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Genetic Diversity and Population Structure of *Petiveria alliacea* in Southern Benin Using Transferable Microsatellite Markers

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Abstract

Petiveria alliacea is a uterotonic plant that effectively helps resolve uterine contractility abnormalities in traditional medicine. However, scientific knowledge of its diversity remains very limited to date. This study aimed to assess the transferability of eleven SSR markers from *Phytolacca acinosa* to *Petiveria alliacea* in order to evaluate its genetic diversity. Genomic DNA was extracted from 28 accessions of *Petiveria alliacea* sampled in southern Benin for PCR amplification using eleven pairs of SSR primers derived from *Phytolacca acinosa*. UPGMA and PCoA analyses were performed using NTSYS version 2.11a software to assess the genetic structure within the collection. Among the 11 markers studied, nine were transferable (81.82%), and six were polymorphic (54.55%). A total of 25 alleles were observed, with an average of 4.16 alleles per locus. The expected heterozygosity (He) ranged from 0.227 to 0.846, with an average of 0.633, while the observed heterozygosity (Ho) ranged from 0.150 to 1.000, with an average of 0.670. The mean fixation index (Fis) was −0.005. The dendrogram constructed using the UPGMA method revealed two main groups at a similarity rate of 73%, which further divided into four subgroups at a similarity rate of 65%. The studied collection exhibited high genetic diversity. It is therefore urgent that these data be used as a basis for establishing conservation and domestication programs to ensure the efficient and sustainable use of the species.

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Keywords

Petiveria alliacea, *Phytolacca acinosa*, Transferability, SSRs or Microsatellites, South Benin

1. Introduction

Medicinal plants have been used to treat various ailments since time immemorial, often containing natural compounds with recognized therapeutic properties [1]. Modern medicine sometimes draws on these plant resources, as global interest in traditional medicine continues to grow. Although traditional medical practices in Africa require significant improvement compared to those in India or China [2], their use is increasing among approximately 80% of the African population living in rural areas due to the high costs of modern medical services [3]. In addition, some people living in urban areas and even industrialized countries also turn to nature for their healthcare needs [4]. With over 200,000 plant species recorded in tropical African nations, of which 5,400 are used in traditional medicine [5], natural medicine and pharmacopeia remain the primary users of these plants, with 70% of the populations in Third World countries relying on them [4].

In Benin, for example, more than 507 species are used to treat various human ailments [6]. *Petiveria alliacea*, a herbaceous plant of the *Phytolaccaceae* family, is one of these medicinal plants [7]. In Benin, it grows near houses and is widely used in folk medicine [8]. It is commonly used in southern Benin for its uterotonic properties during difficult deliveries, as it triggers contractions after being triturated and applied by massage on the stomach and hips [9] [10]. According to Ayedoun *et al*. [8], the oil obtained from the root of *Petiveria alliacea* contains chemical compounds such as benzaldehyde (48%), dibenzyl disulfide (23.3%), dibenzyl trisulfide (9.4%), and cis- and trans-stilbene (8.1%). Thus, *Petiveria alliacea* exhibits antimicrobial properties, and the ethanolic extract of its leaves is effective in limiting the growth of certain microorganisms, including *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Rhizopus* sp., and *Aspergillus niger* [11].

Despite the use of uterotonic plants in many developing countries, numerous women continue to suffer from childbirth-related complications [12]. This situation could be attributed to the lack of in-depth scientific knowledge regarding the diversity, safety, efficacy, and mechanisms of action of these plants. Given the multiple pressures exerted by human activities on the environment, it is essential to implement a program aimed at exploring the genetic potential of these plants to prevent their disappearance. Indeed, a species with high genetic diversity is more likely to adapt to environmental changes. Additionally, studying genetic diversity could lead to the discovery of rare alleles that may be associated with this uterotonic property [13]. They offer thereby opportunities for genetic improvement and provide valuable information for effective conservation.

Although understanding the phenotypic diversity of species is essential, it is now recognized that environmental factors influence phenotypic traits. This explains the growing interest in using molecular genetic evaluation to study plant genetic resources. Molecular genetic markers are the most suitable tools for diversity studies [14]. Among them, microsatellite markers, or SSRs, are considered the most appropriate for genetic diversity analysis [15]. However, no specific SSR markers have yet been developed for the species in question. Due to the complexity and high cost of developing species-specific SSR markers, the use of cross-species markers between genetically related species appears to be a viable strategy. Provan *et al*. [16] highlighted the potential of inter-species SSR marker amplification for genetic research. High genetic conservation has been suggested not only among species within the same genus but also potentially between genera within the same family [17]. Indeed, *Petiveria alliacea* and *Phytolacca acinosa*, belonging to the same family (Phytolaccaceae), have a chromosome number of 2n = 34 and 2n = 36, respectively. This chromosomal similarity suggests a certain level of genetic compatibility and a close phylogenetic relationship. Both species contain compounds with recognized medicinal properties and are used in traditional medicine for their therapeutic effects [18]. Genetic markers would be useful not only for the species Phytolacca acinosa but also for assessing genetic diversity and providing information on the genome of related species like *Petiveria alliacea* to ensure its sustainable use [19]. However, this technique could be subject to low transfer rates due to amplification inefficiency caused by variations in the flanking sequences. Additionally, reduced polymorphism could be observed, as even if the markers are amplified, their level of polymorphism may be lower in the non-target species.

This study will therefore utilize microsatellite markers specific to *Phytolacca acinosa*, a species from the same family as *P. alliacea*, to contribute to the molecular characterization of *P. alliacea* accessions collected in southern Benin. The goal is to identify the most promising subgroups for future exploitation of plant genetic resources with uterotonic properties.

2. Materials and Methods

2.1. Biological Material

P. alliacea is primarily found in tropical regions. In Benin, the plant is widespread in humid and forested areas with high rainfall, particularly in the southern part of the country, such as Atlantique, Mono, Couffo, Plateau, etc. The biological material utilized in this study comprises twenty-eight (28) accessions of *P. alliacea*, collected from various regions of southern Benin. Specifically, the samples were gathered from the departments of Atlantique, Ouémé, Plateau, Mono, and Couffo (**Figure 1**). Leaf samples were obtained from the following communes: Abomey-Calavi, Zè, Toffo, Kpomassé, Tori-Bossito (Atlantique); Adjohoun, Akpro-Misserété, Adjarra, Dangbo (Ouémé); Kétou, Pobe, Sakété (Plateau); Comè, Bopa, Athiémé (Mono); and Toviklin and Klouékanmè (Couffo) (**Table 1**).

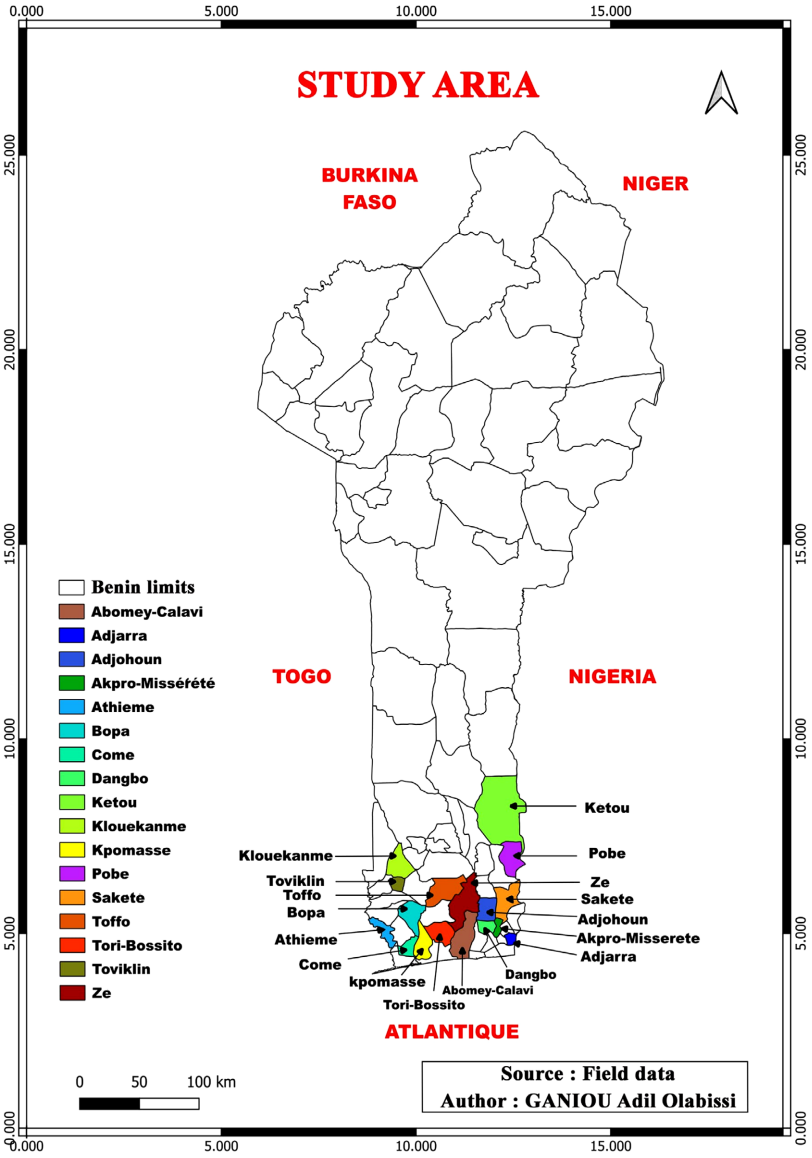

Figure 1. Sampling areas.

Table 1. List of different accessions of *P. alliacea* collected in southern Benin.

Departments	Communes	Villages	Access codes
Atlantique	Abomey-Calavi	Ouedo	CAL/OUE
	Toffo	Coussi	TOF/COU
	Ze	Hekanme	ZE/HEN
	Ze	Dangbo	ZE/DAN
	Tori-Bossito	Zounme	TOR/ZOU
	Kpomasse	Gogotinkpon	KPO/GOG
	Toffo	Doudji	TOF/DOU
	Kpomasse	Kpomasse	KPO2

Continued

Oueme	Adjohoun	Gbada	ADJO/GBA
	Akpro-Misserete	Abogome	ABO
	Adjarra	Honvie	HON
	Dangbo	Gbeko	DAN/GBE
Plateau	Ketou	Kpankou	KPAN
	Pobe	Chinangbore	POCH
	Ketou	Ilara	ILA
	Ketou	Atchoubi	KET/AT
	Ketou	Oloka	ORLO
	Sakete	Yoko	SAK/YOK
Mono	Come	Oumako	COM/OUMA
	Come	Come center	COM/CENT
	Come	Come center	COM/CEN
	Come	Oumako	COM/OUM
	Bopa	Dassatingo	BOP/DAS1
	Athieme	Kpinnou	ATH/KPI
	Athieme	Kpinnou	ATH/KPI2
	Bopa	Dassatingo	BOP/DAS
Couffo	Toviklin	Doko	TOV/DOK
	Klouekanme	Tchikpe	KOU/TCHI

2.2. Method

2.2.1. Leaf Collection

For each accession included in this study, three to four young leaves of *P. alliacea* were collected using scissors, wrapped in aluminum foil, and labeled. The samples were then stored in a cooler with ice and transported to the laboratory of Molecular Biology and Bioinformatics Applied to Genomics for DNA analysis.

2.2.2. Genomic DNA Extraction

DNA extraction involved isolating deoxyribonucleic acid (DNA) from cells or tissues for each sample. The extracted DNA was used to assess genetic variability within the accessions. For this study, 0.2 g of young, fresh leaves from each sample were weighed using a precision electronic balance, following the protocol described by Gawel and Jarret [20], as modified by Djedatin *et al.* [21]. A 2% CTAB (Cetyltrimethylammonium bromide) solution was used for extraction. The weighed young leaves were placed into a porcelain mortar, and 1000 µl (500 µl × 2) of CTAB preheated to 65˚C was added. The leaves were then manually ground with a porcelain pestle to dissociate the tissues and cells. The resulting homoge-

nate was transferred to a 2 ml Eppendorf tube, and 50 µl of SDS (20% Sodium Dodecyl Sulfate) was added, followed by gentle shaking. The tubes containing the homogenates were incubated at 65˚C in an oven for 45 minutes and then allowed to cool to room temperature. After cooling, 750 µl of CIA (Chloroform Isoamyl Alcohol) in a 24:1 ratio was added to each tube, followed by gentle agitation by manual inversion for 5 minutes. The samples were then centrifuged at 4500 rpm for 15 minutes. The supernatant from each sample was collected into a 1.5 ml Eppendorf tube. To precipitate the DNA, 800 µl of cold isopropanol (−20˚C) was added to each collected supernatant and gently mixed by manual inversion. The samples were incubated at −20˚C for 30 minutes, vortexed, and then centrifuged at 4500 rpm for 10 minutes. The isopropanol was decanted, leaving the DNA pellet in the tube. The pellet was washed with 500 µl of 70% ethanol, followed by centrifugation at 4500 rpm for 5 minutes, then drained to dryness. To ensure the purity of the DNA, the washing procedure was repeated three times. After the final wash, the tubes were left to dry at room temperature for at least eight hours. Once dry, the DNA pellets were suspended in 100 µl of sterile distilled water and stored at −20˚C. The quality and concentration of the genomic DNA were assessed by 1% agarose gel electrophoresis and visualized under a UV transilluminator. DNA concentrations were adjusted by dilution as needed.

2.2.3. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is an in vitro gene amplification technique that enables the replication of specific regions of the genome in large quantities, with a high multiplication factor. This method allowed us to amplify and copy targeted DNA sequences using primers designed to detect the presence of these sequences. In this study, eleven (11) *P. acinosa*-specific SSR markers were used to genotype the *P. alliacea* accessions. The sequences and repeat motifs of these markers are presented in Table 2 below. The PCR reagents used in this study are outlined in Table 3. Amplification was carried out using the program outlined in Table 4. This program was applied to all eleven (11) primer pairs, with the exception of the hybridization temperature, which was adjusted according to the specific requirements of each marker. The fragments are separated by migration on 2% agarose gel with 0.5X TBE at 100 V for 45 minutes and visualized on a UV transilluminator.

Table 2. List of microsatellite markers used [19].

Locus	Primer sequences (5'-3')	Hybridization temperatures (˚C)	Type of repetition
SL-34	F: TGTCCACCATAAAACACTT A: CCTCTTTCGCTACTTGC	45	$(ATC)_3$
SL-58	F: CTCCTGAATCTGATGGTGAA A: AGTTGTGCGTGTTGAAGAAG	50	$(ATC)_6$
SL-116	F: AGCCCCATACTCTACATC A: CTCTTCTTTCTTTTCTGTG	47	$(ATC)_5$

Continued

SL-200	F: TCCAACCCCCATCTCAAG A: CAAGATGCCCACCAATGA	52	$(GAT)_5$
SL-287	F: CAAGGAAGAACAAGAGG A: TAGGTGAGAGAAGGAGT	45	$(CAT)_7$
SL-307	F: GCCCCATTTCTTTTATTC A: AAGGGTCTTGGTGTTGAT	45	$(TCA)_4$
SL-324	F: TGGAAAGGTCGCTAATAC A: AAACACAAGGCTTCTGAG	46	$(TCA)_4$
SL-377	F: TGGACCCCTCTGCTACT A: GGACACCTCATCAGTAAA	47	$(TGA)_8$
SL-379	F: ATTTGGGTACTTGGGGAC A: TTTGATTTGGGAGGGACT	47	$(GTT)_4$
SL-385	F: GAATGATGGGGACAAGGA A: TTTGATTTGGGAGGGACT	48	$(ATG)_3$
SL-546	F: CCATCCATTCATCCTTTG A: ATTCACTATTCTTTGGCTTC	46	$(ATC)_4$

Table 3. PCR reagents, volumes and concentrations.

PCR reagents	Volumes (µl)	Concentrations
Buffer (with $MgCl_2$)	2.5	10X
dNTPs	0.75	2 mM
Primer (Forward)	2.5	10 µM
Primer (Reverse)	2.5	10 µM
Taq polymerase	0.1	0.5 U/µl
Sterile distilled water	5.65	
DNA template	6	25 to 35 ng
Total	20	-

Table 4. PCR program.

Program			
Steps	Temperature	Time	Number of cycles
Pre-Denaturation	95˚C	5 min	
Denaturation	94˚C	1 min	35 cycles
Hybridization		1 min	
Elongation	72˚C	2 min	
Final elongation	72˚C	5 min	
Cooling	4˚C	∞	

2.2.4. Data Analysis

Each accession was analyzed by examining the bands obtained at each locus, which were previously recorded as allelic compositions. The presence of a band was coded as 1, and its absence as 0. The scores were recorded in an Excel sheet, forming a data matrix. Several genetic diversity parameters were calculated, including the polymorphism rate (P), number of alleles (Na), average number of alleles per locus (A), observed heterozygosity (Ho), polymorphism information content (PIC), expected heterozygosity (He), and fixation index (Fis) [22].

- **Average number of alleles per locus (*A*)**: This parameter represents the abundance of alleles in a population and is calculated using the formula: $A = 1/L \sum a$ where *a* represents the number of alleles at a locus and **L is** the number of loci studied.
- Polymorphism rate (P): This is the percentage of loci with multiple alleles in the sample. The probability of observing at least two alleles at the same locus is influenced by the frequencies of the different alleles and the sample size. In this study, a locus was considered polymorphic when the frequency of the most frequent allele was less than or equal to 0.95.
- Observed heterozygosity rate ($_o$): This corresponds to the proportion of heterozygous individuals at a given locus. A relatively high rate is considered when it is greater than 50% [23].
- Polymorphism Information Content (PIC): This measures the genetic diversity of a locus and is estimated using the formula: $\text{PIC} = 1 - \sum fi^2$. where fi is the frequency of the i-th allele.
- Expected heterozygosity rate (H_e): This is calculated based on the Hardy-Weinberg equilibrium hypothesis, from the allele frequencies determined for each locus using the formula: $H_e = 1 - \sum pi^2$ with pi the frequency of the i^{th} allele at this locus. A relatively high rate is considered when it is greater than 50% [23].
- Fixation index (Fis): This measures the gap between the observed heterozygosity (H_o) and the expected heterozygosity (H_e). It is also referred to as the panmixia gap and is calculated as: $1 - (H_o / H_e)$. A positive value indicates heterozygote deficiency, while a negative value suggests a heterozygote excess.

Using the (0,1) matrix, Nei's genetic distances [24] were calculated between accession pairs. A dendrogram was then constructed to visualize the structure of the collection, employing the UPGMA method (Unweighted Pair-Group Method using Arithmetic Average) as per the SAHN procedure (Sequential Agglomerative Hierarchical Nested method) of the NTSYS software version 2.11a [25]. NTSYSpc is a system of programs used to find and display multivariate structure data. Many of the methods provided in NTSYSpc are associated with the field of phenetics. However, they are best interpreted as simple multivariate data analysis methods.

Additionally, to confirm potential groupings of the analyzed accessions, Principal Coordinate Analysis (PCoA) was performed using the DCENTER and EIGEN procedures of the same software. This analysis, based on the genetic coor-

dinate matrix, helped in better understanding the genetic differentiation between the identified groups.

3. Results

3.1. SSR Markers

In this study, we first tested eleven (11) *P. acinosa*-specific SSR markers on 28 *P. alliacea* samples to evaluate their amplification efficiency in this species. Figure 2 illustrates the electrophoretic profiles of the SL-379 and SL-546 markers, which were successfully amplified, revealing a total of seven (07) and four (04) alleles, respectively.

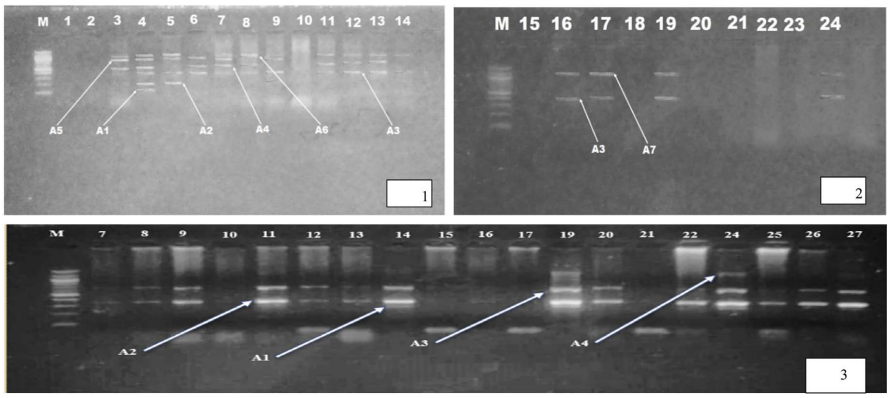

Figure 2. Electrophoretic profiles of SL-379 and SL-546 markers. 1: SL-379 marker showing alleles 1 to 6; 2: SL-379 marker showing alleles 3 and 7; 3: SL-546 marker showing 4 alleles (A1-A4); M: size marker; A: Allele.

Among the tested markers, nine (9) were found to be transferable (Table 5), resulting in a transferability rate of 81.82% (Figure 3). Of these transferable SSR markers, six (6) exhibited polymorphic characteristics, corresponding to a polymorphism rate of 54.55%. Table 5 presents the transferability results of the tested SSR markers, along with the observed allelic variation and Polymorphism Information Content (PIC) for each locus. The PIC values ranged from 0 to 0.846 across all transferable markers, with an average of 0.422. Among them, marker SL-379 was identified as the most informative, displaying a PIC of 0.846, indicating its high potential for assessing genetic diversity among the 28 accessions.

Table 5. Transferability, allelic variation, and PIC of the tested SSR markers.

Order number	Markers tested	Transferable (+/−)	Number of alleles observed	Allele variations	PEAK
1	SL-34	−	−	−	−
P2	SL-58	+	4	4-4	0.750
3	SL-116	+	1	1	0
4	SL-200	−	−	−	−
5	SL-287	+	1	1	0

Continued

6	SL-307	+	2	1-2	0.227
7	SL-324	+	1	1	0
8	SL-377	+	4	1-3	0.660
9	SL-379	+	7	2-6	0.846
10	SL-385	+	4	1-4	0.672
11	SL-546	+	4	1-4	0.644
Total	11	9	28	1-6	0.422

+: Positive, −: Negative, PIC: Polymorphism information content.

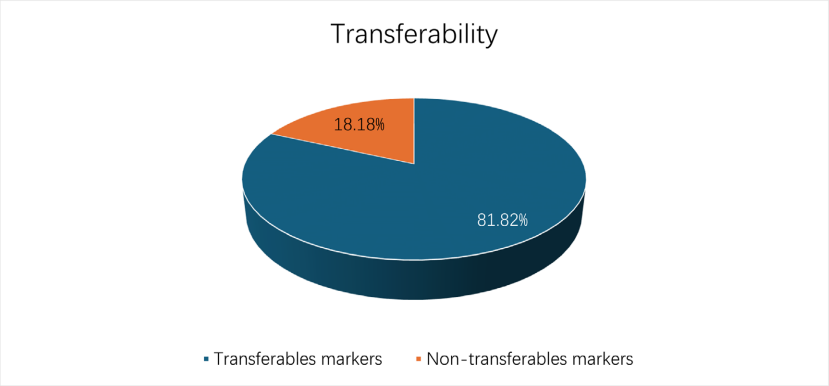

Figure 3. Transferability rate of SSRs markers.

Figure 4 presents the allele frequencies of polymorphic markers at the 1% threshold. The estimated allele frequencies for each locus range from 0.075 to 0.87. Notably, no rare alleles (i.e., those with a frequency below 0.05) were detected among the observed alleles.

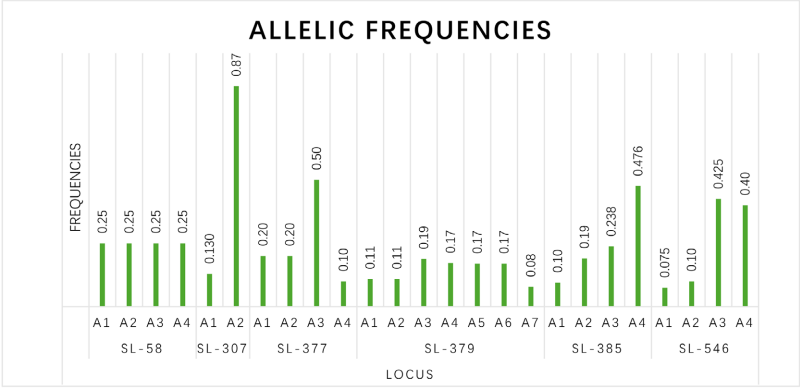

Figure 4. Allele frequencies of polymorphic loci.

3.2. Genetic Structuring Within the Collection

3.2.1. Assessment of Genetic Diversity Parameters

The six (6) polymorphic markers were used to evaluate genetic diversity parame-

ters within the collection (Table 6). A total of 25 alleles were identified across these markers, with the number of alleles per locus ranging from 2 to 7, and an average of 4.16 alleles per locus. The expected heterozygosity (H_e), also known as Nei's genetic diversity, varied from 0.227 to 0.846, with an average of 0.633. Observed heterozygosity (Ho) ranged from 0.150 to 1.000, with an average of 0.670. The fixation index (Fis), representing deviation from panmixia, ranged from -0.46 to 0.50, with an average of −0.005. Among the markers, SL-307, SL-377, and SL-385 exhibited positive Fis values, indicating a heterozygote deficiency. In contrast, SL-58, SL-379, and SL-546 had negative Fis values, suggesting an excess of heterozygotes.

Table 6. Values of genetic diversity parameters according to loci.

No.	Locus	Sample size (N)	Number of alleles (Na)	Effective allele number (Ne)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Fixation index (Fis)
1	SL-58	12	4.000	4.000	1.000	0.750	−0.333
2	SL-307	20	2.000	1.150	0.150	0.227	0.339
3	SL-377	6	4.000	1.660	0.330	0.660	0.500
4	SL-379	16	7.000	4.000	1.000	0.846	−0.182
5	SL-385	10	4.000	2.100	0.600	0.672	0.107
6	SL-546	17	4.000	2.353	0.940	0.644	−0.460
Average	-	13.50	4.166	2.544	0.670	0.633	−0.005

3.2.2. Dendrogram Construction and Principal Coordinate Analysis (PCoA)

The estimated genetic distance matrix was used to construct a dendrogram, revealing two main groups with a similarity rate of 73% (Figure 5). These groups are further divided into four subgroups, sharing a similarity rate of 65%. Each of the two main groups (I and II) consists of 14 accessions, representing 50% of the collection per group. Group I is predominantly composed of accessions from the Atlantique (42.86%) and Mono (28.57%) departments, whereas Group II primarily includes accessions from Ouémé, Plateau, and Mono, each representing 28.57% of the group. A zero genetic distance (e.g., between ILA and ABO) indicates a high similarity at the loci studied, whereas the maximum genetic distance (e.g., between CAL/OUE and KPO2) reflects significant divergence. To further validate the clustering observed in the dendrogram, a Principal Coordinate Analysis (PCoA) was performed based on the genetic coordinate matrix (Figure 6). The PCoA largely confirmed the structuring of the two major groups identified in the dendrogram. Notably, the CAL/OUE accession did not cluster with any of the identified groups, highlighting its distinct genetic composition.

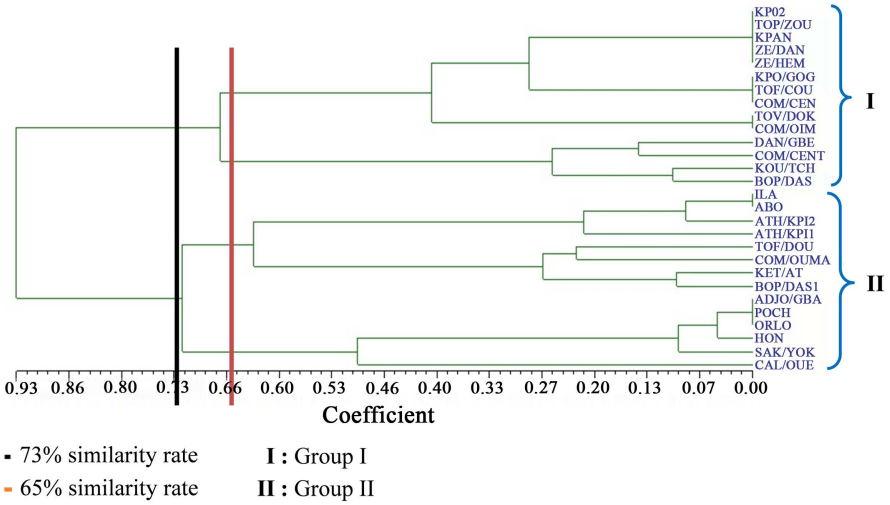

Figure 5. Dendrogram of 28 accessions based on the UPGMA method.

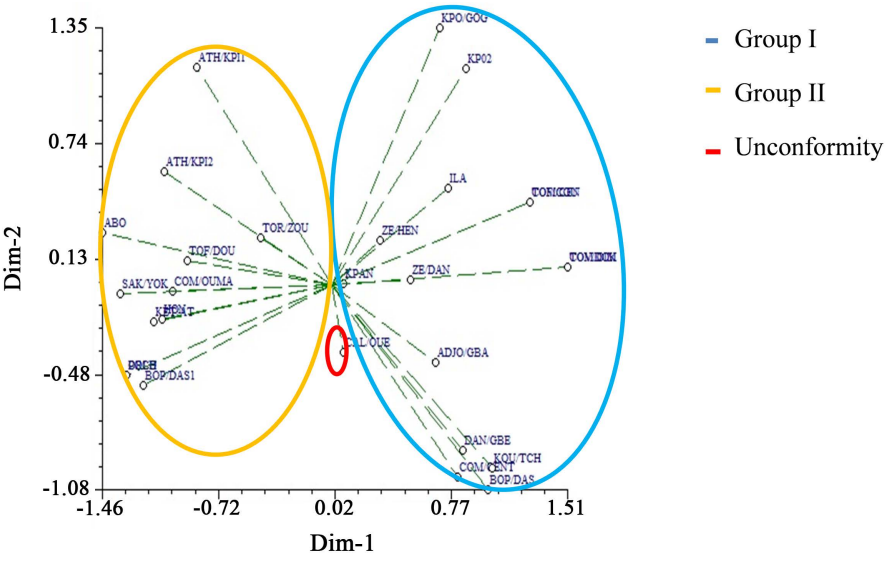

Figure 6. Principal coordinate analysis (PCoA).

4. Discussion

Understanding the genetic structure of a species is crucial for ensuring its sustainable use [26]. This study aimed to evaluate the transferability of eleven (11) SSR markers from *P. acinosa* to *P. alliacea* and to assess the genetic diversity revealed by these markers within the studied collection. Of the 11 microsatellite markers tested, 9 successfully amplified, resulting in a transferability rate of 81.82%. This rate is higher than the 68.97% reported by [27] in their study on SSR marker transferability between *Ricinus communis* and *Euphorbia antiquorum*, as well as the 59.18% observed by [28] between *Manihot esculenta* Crantz and *Hevea brasiliensis* Muell. Arg. The relatively high transferability rate observed in this study highlights the efficiency of *P. acinosa*-derived primers for analyzing the genetic diversity and structure of *P. alliacea* accessions. This result may be attributed to

the presence of conserved genomic regions shared between the two species, which have undergone minimal modification during evolution [29] [30]. The transferability of SSR markers across species or genera primarily depends on the conservation of genomic DNA sequences and the stability of primer binding sites in SSR flanking regions throughout evolution [31]. In this case, the conserved regions identified could provide valuable insights into the phylogenetic relationships between *P. acinosa* and *P. alliacea*. The high transferability rate observed further suggests that the evolutionary divergence between these two species occurred relatively recently [32].

The presence of non-transferable markers (18.18%) could be attributed to genetic divergence between species over evolutionary time and the complexity of their genomes [33]. For instance, SSR marker transferability rates between *Pinus taeda* and *Pinus sylvestris* range from 36% to 53% [34], whereas the rate between *Litchi chinensis* and *Dimocarpus longan* reaches 91.7% [35]. These variations highlight that SSR marker transferability largely depends on the phylogenetic distance between the source and target species [36]. In this study, six (6) markers were identified as polymorphic, with an average of 4.16 alleles per locus. This relatively high allelic count reflects the genetic variability among the accessions studied, indicating allelic enrichment within the collection. Allelic richness in a population is generally influenced by sample size, as the likelihood of identifying new alleles increases with the number of individuals analyzed [37]. Therefore, studying a larger number of accessions beyond the 28 examined here could potentially reveal additional alleles.

The average number of alleles per locus (4.16) observed in this study is higher than the 2 alleles per locus reported by [38] in *Vigna unguiculata* L. using two SSR markers across 20 accessions. Similarly, it surpasses the results of [39], [40], and [41], who reported average allele counts of 1.31, 2, and 3.8 per locus, respectively. However, this value is lower than the 6.4 alleles per locus observed by Mantello *et al*. [15] in a cross-transferability study of SSR markers between *Hevea brasiliensis* and wild *rubber* species, as well as the 5.6 alleles per locus reported by [42] in a study on the genetic variability of wild *Jatropha curcas* in northwestern Mexico. These differences may be attributed to variations in the number of accessions and polymorphic markers analyzed. A greater number of polymorphic markers and accessions typically leads to the detection of more alleles, thereby providing a more comprehensive view of genetic diversity.

The analysis of the genetic diversity within *P. alliacea* based on microsatellite markers provides valuable insights into the population structure and genetic variation. The polymorphism information content (PIC) value of 0.422 indicates a relatively low level of polymorphism, likely influenced by the presence of three monomorphic markers (SL-116, SL-287, and SL-324), which do not contribute to genetic diversity. Despite this, the study demonstrates that microsatellite markers from *P. acinosa* are transferable to *P. alliacea*, offering potential tools for genetic diversity assessments in *P. alliacea* populations.

Ho and He values can arise from various biological mechanisms, including natural selection, the introduction of new alleles through migration, and mutations. The genetic variation from heterozygosity enhances a species' ability to adapt to its environment. Individuals carrying different alleles have a greater chance of surviving and reproducing in changing conditions. This, in turn, enables the species to respond to selective pressures and evolve. In this study, the expected heterozygosity (H_e = 0.633) being lower than the observed heterozygosity (H_o = 0.670) suggests a negative deviation from Hardy-Weinberg equilibrium, pointing to excess heterozygosity within the population. This excess, indicated by the negative fixation index (Fis = −0.005), could be due to several factors, including hybridization events, even though *P. alliacea* is primarily autogamous. It appears that rare instances of hybridization might increase heterozygosity, possibly due to selective pressures that favor heterozygous genotypes in some cases, despite the predominantly self-fertilizing nature of the species. This aligns with findings by [43] about the outcrossing potential in highly self-pollinating species.

The dendrogram analysis, which clusters accessions into two distinct genetic groups, suggests that geographic origin may not play a significant role in shaping the genetic structure of the species. This lack of clear geographical separation could reflect a large common genetic base among accessions, as noted by [22]. Identification of accessions like KPO2, TOR/ZOU, ZE/DAN, KPAN, and ZE/HEN as genetically identical or closely related further supports the possibility of shared genetic material, possibly from a limited number of founding individuals or extensive gene flow. The discordance observed between the classification of the CAL/OUE accession in the dendrogram and the principal coordinates analysis (PCoA) could be attributed to the limited number of markers used in the study. This highlights the need for more markers or additional genetic tools to improve resolution and classification accuracy.

Overall, the study suggests a relatively high level of genetic variability within the *P. alliacea* accessions, despite the challenges posed by the species' self-pollination. These results open up further possibilities for understanding the genetic structure and evolutionary dynamics of *P. alliacea*.

5. Conclusion

The findings from this study significantly contribute to the understanding of *Petiveria alliacea* (commonly known as an important uterotonic plant) by revealing its genetic diversity for the first time. The successful transfer of microsatellite markers from *P. acinosa* to *P. alliacea* with a high polymorphism rate is a breakthrough, as it enables the identification of genetic variation within *P. alliacea*, a species previously lacking species-specific molecular markers. This discovery not only provides a better understanding of the genetic structure of *P. alliacea* but also highlights the potential of SSR markers for exploring both intra- and interspecific genetic diversity. The ability to compare genetic diversity across different species is essential for studying evolutionary relationships and understanding how these

plants evolve and adapt over time. The observed genetic diversity within the studied population emphasizes the importance of preserving this variation, especially in the context of conservation efforts. Given that *P. alliacea* has medicinal significance, understanding its genetic variation could help in the sustainable use and conservation of the species in Benin and beyond. The research could serve as a foundation for future studies exploring the genetic diversity of *P. alliacea* populations across different regions of the country. Incorporating these findings into conservation strategies is vital, as maintaining genetic variation is key to preserving the resilience and adaptability of plant species. This study could play a crucial role in ensuring that *P. alliacea* populations are protected and managed effectively, securing their long-term survival and medicinal value.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Dassou, H.G., Yédomonhan, H., Adomou, A.C., Ogni, C.A., Tossou, M.G. and Akoegninou, A. (2015) Facteurs socioculturels et environnementaux déterminant la connaissance ethnovétérinaire au Bénin. *Afrique Science*, **11**, 335-360.

[2] Turner, N.J. and Turner, K.L. (2008) "Where Our Women Used to Get the Food": Cumulative Effects and Loss of Ethnobotanical Knowledge and Practice; Case Study from Coastal British Columbia. *Botany*, **86**, 103-115. https://doi.org/10.1139/b07-020

[3] Igoli, J., Ogaji, O., Tor-Anyiin, T. and Igoli, N. (2005) Traditional Medicine Practice Amongst the Igede People of Nigeria. Part II. *African Journal of Traditional, Complementary and Alternative Medicines*, **2**, 134-152. https://doi.org/10.4314/ajtcam.v2i2.31112

[4] Sofowora, A. (2010) Plantes médicinales et médecine traditionnelle d'Afrique. KARTHALA Editions.

[5] Neuwinger, H.D. (2000) Médecine traditionnelle africaine: Dictionnaire de l'utilisation et des applications des plantes. Medpharm Scientific Publishers, 589 p.

[6] Adjanohoun, E., Adjakidjè, V., Ahyi, M.R.A., Aké Assi, L., Akoègninou, A., Dalmeda, J., Akpovo, F., Boukef, K., Chadaré, F., Cusset, G., Dramane, K., Eyme, J., Gassita, J.N., Gbaguidi, N., Goudoté, E., Guinko, S., Houngnon, P., Issa, L., Keita, A., Kiniffo, H.V., Koné Bamba, D., Musampa Nseyya, A., Saadou, N., Sodogandji, T.H., De Souza, S., Tchabi, A., Zinsou Dossa, C. and Zohoun, T. (1989) Contribution aux

études ethnobotaniques et floristiques en République Populaire du Bénin. ACCT, 895.

[7] Cronquist, A. (1968) The Evolution and Classification of Flowering Plants. New York Botanical Garden Press.

[8] Ayedoun, M.A., Moudachirou, M., Sossou, P.V., Garneau, F., Gagnon, H. and Jean, F.-(1998) Volatile Constituents of the Root Oil of *Petiveria alliacea* L. from Benin. *Journal of Essential Oil Research*, **10**, 645-646. https://doi.org/10.1080/10412905.1998.9700996

[9] Ouensanga, C. (1983) Medicinal Plants and Creole Remedies. Ed Désormeaux.

[10] Oluwole, F.S. and Bolarinwa, A.F. (1998) The Uterine Contractile Effect of *Petiveria alliacea* Seeds. *Fitoterapia*, **69**, 3-6.

[11] Olomieja, A.O., Olanrewaju, I.O., Ayo-Ajayi, J.I., Jolayemi, G.E., Daniel, U.O. and Mordi, R.C. (2021) Antimicrobial and Antioxidant Properties of *Petiveria alliacea*. *IOP Conference Series: Earth and Environmental Science*, **655**, Article ID: 012015. https://doi.org/10.1088/1755-1315/655/1/012015

[12] Elemo, O.O., Akinyede, A.A., Oreagba, I.A., Nicholas-Okpara, V.A. and Unuofin, J.O. (2022) Uterine Contractile Activity and Abortifacient Effect of the Aqueous Extract of Euphorbia Heterophylla L. Leaves in Albino Rats. *Pharmacology and Toxicology of Natural Medicines*, **2**, 1-11. https://doi.org/10.52406/ptnm.v2i1.14

[13] Dissanayake, R., Braich, S., Cogan, N.O.I., Smith, K. and Kaur, S. (2020) Characterization of Genetic and Allelic Diversity Amongst Cultivated and Wild Lentil Accessions for Germplasm Enhancement. *Frontiers in Genetics*, **11**, Article 546. https://doi.org/10.3389/fgene.2020.00546

[14] Adoukonou-Sagbadja, H., Wagner, C., Ordon, F. and Friedt, W. (2010) Reproductive System and Molecular Phylogenetic Relationships of Fonio Millets (*Digitaria spp.*, Poaceae) with Some Polyploid Wild Relatives. *Tropical Plant Biology*, **3**, 240-251. https://doi.org/10.1007/s12042-010-9063-0

[15] Mantello, C.C., Suzuki, F.I., Souza, L.M., Gonçalves, P.S. and Souza, A.P. (2012) Microsatellite Marker Development for the Rubber Tree (*Hevea brasiliensis*): Characterization and Cross-Amplification in Wild Hevea Species. *BMC Research Notes*, **5**, Article No. 329. https://doi.org/10.1186/1756-0500-5-329

[16] Provan, J., Powell, W. and Waugh, R. (1996) Microsatellite Analysis of Relationships within Cultivated Potato (*Solanum tuberosum*). *Theoretical and Applied Genetics*, **92**, 1078-1084. https://doi.org/10.1007/bf00224052

[17] Kresovich, S., Szewc-McFadden, A.K., Bliek, S.M. and McFerson, J.R. (1995) Abundance and Characterization of Simple-Sequence Repeats (SSRs) Isolated from a Size-Fractionated Genomic Library of *Brassica napus* L. (Rapeseed). *Theoretical and Applied Genetics*, **91**, 206-211. https://doi.org/10.1007/bf00220879

[18] Umer, F., Lovleen, and Manjit, I.S.S. (2011) Meiotic Analysis in a Natural Autotetraploid of *Phytolacca acinosa* Roxb. *Chromosome Science*, **14**, 63-66.

[19] Cheng, Y., Li, P., Yang, Y., Zhang, J., Yan, F. and Wang, H. (2017) Microsatellites for *Phytolacca acinosa* (Phytolaccaceae), a Traditional Medicinal Herb. *Applications in Plant Sciences*, **5**, Article ID: 1700028. https://doi.org/10.3732/apps.1700028

[20] Gawel, N.J. and Jarret, R.L. (1991) A Modified CTAB DNA Extraction Procedure for *Musa* and *Ipomoea*. *Plant Molecular Biology Reporter*, **9**, 262-266. https://doi.org/10.1007/bf02672076

[21] Djedatin, G., Nanoukon, C. and Sedah, P. (2021) DNA Extraction Methodology Following the CTAB Protocol. Laboratory of Molecular Biology and Bioinformatics Ap-

plied to Genomics. National School of Applied Biosciences and Biotechnologies.

[22] Ahmed, M.O., Salem, F.B., Bedhiaf, S. and M'Naouer, D. (2010) Analysis of Molecular Genetic Diversity of Dromedaries (*Camelus dromedarius*) in Tunisia. *Biotechnology, Agronomy and Society and Environment*, **14**, 399-408.

[23] Loukou, N.E., Yapi-Gnaore, C.V., Touré, G., Coulibaly, Y., Rognon, X., Kayang, B., N'guetta, A.S.P., *et al.* (2009) Assessing the Diversity of Indigenous Chicken from Two Agroecological Zones of Côte d'Ivoire Using Microsatellite Markers. *Journal of Animal and Plant Sciences*, **5**, 425-436.

[24] Nei, M. (1972) Genetic Distance between Populations. *The American Naturalist*, **106**, 283-292. https://doi.org/10.1086/282771

[25] Rohlf, F.J. (1992) NTSYS-pc Numerical Taxonomy and Multivariate Analysis System, vs 2.11a. Exeter Publication.

[26] Adoukonou-Sagbadja, H., Missihoun, A.A., Sedah, P., Dagba, R.A., Kinhoegbe, G., Ahanhanzo, C. and Agbangla, C. (2014) Variabilité génétique des accessions d'igname *Dioscorea alata* L. introduites au Bénin à partir des Îles du Sud-Pacifique. *Journal of Applied Biosciences*, **73**, 5966-5978.

[27] Dharajiya, D.T., Shah, A., Galvadiya, B.P., Patel, M.P., Srivastava, R., Pagi, N.K., *et al.* (2020) Genome-Wide Microsatellite Markers in Castor (*Ricinus communis* L.): Identification, Development, Characterization, and Transferability in Euphorbiaceae. *Industrial Crops and Products*, **151**, Article ID: 112461. https://doi.org/10.1016/j.indcrop.2020.112461

[28] Whankaew, S., Kanjanawattanawong, S., Phumichai, C., Smith, D.R., Narangajavana, J. and Triwitayakorn, K. (2011) Cross-Genera Transferability of (Simple Sequence Repeat) SSR Markers among Cassava (*Manihot esculenta* Crantz), Rubber Tree (*Hevea brasiliensis* Muell. Arg.) and Physic Nut (*Jatropha curcas* L.). *African Journal of Biotechnology*, **10**, 1768-1776.

[29] Kpatènon, M.J., Salako, K.V., Santoni, S., Zekraoui, L., Latreille, M., Tollon-Cordet, C., *et al.* (2020) Transferability, Development of Simple Sequence Repeat (SSR) Markers and Application to the Analysis of Genetic Diversity and Population Structure of the African Fan Palm (*Borassus aethiopum* Mart.) in Benin. *BMC Genetics*, **21**, Article No. 145. https://doi.org/10.1186/s12863-020-00955-y

[30] Ben Romdhane, M., Riahi, L., Yazidi, R., Mliki, A. and Zoghlami, N. (2022) Cross Transferability of Barley Nuclear SSRs to Pearl Millet Genome Provides New Molecular Tools for Genetic Analyses and Marker Assisted Selection. *Open Agriculture*, **7**, 668-678. https://doi.org/10.1515/opag-2022-0132

[31] Zhang, C., Jia, C., Liu, X., Zhao, H., Hou, L., Li, M., *et al.* (2022) Genetic Diversity Study on Geographical Populations of the Multipurpose Species *Elsholtzia stauntonii* Using Transferable Microsatellite Markers. *Frontiers in Plant Science*, **13**, Article 903674. https://doi.org/10.3389/fpls.2022.903674

[32] Adoukonou-Sagbadja, H., Wagner, C., Dansi, A., Ahlemeyer, J., Daïnou, O., Akpagana, K., *et al.* (2007) Genetic Diversity and Population Differentiation of Traditional Fonio Millet (*Digitaria spp.*) Landraces from Different Agro-Ecological Zones of West Africa. *Theoretical and Applied Genetics*, **115**, 917-931. https://doi.org/10.1007/s00122-007-0618-x

[33] Barreneche, T., Casasoli, M., Russell, K., Akkak, A., Meddour, H., Plomion, C., *et al.* (2003) Comparative Mapping between *Quercus* and *Castanea* Using Simple-Sequence Repeats (SSRs). *Theoretical and Applied Genetics*, **108**, 558-566. https://doi.org/10.1007/s00122-003-1462-2

[34] González-Martínez, S.C., Robledo-Arnuncio, J.J., Collada, C., Díaz, A., Williams, C.G., Alía, R., *et al.* (2004) Cross-Amplification and Sequence Variation of Microsatellite Loci in Eurasian Hard Pines. *Theoretical and Applied Genetics*, **109**, 103-111. https://doi.org/10.1007/s00122-004-1596-x

[35] Viruel, M.A. and Hormaza, J.I. (2003) Development, Characterization and Variability Analysis of Microsatellites in Lychee (*Litchi chinensis* Sonn., Sapindaceae). *Theoretical and Applied Genetics*, **108**, 896-902. https://doi.org/10.1007/s00122-003-1497-4

[36] Rossetto, M. (2001) Sourcing of SSR Markers from Related Plant Species. In: Henry, R.J., Ed., *Plant genotyping*: *The DNA fingerprinting of plants*, CABI Publishing, 211-224. https://doi.org/10.1079/9780851995151.0211

[37] Foulley, J.L. and Ollivier, L. (2006) Genetic Diversity and Allelic Richness: Concepts and Application to Bovine Breeds. *Rencontres autour des Recherches sur les Ruminants*, **13**, 227-230.

[38] Wamalwa, E.N., Muoma, J. and Wekesa, C. (2016) Genetic Diversity of Cowpea (*Vigna unguiculata* (L.) Walp.) Accession in Kenya Gene Bank Based on Simple Sequence Repeat Markers. *International Journal of Genomics*, **2016**, Article ID: 8956412. https://doi.org/10.1155/2016/8956412

[39] Kouakou, C.K., Roy-Macauley, H., Gueye, M.C., Otto, M.C., Rami, J.F., Cissé, N. and Pasquet, R.S. (2007) Genetic Diversity of Traditional Cowpea Cultivars [*Vigna unguiculata* (L.) Walp.] in Senegal: A Preliminary Study. *Plant Genetic Resources Newsletter*, **152**, 33-34.

[40] Mafakheri, K., Bihamta, M.R. and Abbasi, A.R. (2017) Assessment of Genetic Diversity in Cowpea (*Vigna unguiculata* L.) Germplasm Using Morphological and Molecular Characterisation. *Cogent Food & Agriculture*, **3**, Article ID: 1327092. https://doi.org/10.1080/23311932.2017.1327092

[41] Asare, A.T., Gowda, B.S., Galyuon, I.K.A., Aboagye, L.L., Takrama, J.F. and Timko, M.P. (2010) Assessment of the Genetic Diversity in Cowpea (*Vigna unguiculata* L. Walp.) Germplasm from Ghana Using Simple Sequence Repeat Markers. *Plant Genetic Resources*, **8**, 142-150. https://doi.org/10.1017/s1479262110000092

[42] Araiza-Lizarde, N., Angulo-Escalante, M.Á., Reynoso-Granados, T., Cruz-Hernández, P., Calderón-Vázquez, C.L. and Alcaraz-Melendez, L. (2021) Genetic Variability of Wild *Jatropha curcas* in Northwest Mexico. *Tropical and Subtropical Agroecosystems*, **24**, 1-7.

[43] Baudoin, J.P., Demol, J., Louant, B.P., Maréchal, R., Mergeai, G. and Otoul, É. (2002) Plant Improvement. Application to the Principle Species Cultivated in Tropical Regions. Les presses agronomiques de Gembloux, 581 p.