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GENETIC DIFFERENTIATION IN A COLLECTION OF CUCUMEROPSIS MANNII NAUDIN (CUCURBITACEAE) FROM CÔTE D'IVOIRE BY USING ISSR MARKERS

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ABSTRACT

Thirty accessions from three cultivars (small seeds, medium seeds and large seeds), collected from center, east, north and south of Côte d'Ivoire, were analyzed for genetic differentiation. Five primers (Sola1, Sola4, Sola7, Sola8 and B4) were used to identify ISSR markers in C. mannii. These primers revealed a total of 55 bands of which 52 bands (93.06 %) are polymorphic. Molecular Analysis of Variance (AMOVA) indicates a weak genetic differentiation of the three cultivars (Fst = 0.048 or 5 %; p = 0.01). When considering the collection area, a weak differentiation between accessions was observed (Fst = 0.027 or 3 %; p = 0.01). On the other hand, a strong genetic differentiation was observed between accessions of the same cultivar (Fst = 0.268 or 24 %; p = 0.01) and between individuals of the same accession, for which a highly significant differentiation (Fst = 0.261 or 74 % ; p = 0.01) was observed.

For the constitution of a gene bank, it is necessary to keep many individuals in a limited number of accessions from few regions. Indeed, the dendrogram of C. mannii indicates a weak differentiation between cultivars and therefore their belonging to the same common ancestor.

Keywords: Cucumeropsis mannii; Cucurbitaceae, genetic diversity; ISSR; polymorphic.

1. INTRODUCTION

The conservation and efficient management of plant genetic resources remains an important research challenge. In many countries of the South, agricultural production systems still rely on a wide range of varietal diversity that forms the basis for agricultural and socio-cultural development of communities. In addition, local plant genetic resources possess hardiness traits that are more favorable to stable and sustainable yields under current climate change conditions (Sadiki et al., 2010). In Sub-Saharan African countries, we are increasingly witnessing changes in agricultural systems oriented towards massive exploitation of export crops that are more economically profitable to the detriment of local species, some of which are seriously threatened with extinction. This is the case of the oleaginous cucurbits called "pistachio" or "egussi", which have long been absent from improvement programs and qualified as "minor crops" by the public authorities and the scientific community.

In Côte d'Ivoire, studies by Zoro Bi et al. (2006) showed that there are five species of oilseed cucurbits: *Citrullus lanatus* (Thunberg) Matsumara and Nakai, *Cucurbita moschata* Duchesne, *Cucumis melo* L. spp. agrestis Naudin, *Lagenaria siceraria* (Molina) Standley and *Cucumeropsis mannii* (Naudin). *Cucumeropsis mannii* is the second most widely distributed

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species. This species has a very high agromorphological diversity that is a potential source of genetic diversity, useful for plant breeding (Demol et al., 2001). According to Zoro Bi et al. (2006), there are three cultivars based on seed size within the species: small-seeded, medium-seeded, and large-seeded cultivars.

Despite its great importance, there is still no efficient program for the conservation and preservation of this species in Côte d'Ivoire. Relatively recent information on *Cucumeropsis mannii* has focused on botanical description (Akoegninou et al., 2006), agronomic evaluation (Achigan et al., 2006; Zoro Bi et al., 2006), biochemical composition (Badifu, 1993; Loukou et al., 2007) and enzymatic characterization (Koffi et al., 2008). To our knowledge, there is no study on the molecular characterization of Ivorian cultivars of *Cumeropsis manni*. Such a study can bring more precision to the genetic parameters. This will help to define the optimal sample size to be kept in the genebank and to implement a more efficient management strategy of the *C. mannii* genetic resources. In particular, the results of such studies will help to plan and direct survey missions, determine conservation and regeneration strategies to set up genetic improvement schemes.

The present study aims to evaluate the genetic diversity of local cultivars of *C. mannii*, to refine the analysis of the diversity and genetic structuring of the collection of the University Nangui Abrogoua. Specifically, it will involve the characterization of the genetic diversity of the collection of the University Nangui Abrogroua and the evaluation of genetic differentiation within and between Ivorian cultivars of *C. mannii* using ISSR markers.

2.MATERIALS AND METHODS

2.1. Materials

Plant materials were selected from a collection of *Cucumeropsis mannii* of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). The seed samples of thirty accessions were collected mainly in four geographical zones (South, East, North, and Centre) of Côte d'Ivoire (Table 1).

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Table 1. Characteristics of 30 *Cucumeropsis mannii* accessions used for genetic diversity analysis

N°	Accessions	Cultivar	Collection site	Collection zone	N°	Accessions	Cultivar	Collection site	Collection zone
1	NI128 (5)	Small	Manfla	Centre	16	NI385 (5)	Large	Zuénoula	Centre
2	NI265 (5)	Small	Soko	Est	17	NI189 (5)	Large	Manfla	Centre
3	NI173 (5)	Small	Assiè-koumassi	Est	18	NI129 (5)	Large	Manfla	Centre
4	NI154 (6)	Small	Assiè-koumassi	Est	19	NI281 (5)	Large	Bouaké	Centre
5	NI184 (5)	Medium	Agoua	Est	20	NI389 (5)	Large	Alépé	Est
6	NI270 (5)	Medium	Soko	Est	21	NI352 (5)	Large	Flakiè	Est
7	NI181 (5)	Medium	Amanda	Est	22	NI349 (5)	Large	Flakiè	Est
8	NI126 (5)	Medium	Assabri	Est	23	NI364 (5)	Large	Tefrô	Est
9	NI312 (5)	Medium	Laoudiba	Est	24	NI363 (5)	Large	Tefrô	Est
10	NI273 (5)	Medium	Soko	Est	25	NI351 (5)	Large	Flakiè	Est
11	NI387 (5)	Medium	Korhogo	Nord	26	NI317 (5)	Large	Laoudiba	Est
12	NI196 (5)	Medium	Korhogo	Nord	27	NI369 (5)	Large	Tefrô	Est
13	NI195 (5)	Medium	Korhogo	Nord	28	NI311 (5)	Large	Djomponé	Est
14	NI089 (5)	Medium	Abidjan	Sud	29	NI316 (4)	Large	Laoudiba	Est
15	NI130 (5)	Medium	Lamikro	Sud	30	NI381 (5)	Large	Kor hogo	Nord

Sample is composed by 15 accessions of large seeded cultivar, 11 accessions of the medium seeded cultivar and four accessions of the small seeded cultivar (Figure 1). Five seeds per accession, in total 150 seeds were analyzed.

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Figure 1. Seeds of the three cultivars of *Cucumeropsis mannii*. A: small seeds; B: medium seeds; C: large seeds (Koffi et al., 2006).

2.2. Methods

A total of 150 seeds, or five individuals per accession, were selected and sown. Then, the young leaves were collected and put in Eppendorff tubes to be immediately preserved in ice. The sampled leaf fragments were stored in a freezer at -80° C until the time of extraction.

2.2.1. ADN extraction

Genomic DNA was extracted from leaf fragments following the method of Dellaporta et al. (1983) with slight modifications. Leaf fragments of approximately 300 g were ground directly in Eppendorff tubes in sterilized Fontainebleu sand. Then, 800 µL of extraction buffer with 150 µL of SDS (10%) and 4 µL Beta mercaptoethanol were added to the grind. The extraction buffer consisted of 1% Polyvinylpyrrimidone (PVP) and 100 mM Tris-HCl pH 8.0. The solution was incubated in a water bath at 65° C for at least 30 min with inversion shaking every 10 min. This step allows the lysis of the nuclear membranes and the denaturation of the DNA-associated proteins. After that, 250 ml of sodium acetate (5 M) is added, and the solution is cooled in ice for 20 min. DNA was extracted once with 500 µL of 70° C isopropanol following centrifugation at 12,000 revolutions per minute (rpm) for five min at room temperature. The supernatant (800 µL) was collected in an Eppendorff tube and supplemented with 800 µL of 70°C isopropanol for a second DNA extraction. Subsequently, the DNA was precipitated with 350 µL of isopropanol for 1 h at -20°C before further cold centrifugation (4°C) for 10 min. DNA was wrung out by inverting the Eppendorff tube onto absorbent paper after removal of the supernatant. Finally, the DNA was washed twice with 500 µL of 70% ethanol and stored at -20° C in 100 µL of Tris-EDTA (TE) buffer consisting of 10 mM Tris-HCl pH 8.0 and 1 mM EDTA pH 8.0, until the time of amplification

The extracted DNA was quantified by performing 0.8% agarose gel electrophoresis subjected to 90 V for 1 h. TBE electrophoresis buffer pH 8.0 (40mM Tris-Hcl, 20 mM Boric acid, 1 mM EDTA pH 8.0) was used for this purpose. The actual quantification was performed using the Smart LadderTM standard which gives bands whose migration distance and intensity can be correlated to a length (base pair) and concentration of DNA.

2.2.2. PCR and electrophoresis conditions

Five ISSR primers (Eurogentec SA, Seraing, Belgium) were tested for PCR. Of these, primers Sola 7, Sola 8 and B 4 are labeled at the 3' end while Sola 1 and Sola 4 are labeled at the 5' end. In addition, except for the Sola 1 primer which has a microsatellite motif consisting of three

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nucleotides (ACA), the others have di-nucleotide motifs. Hybridization temperatures range from 50°C for Sola 1 to 55°C for Sola 4 and Sola 8 (Table 2).

Table 2. ISSR primers used and their hybridization temperatures

Primers	Séquences (5' 3')	Annealing temperature
Sola 1	BDB-$(ACA)_5$	50 °C
Sola 4	VHV-$(GT)_7$-G	55 °C
Sola 7	$(AG)_8$-YT	51 °C
Sola 8	$(GA)_8$-YC	55 °C
B4	$(GA)_8$-C	51 °C

Polymerase Chain Reaction (PCR) amplifications were performed in a reaction volume of 10 µL. This consisted of 0.2 µL (1 Unit) of Taq DNA polymerase (Eurogentec), 1 µL of PCR buffer (Eurogentec), 0.4 µL of 2 mM dNTPs (Eurogentec), 0.16 µL of ISSR primers, 1.5 µL (25 µM) of DNA extract, and 6.44 µL of sterile distilled water.

Five primers were used in this study. Primers with the same hybridization temperature were tested simultaneously on the same gel. Thus, for primers Sola 1, Sola 7 and B 4 of hybridization temperature 51° C. A total of 32 individuals are selected per test until all samples are analyzed. The same is true for Sola 4 and Sola 8 (55° C) where 48 individuals are analyzed per trial.

Each PCR run included a denaturation phase of the extracted double-stranded DNA (5 min at 95°C), 36 cycles of [30 s at 95°C (DNA denaturation), 45 s at a temperature that varied depending on the primer (primer hybridization), 2 min at 72°C (elongation)] and 5 min at 95°C (final elongation).

Each amplification product was analyzed by performing electrophoresis at 90 V for 1 h 45 min on agarose gel (Molecular grade II from Eurogentec) with TBE buffer. The gel was stained by addition of 1.4 µL/100 mL of 0.7 mM ethidium bromide concentration just before solidification. The amplified fragments were visualized using the Vilber Lourmat documentation system with a UV transilluminator, a cage equipped with a camera, and a viewing screen with a 3.5-inch floppy disk drive.

2.2.3. Data collection and analysis

From the gel images, the phenotype of each individual at each locus was scored using the binary method (1 when the band is present and 0 when the band is absent). The data matrix generated for each species was then used to calculate diversity and genetic structure indices. These include the percentage of polymorphic bands, the Nei diversity index, and the rate of gene differentiation.

Average PIC values were calculated per locus and per primer. They will be used to assess the ability of the primers to reveal genetic diversity within the 150 samples analyzed. The means of these indices were compared using the non-parametric Mann-Whitney test, using Statistica, (2005).

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The rate of gene differentiation (Fst) between accessions was tested according to cultivars and collection areas by Molecular Analysis of Variance or AMOVA using GenAlEx software, version 6.3 (Peakall and Smouse, 2006). Genetic distances between different pairs of accessions were calculated to construct a dendrogram from the resulting distance matrix. Discriminant factor analysis was performed with XLSTAT software to determine the level of clustering of different accessions based on cultivars. Phylogenetic trees or dendrograms were drawn using respectively XLSTAT for the phylogeny between accessions and Darwin version 5.0.158 (Perrier and Jacquemard, 2006) for the phylogenetic relationships between the 150 samples analyzed. The robustness of the dendrograms was tested by 1000 bootstraps.

3. RESULTS

3.1. Marker polymorphism rate

A total of five primers were used in this study to reveal ISSR markers in *Cucumeropsis mannii*. Figure 2 shows the electrophoretic profile of 16 individuals from PCR amplification using the Sola 8 primer. The molecular weight of the loci (bands) ranges from 200 to 1500 base pairs. In this Figure 3, for example, the 1000 base pair locus that is present in individuals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, and 16, but absent in individuals 13 and 15, is polymorphic. However, the 400 base pair locus is said to be monomorphic because it is simultaneously present in all 16 individuals analyzed.

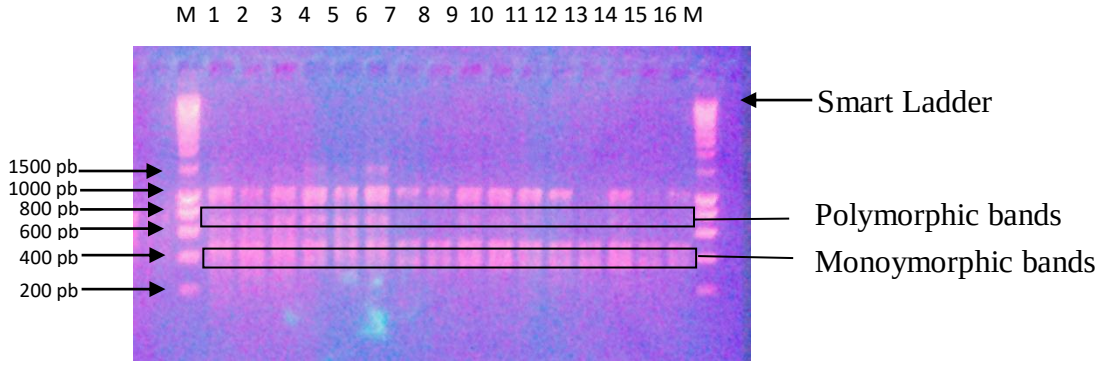

Figure 2. Electrophoretic profile of 16 individuals amplified by the Sola 8 primer in *Cucumeropsis mannii*. M channels indicate the molecular weight marker (Smart Ladder): channels (1-2): NI184 (medium seeds); channels (3-7): channels NI189 (large seeds); channels (8-12): NI270 (medium seeds); channels (13-16): NI196 (medium seeds).

Primers revealed a total of 55 bands with an average of 11 bands per primer. The lowest number of bands was observed with the Sola 8 primer which revealed 8 bands while the Sola 4 and Sola 7 primers gave the highest number of bands (13 bands). Of the 55 bands amplified, 52 showed polymorphisms in the range of 93.06 %. The average number of polymorphic bands per primer was 10.5 (Table 3). The average PIC values per polymorphic loci ranged from 0.13 ± 0.10 for Sola 4 to 0.39 ± 0.11 for the Sola 7 primer, with an average of 0.27 ± 0.11 for all primers used. Highest PIC values are obtained from primers Sola 7 and Sola 8 (Table 3).

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Table 3. Polymorphism indices of ISSR primers in *Cucumeropsis mannii*

Primers	Sequence (5'-3')	N	NP	%P	PIC
Sola 1	BDB-$(ACA)_5$	9	7	77,8	0,23 ± 0,20
Sola 4	VHV-$(GT)_7$-G	13	13	100	0,13 ± 0,10
Sola 7	$(AG)_8$-YT	13	13	100	0,39 ± 0,11
Sola 8	$(GA)_8$-YC	8	7	87,5	0,32 ± 0,20
B4	$(GA)_8$-C	12	12	100	0,18 ± 0,26
Total		55	52		
Mean		11	10,5	93,06	0,28 ± 0,11

N: Numbers of bands**; NP:** Number of Polymorphic bands**; %P:** Percentage of Polymorphic bands; **PIC**: Polymorphic Information Content

3.2. Genetic diversity of *Cucumeropsis mannii*

In order to verify the possible grouping of the examined accessions according to cultivars, the Discriminant Factor Analysis (DFA) was performed. The results show that the 150 individuals from the different accessions are grouped into three main clusters (Figure 3). The first two axes completely discriminate the 150 individuals into three groups (100% variability, i.e., 57.83% variation for axis 1 and 42.17% for axis 2). The first group is essentially made up of individuals of the large-seeded cultivar (colored blue) that are grouped to the left of the axis 1 plane, on either side of the negative part of axis 2. The second group, colored green and located to the right of the plane, on either side of the positive part of factorial axis 2, includes individuals belonging mainly to the medium-seeded cultivar and a few individuals of the large-seeded cultivar. The third group isolated at the bottom of the plane, on either side of the negative part of the factorial axis 1, is formed mainly by individuals of the small-seeded cultivar (orange color). It should also be noted that, the scatterplots of the GI and GII groups are relatively highly interleaved while those of the GII and GIII or GI and GIII groups are very slightly interleaved.

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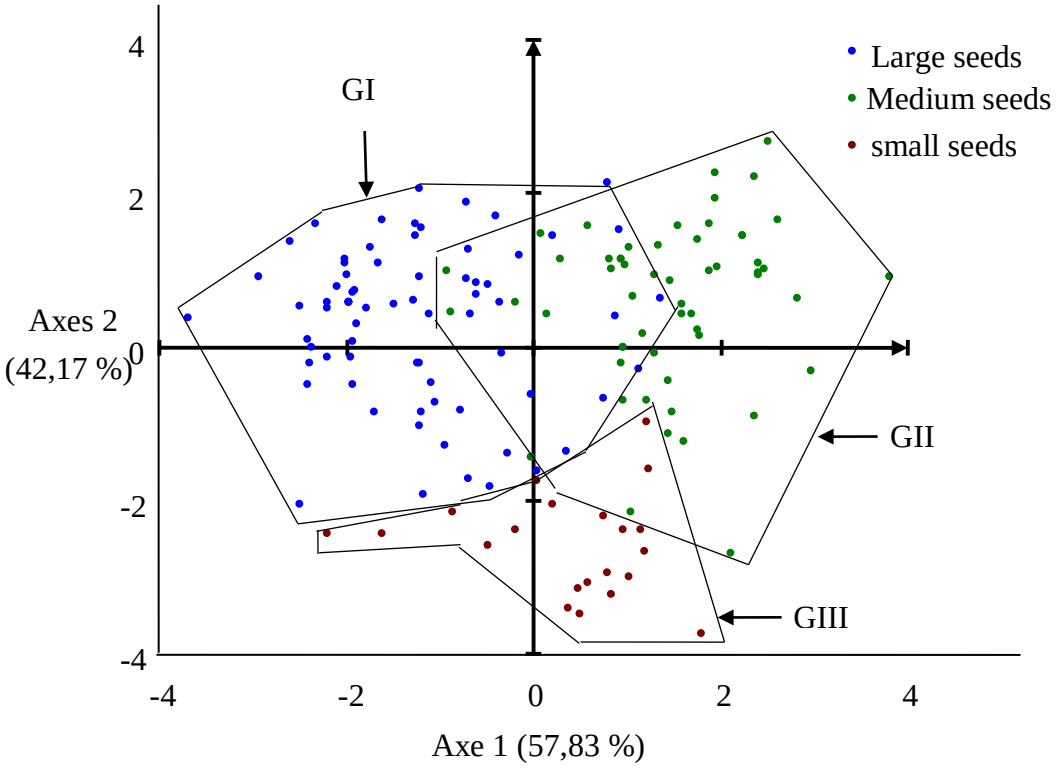

Figure 3. Highlighting groups of individuals identified from the SFM on *Cucumeropsis mannii.*

Molecular Analysis of Variance (AMOVA) was performed to assess the degree of differentiation of accessions by cultivar and by collection area (Table 4). These results indicate that there is little gene differentiation among the three cultivars of *C. mannii* (*Fst* = 0.048 or 5%; *P* = 0.01). On the other hand, a strong differentiation is observed between accessions of the same cultivar (*Fst* = 0.268 or 24 %; *p* = 0.01). The same was true for individuals analyzed by accession, for which differentiation was highly significant (*Fst* = 0.261 or 74 % ; *p* = 0.01).

At the level of the collection areas of the different accessions, the AMOVA results show that within each area there is a significant difference between accessions (*Fst* = 0.256 representing 27% and *P* = 0.01). There is also a significant difference between individuals within an accession (*Fst* = 0.261 representing 74% and *p* = 0.01). However, when the analysis was carried out using the area of origin as a criterion, a very weak gene differentiation between the accessions analyzed was observed (*Fst* = 0.027 or 3 %; *p* = 0.01).

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Table 4. Molecular Analysis of Variance (AMOVA) testing genetic differentiation of 30 accessions of *Cucumeropsis mannii* by cultivars and collection zone.

Level of diversity	Source of variation	DL	SCE	CM	F	%F	*Fst*	*p-value**
Cultivars	between cultivars	2	84,45	42,22	0,65	5	0,05	0,01
	between accessions per cultivar	27	702,44	26,01	3,24	24	0,27	0,01
	between individual per accession	120	1179,05	9,83	9,83	74	0,26	0,01
Zones	between zones	3	68,33	22,78	0,36	3	0,03	0,01
	between accessions per zone	26	718,55	27,64	3,56	27	0,26	0,01
	between individual per accession	120	1179,00	9,83	9,83	74	0,26	0,01

***** Obtained after 99 random permutations. DL: Degree of freedom; SCE: Sum of squares of deviations; CM: Mean square of deviations; F: Variance; % F: Percentage of variation; Fst: Gene differentiation rate.

3.3. Phylogeny

The dendrogram made from the genetic distances between accessions, using the Ward method (Ward, 1963), revealed three main groups of accessions (Figure 4). The groupings of accessions were made independently of cultivar membership. Indeed, within the three groups, the presence of small-seeded, medium-seeded and large-seeded cultivars is observed. For example, in group C, a grouping of accessions NI128, NI154 and NI173 (small seeds), NI129, NI316, NI317 and NI369 (large seeds), and NI312, NI273 and NI196 (medium seeds) was observed. Group B is composed of accessions of the medium-seeded and large-seeded cultivar. In group A, there is only one accession of the small-seeded cultivar (NI265). This accession is paired with accessions NI349, NI352, NI364, NI385 and NI389 of the large seeded cultivar and NI181, NI130, NI195 (medium seeded). In addition, it should be noted that the groupings are made independently of the collection areas. Indeed, in the different groups, almost all regions are represented, thus confirming the weak differentiation between zones revealed by the AMOVA.

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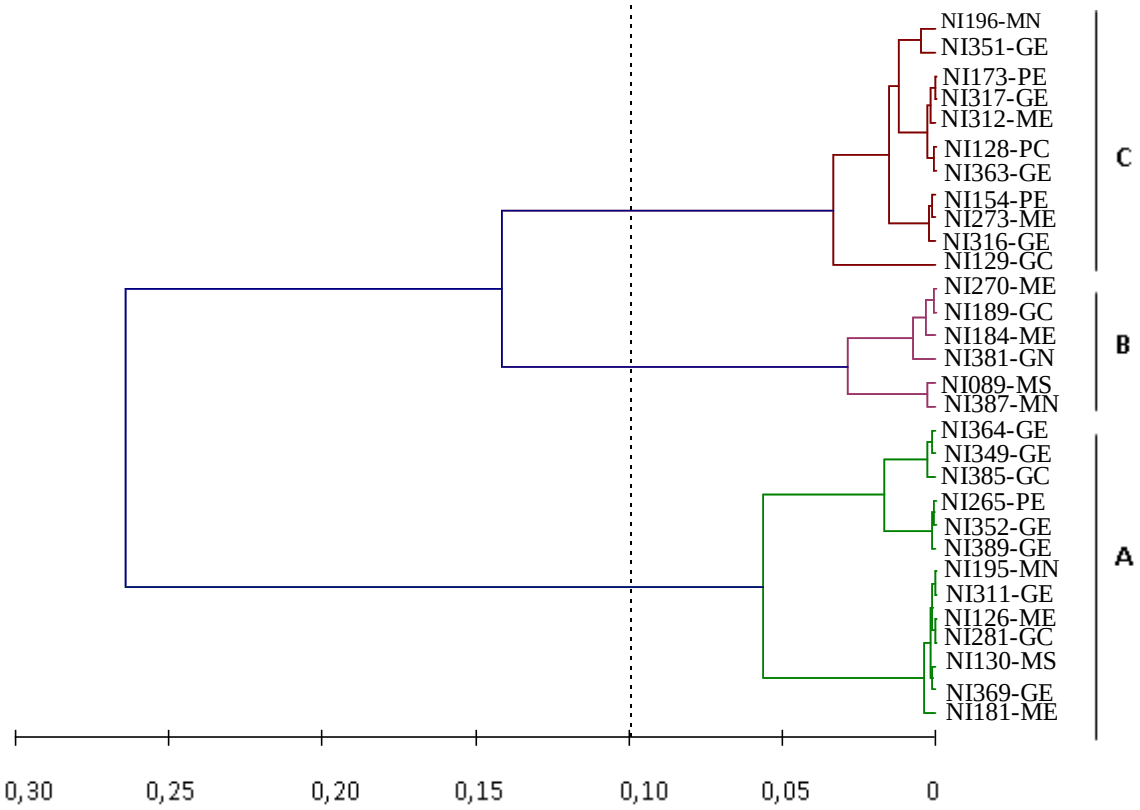

Figure 4. Dendrogram obtained by Ward's method (Ward, 1963) for genetic differentiation, based on ISSR markers, among 30 accessions of *Cucumeropsis mannii*. Accession numbers are followed by cultivar type (P: small seeds; M: medium seeds; G: large seeds) and region of origin (C: Central; E: Eastern; N: Northern; S: Southern).

The molecular data collected allowed the establishment of phylogenetic relationships between 150 individuals from three cultivars of *Cucumeropsis mannii* analyzed. Figure 5 shows that the distribution of individuals is not based on cultivars or collection area. The UPGMA dendrogram grouped the 150 individuals into three main groups (A, B, and C) separated by relatively low boostraps values (287 to 285) and in which each cultivar is represented. In group A, for example, individuals from the same cultivar are scattered. Group B consists mainly of individuals of the large-seeded cultivar with 17 representatives, four representatives of the medium-seeded cultivar, and one individual of the small-seeded cultivar (Table 5).

Group C contains the largest number of individuals (105 individuals). This group is made up of several subgroups in which some samples of the same cultivar or accession are isolated. This is the case of individuals from accessions NI126 (medium seeds) and NI265 (small seeds) which are all grouped in the same subgroup.

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Table 5. Composition of the three groups highlighted by the UPGMA dendrogram

Groups	Individual number			
	Large seed	Medium seed	Small seed	Total
A	9	8	6	23
B	17	4	1	22
C	48	43	14	105

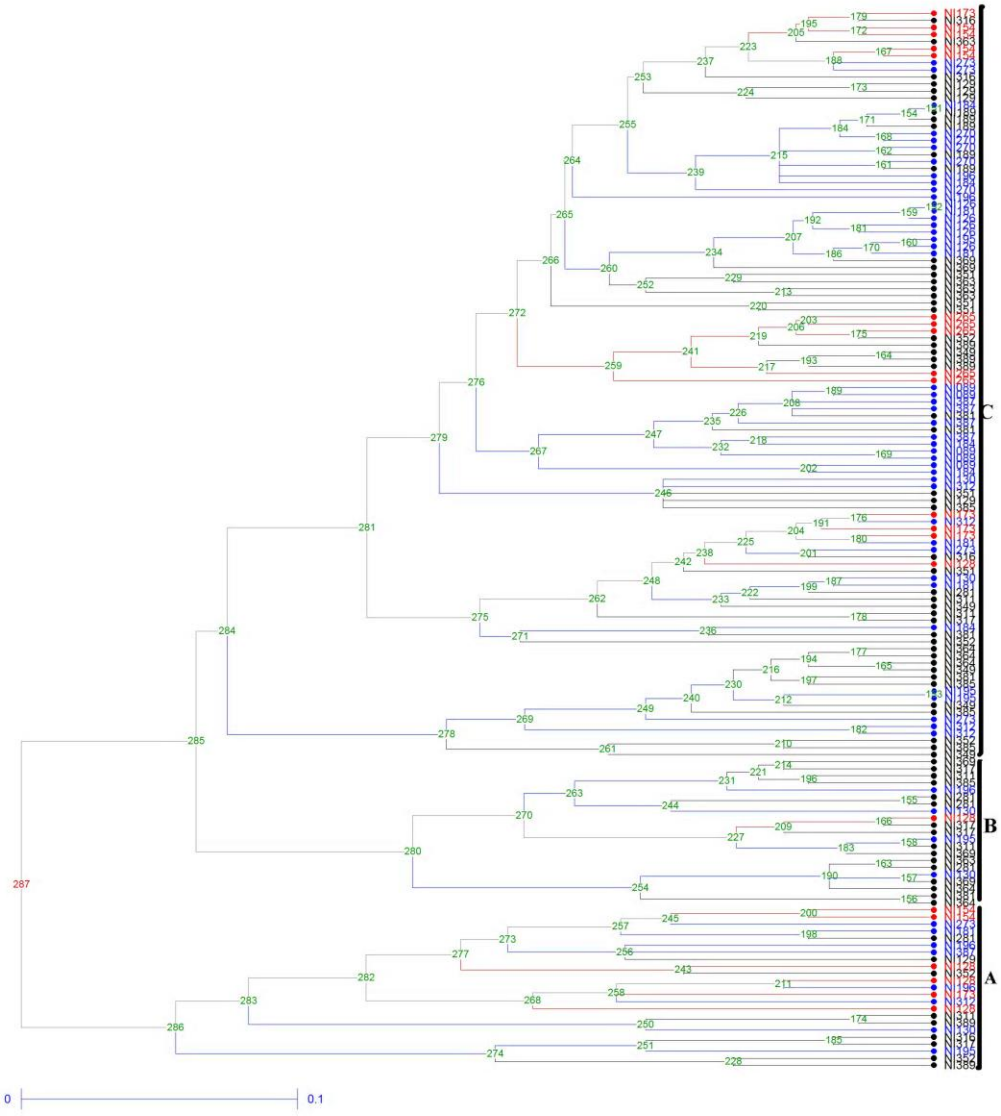

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Figure 6. Dendrogram obtained by UPGMA for genetic differentiation, based on ISSR markers, between 150 individuals from three cultivars of *Cucumeropsis mannii* from the genetic distance of Sokal and Michener. Large seeds (back) ; Medium seeds (blue) ; Small seeds (red)

4. DISCUSSION

Five primers (B 4, sola 1, Sola 4, Sola 7, and Sola 8) were applied to 30 accessions of *Cucumeropsis mannii* to assess the genetic diversity of this species. In a similar study involving another cucurbit, *Benincasa hispida* (Thunb.) Cogn. five ISSR primers were used by Verma et al. (2007). The primers used revealed 55 bands with 93.056% polymorphic bands. These results are similar to those obtained from ISSR markers in other cucurbits. Indeed, in *Cucurbita pepo*, *Lagenaria siceraria* and *Citrullus lanatus*, 74% (Paris et al., 2001), 96.4% (Koffi et al., 2008), and 97.6% (Djè et al., 2010) of polymorphic bands were observed respectively.

The results show that ISSR markers are highly polymorphic and can, therefore, be used to efficiently reveal genetic variability in *C. mannii*. The highest average ICP values per loci, obtained with primers Sola 7 and Sola 8, indicate that the latter have a higher level of polymorphism than the other primers. Indeed, according to Blair et al. (1999), di-nucleotide repeats are much more frequent in the genome than tri- and tetra-nucleotide repeats. This would favor the binding of primers with di-nucleotide motifs (Sola 7 and Sola 8) during amplification of ISSR markers. These results are like those of Balfourier et al. (2006) who, following work on wheat, showed that primers with di-nucleotide motifs revealed greater diversity than those with tri- and tetra-nucleotide motifs. However, our results are contrary to those of Tahi, (2006). Indeed, this author showed that the ISSR primers used for the study of genetic diversity of *Citrullus lanatus* present statistically identical average values of the PIC whatever the basic motif.

The different analyses carried out in this study showed little differentiation between collection areas and between cultivars. However, a strong differentiation of accessions and individuals per area and per cultivar was observed. The low genetic variability between regions (Fst = 0.027 or 3%; p = 0.01) could be explained by a significant gene flow between cultivation areas. Indeed, to grow crops, farmers most often exchange seeds between neighbors or simply buy seeds on the market. Thus, the same genotype could easily be dispersed in different regions at the same time. This same finding was made by Romao, (2000) in melon from Northeast Brazil and in *Citrullus lanatus* from Côte d'Ivoire (Djè et al., 2010; Minstart et al., 2011). Furthermore, our results are consistent with those of Koffi et al. (2008) who argue that the Ivorian cultivars of *Cucumeropsis manni* are derived from a common ancestor.

The low genetic differentiation observed between cultivars could be related to farmers' agricultural practices. Most often after the harvest, some seeds are stored for the following season. During this storage period, the seeds, poorly preserved, lose their germinative powers. This would constitute a loss of a certain number of genotypes and would contribute to the decrease of genetic diversity for the following generations. Also, the phenomena of genetic drift, founder effect, and directed selection of seeds that favor one or few genotypes, would lead to a great loss of genetic diversity in small populations such as cucurbits grown by women in Côte d'Ivoire (Behera et al., 2008; Djè et al., 2010; Jian et al., 2010). These results are consistent with those obtained by Koffi et al. (2008), who based on enzymatic data indicated high genetic similarity among *C. mannii* cultivars.

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Also, although the SFM showed a classification of accessions into three main groups that are relatively intertwined, the phylogenic study using Ward's method (Ward, 1963), indicates that the grouping of accessions was done independently of cultivars and collection areas. Similarly, the classification of individuals by the UPGMA method indicates that the groupings are not made according to cultivars or collection areas. These results could be the consequence of the strong differentiation observed between individuals of the same accessions (*Fst* = 0.261; *p* = 0.01). Thus, it seems that the differences observed between individuals are not related to the environments but come from variation in the genetic constitution of the plants (Demarly, 1977). Indeed, this diversity could be explained by the reproductive regime of this species. *C. mannii* is a monoecious and allogamous plant. Each individual, because of cross-fertilization, may have a totally different genetic makeup from the other individuals. In fact, according to Hamrick and Godt, (1990), in plants that are essentially cross-pollinated, up to 90% of the variability can be observed at the intra-population level; that is, at the level of the individuals that make up the population.

For the establishment of a gene bank representative of the diversity of the species, the collection can be done by considering many individuals and a limited number of accessions from few regions. For the establishment of a core collection from the gene bank of the University of Nangui Abrogoua, it will be necessary to keep a small number of accessions with many individuals for each of the selected accessions.

5. CONCLUSION

The implementation of any conservation program for plant genetic resources requires the characterization of the genetic diversity of the seeds to be conserved. This characterization must be able to provide the information necessary to collect the germplasm that is most representative of the allelic richness of the species whose genetic diversity is to be conserved. In this study, ISSR molecular markers were used to assess the genetic diversity of *C. mannii*.

The high level of polymorphism in the amplified bands indicates that ISSR markers are efficient in revealing diversity in *C. mannii*. It was shown that there was little differentiation between cultivars in the collection at the University of Nangui Abrogoua. The high genetic diversity, both between accessions and between individuals analyzed, suggests that individual accessions of *C. mannii* constitute valid genetic units for conservation. Thus, to better understand the full structure of genetic diversity within local *C. mannii* cultivars, a small number of accessions and a large number of individuals per accession must be considered. This will allow to have, in an optimal way, a well representative sample of the genetic diversity.

Thus, a small number of accessions per cultivar would be sufficient to conserve a significant proportion of the diversity present in situ. For the establishment of a core collection from the Nangui Abrogoua University gene bank, it will be necessary to keep a small number of accessions each containing many seeds.

To better assess the number and location of populations to be conserved, it would be useful to examine groups of more related accessions and define a stratified sampling strategy based on these groups. This work would require characterization of populations of the species under consideration by collecting identifying data from collection sites such as geographic coordinates, climate, soil, and surrounding vegetation. It will also be necessary to implement a permanent regeneration plan for accessions stored in the Nangui Abrogoua University gene bank. This will allow for the long-term conservation of existing diversity.

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