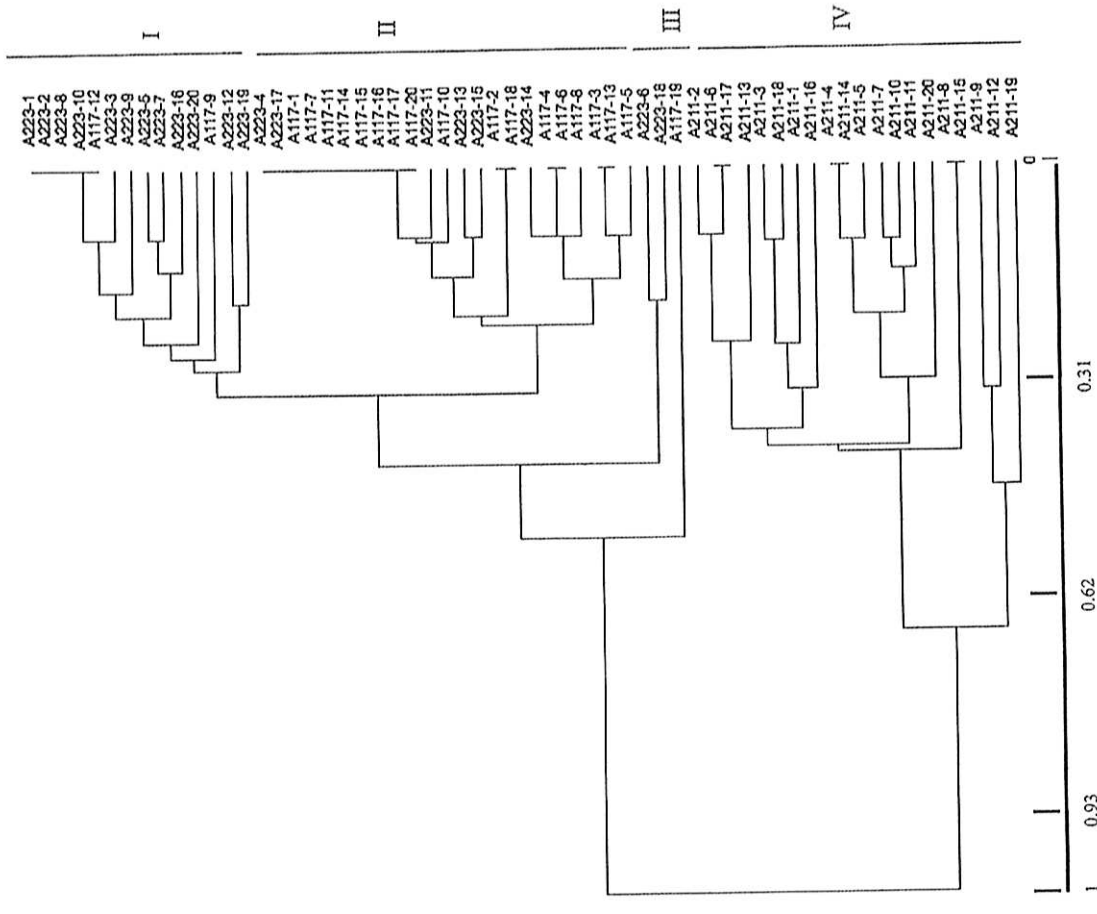


Fig. 4 Assignments tests when the estimated number of subpopulations (parameter k) is set to 3. The test is realized with the entire set of accessions [No. 211, from Alépé (*southern part*), wîlèwè morphotype: No. 223, from Alépé (*southern part*), bebu morphotype], by means of the Structure

software (Pritchard et al. 2000). For $k = 2$, the 2 subpopulations includes the accession 211 on one side and the accessions 223 and 117 on the other side. For $k > 3$, the subpopulations resulting from the Ward criterion are not structured anymore

Table 3 Analysis of molecular variance (AMOVA) of accessions of *C. lanatus* oleiginous type from Ivory Coast

Source	df	SS	MS	Est. var.	%D	P value*
Among pops	2	71.185	35.593	1.768	88	0.01
Within pops	57	13.222	0.232	0.232	12	
Total	59	84.407	35.825	2.000		

df degrees of freedom, SS sum of square, MS mean squares, Est. var. estimated variance, %D distribution of total variance

* Number of permutations: 999

- all the individuals from accession 211,
- all individuals from accession 117 except # 9 and 12,
- all the remaining individuals from accession 223 except # 13.

Some discrepancies between the two methods (MCA and UPGMA) are observed for the individuals 223-6, 223-18 and 117-19 that in the middle of cluster III in Fig. 3 but are not separated in Fig. 2. (We checked that even the third principal axis of the MCA does not separate them.)

Furthermore, the general separation of all accessions was established by molecular variance analysis (Table 3), which showed a genetic differentiation between accessions (88%, P -value = 0.01).

Discussion

The small number of alleles identified by the 9 SSR markers showed that individuals were not very polymorphic. A comparison can be made with *Cucumis melo* L., on which 25 SSR markers have been applied (Ritschel et al. 2004). These markers revealed 92 different alleles, which indicated a substantially higher polymorphism than in the case of *Citrullus lanatus*.

More quantitatively, an indication of allele diversity and frequency among the varieties is provided by the PIC values (Kwon et al. 2007). The average was relatively small: 0.37, with a range from 0.06 to 0.56. This can be compared with the PICs of other species available in the literature: *Citrullus lanatus* has an average of 0.53 and a range from 0.22 to 0.79 in the study of Joobeur et al. (2006) and an average of 0.34 and a range from 0.15 to 0.66 in the study of Kwon et al. (2007). *Cucumis melo* has an average of 0.45 and a range from 0.28 to 0.65 (Ritschel et al. 2004).

The GD revealed a large variability in the dispersion of plants of different accessions. The dispersion of accession 211 was the largest (GD = 0.25), followed by accession 223 (GD = 0.16). Accession 117, which is the only one cultivated in the center of Ivory Coast, had the lowest dispersion (GD = 0.13). The selection of the seeds (arising from a single fruit or a sample taken from several fruits from the same field), the seed flow) between farmers are possible factors that could account for the different genetic variability between accessions. More information about farming systems related to these accessions is needed to explain the difference of variability.

There was a lack of observed heterozygosity compared to the Hardy–Weinberg equilibrium (The F_{is} value for the sample including all the fields was significantly positive). This might suggest the presence of geitonogamy. This assumption is also corroborated by the fact that geitonogamy has been demonstrated in commercial watermelon cultivars (Ferreira et al. 2000; Loehrlein and Ray 2007). However, more statistics and studies are necessary to confirm this hypothesis.

It is also worth noting that genetic patterns corresponding to different accessions were quite well separated, as can be seen with the AMOVA. However, there was no correspondence between the morphological and molecular classification. On the first hand, accession 223 was closer to accession 117—although these two accessions are morphologically well separated—than accession 211 (which share almost the same morphology with accession 117). On the second hand, accessions 117 and 211 display a rather different genetic pattern (MCA, UPGMA). This is not too surprising as it has been demonstrated that the thick and fleshy pericarp phenotype, our morphotype «bebu»—is controlled by a single recessive gene (Gusmini et al. 2004). This shows that the morphological classification of this particular species is not relevant at the genetic level. This feature is certainly not reported in all plant species. In the case of *Oxalis tuberosa* Mol. for example, it is known that the correlation between morphological markers and molecular markers systems is weak but significant (Pissard et al. 2008).

In order to explain the low genetic diversity of *C. lanatus*, Dane and Liu (2007) demonstrated that the watermelon varieties have diverged into small populations. Moreover the domestication—and the

resulting selection—may have reduced the genetic diversity from the original watermelon crops.

Conclusion

The purpose of this work was to study the genetic diversity of *C. lanatus* in Ivory Coast. One of the main results of our analysis is the low genetic diversity observed from a sample of three accessions. Our analysis also provides the first evidence that the morphological variability does not match the genetic variation pattern in *C. lanatus*. This feature has to be taken into account if one wants to establish a seed bank in order to preserve or rationally exploit the genetic diversity. Our analysis has also shown that genetic dispersion can fluctuate significantly from one accession to another. Further studies with more plants are required to extend our knowledge of the genetic variability, both in a given accession and among different accessions. In the future, we also plan to shed light on the current ambiguities associated with the classification of *C. lanatus* in the Ivory Coast.

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