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Transferability of Some Nuclear Microsatellite (SSRs) Markers from Related Species in *Parkia biglobosa*

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Abstract: Molecular DNA markers, particularly microsatellites (SSRs), are important tools for plant genetic resources characterization. The present study examined the transferability of nine polymorphic microsatellite markers developed for *Parkia panurensis* in *Parkia biglobosa* from Northern Benin, favorite area of *Parkia biglobosa*. Fourty (40) accessions have been considered. Five microsatellite loci (Parpan 3, Parpan 4, Parpan 9, Parpan 13 and Parpan 15) showed good amplifications in *Parkia biglobosa*. The size of the amplified markers, ranging from 100 to 200 bases pairs, was similar to those previously reported. Forest tree species are too numerous to benefit all a significant investment in molecular biology. Also, the present study has shown that the transfer of markers between species is possible. The transferability is possible for the species *Parkia biglobosa*, too. The microsatellites identified in this study are important to analyse the genetic structure and diversity of natural populations of *Parkia biglobosa* in Benin particularly and in the world generally. They are also important to analyse the evolution of species with phylogeny construction, to analyse the genome with its applications in genetic improvement and genetic identification of given material. For these different studies, the microsatellites of the present study could be completed to those defined for *Parkia biglobosa*.

Keywords: Microsatellites, *Parkia biglobosa*, Transferability, Related Species

1. Introduction

Parkia biglobosa (Jacq.) R. Br. Ex G. Don is an important species which occupies a crucial place in the life of the people of Sahelian and Sudanian Africa areas. The fruits are consumed, especially their seeds which, after a long preparation, provide a condiment used in traditional dishes cooking in Africa. In several African countries, this condiment plays an important role in the diet of the population. *P. biglobosa* occupies the fourth position among eighteen important food woody species to be preserved [1] and the fifth of the thirty one most used medicinal woody species in Beninese traditional medicine [2]. However, this species is seriously threatened. In Benin, its stands are growing old, while its natural regeneration is very low to ensure its renewal [3-5]. A decrease of its distribution area in Benin was reported [6]. Taking into account the socio-economic and cultural importance of this species, populations tend to abusively harvest fruit production, limiting the regeneration of this species [2, 7]. This worrying situation requires that measures aimed at preserving this species must be taken. The effective conservation and utilization of genetic resources of *P. biglobosa* require an accurate assessment of the pattern of its genetic variation.

The genetic diversity in plants can be measured through diverse morphological, biochemical, and molecular DNA (Deoxyribonucleic Acid) markers. In West Africa, few data

currently exist on the genetics of *P. biglobosa* [8]. The only one study devoted to *P. biglobosa*'s genetic diversity analysis used the enzymatic electrophoresis to determine intra- and inter-population variations, the structure and genetic distances between sixty four natural populations of *P. biglobosa* sampled across 11 countries of the western and central Africa [7]. However, an important limitation of the isozymes lies in the fact that these types of markers are based on gene products. As such and like morphological traits, these markers can be influenced by environmental factors; the differences in expression can bring confusion in the interpretation of the results. The genes can be expressed differently during the various stages of development or in different tissues. Nowadays, DNA molecular tools are mostly used as they are not subject to the influences of the environment. Their interest lies in their important polymorphism directly detectable at DNA level. The use of molecular markers compensates the ignorance of the genes responsible of the studied characters. The analysis can be performed at any stage of the development of the plant, and even better for some markers such as the microsatellites, they have the potential to exist in unlimited number, covering the entire genome. Among the most used DNA-based molecular markers [Restriction Fragment Length Polymorphism (RFLP), microsatellites, Single Nucleotide Polymorphism (SNP), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP)], the microsatellites together with RFLP and SNP are codominant markers [9, 10]. The microsatellites are one of the most powerful markers type which reveals high polymorphism [10]. In addition to their distribution in the whole genome, the interest in microsatellites lies in their extremely high polymorphism. They constitute excellent genetic markers, locus-specific, co-dominants and highly polymorphic. They essentially mark the non coding regions; however, their distribution is fairly uniform on the genome. They are easily manipulated by PCR (Polymerase Chain Reaction). The visualization protocols can be partially automated. Therefore, they are more frequently used for many genetic studies.

Despite their many advantages, the development of the microsatellite markers is a heavy work. According to conventional protocols, a genomic library should be achieved as well as its screening to identify clones that carry microsatellite motifs. These clones must then be sequenced to further define the primers. The conditions of amplification and of polymorphism revelation must also be developed. The laborious procedures of cloning and screening used in the development of microsatellites could nonetheless be circumvented by the use of microsatellite markers of closely related taxa. Luettmann *et al.* (2010) have developed for *Parkia panurensis* a series of nine nuclear microsatellite loci, polymorphic and which have shown a very good amplification on six (6) other species of the genus *Parkia* (*P. ingneiflora*, *P. multijuga*, *P. nitida*, *P. plathycephala*, *P. bahiae* and *P. pendula*) [11]. The aim of this study, is to test the potential of transfer of these microsatellite markers for the species *Parkia biglobosa* in order to identify the positive ones that can be further used in genetic characterization of natural populations of *Parkia biglobosa*. The importance of this study is to show that the transferability is possible for the species *Parkia biglobosa*, too. For studies of population genetics and reproductive biology of *Parkia biglobosa*, the microsatellites of the present study could be completed to those defined for *Parkia biglobosa* in 2014 [12]. Specifically, the microsatellites of the present study and those defined for *Parkia biglobosa* in 2014 by Lassen *et al.* could be used to analyse the evolution of species with phylogeny construction, to analyse the genome with its applications in genetic improvement and genetic identification of given material.

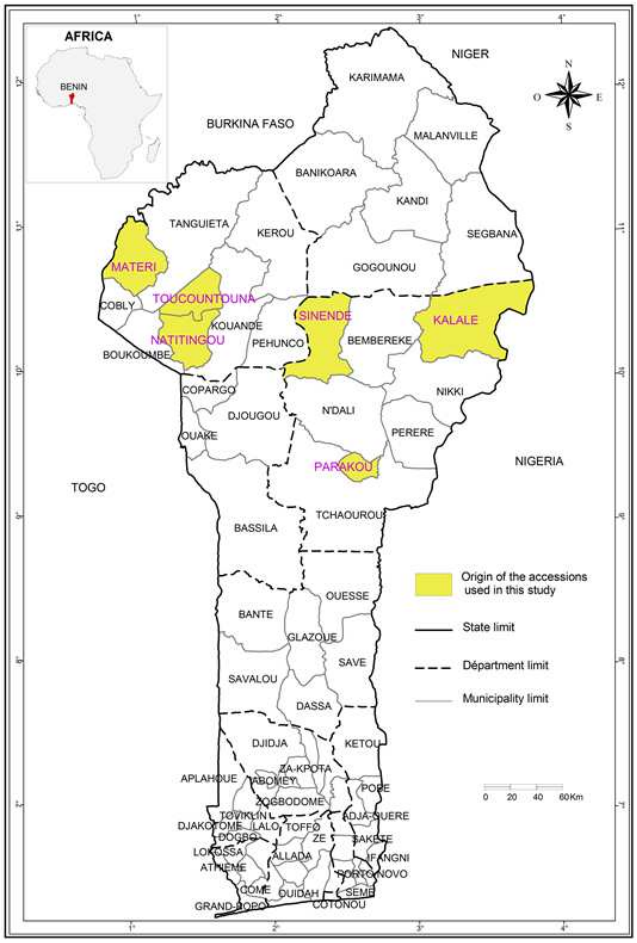

Figure 1. *Origin of the accessions used in this study.*

2. Methods

2.1. Extraction and Dosage of Genomic DNA

Genomic DNA was extracted from young leaves of *Parkia biglobosa* from Northern Benin (Figure 1), his favourite area, according to the Protocol described by Gawel and Jarret (1991) [13] slightly adjusted to local laboratory conditions by Agbangla *et al.* (2002) [14] using Mixed Alkyl Trimethyl Ammonium Bromide (MATAB) as lyses buffer. Fourty (40) accessions have been considered. To check the success of DNA extraction, 2µL of extracted DNA samples are visualized by electrophoresis on 1% agarose gel. This

electrophoresis allowed evaluating the quality and the concentration of extracts of DNA obtained. After this verification, the samples of extracted DNA were diluted and conserved at -20°C for further analyses.

2.2. Amplification by Polymerase Chain Reaction (PCR)

Nine microsatellite markers, published by Luettmann *et al.* (2010) [11], were tested in this study on *P. biglobosa*. The table 1 presents the characteristics (name of loci, sequences, repeat motif, size range, GenBank accession number) of the nine loci nuclear microsatellites used in this study. Dilutions of DNA extracts were made. PCR reactions were performed in a 25µL volume of ultra pure water containing 10 to 30ng of template DNA, 1 X PCR buffer PCR (10X), 0.2µM of each primer (Forward primer and Reverse primer sequences), 0.2mM of each Deoxyribonucleotide triphosphate (dNTP), 2.0mM $MgCl_2$, 1 unit/µL Taq polymerase and 0.5% Bovine Serum Albumin (BSA) (20mg/ml). The PCR was performed using a thermal cycler (PTC – 100TM Programmable Thermal Controller MJ Research, Inc). The PCR conditions are reported by Luettmann *et al.* (2010) [11]: 5 minutes at 95°C for initial denaturation phase, followed by 33 cycles at 95°C for 45s, different annealing temperatures ranging from 54 to 60°C for 45s depending on the specific primer pair used, elongation time at 72°C of 40s and final extension at 72°C for 10 minutes. The annealing temperatures of the specific primer pairs for best amplification are summarized in table 1. The PCR products were migrated by electrophoresis on 2% agarose gel to evaluate the quality of amplifications.

Table 1. *Characteristics of the nine nuclear microsatellites identified by Luettmann et al. (2010) [11] and used in this study.*

Locus	Primer sequences (5' - 3')	Annealing temperature (°C)	Repeat motif	Allele size range (bp)	GenBank accession no.
Parpan 3	F: FAM-CACGTTAATTCAATCAAAATGGTG R: TTTTGCCTTTTTCGGACTTG	56.5	$(GT)_{15}$	155-209	GU735073
Parpan 4	F: TAMRA-TTGATGGGAGTGGGAAAAAG R: CAGGAGGTGGTCTCTTCAGG	54.0	$(GT)_{13}(GA)_{15}$	148-210	GU735074
Parpan 5	F: FAM-CTCAATAAGATACCCTTTACATTGC R: TTGAATCGAGGAATGAGATTATTG	60.0	$(CA)_{17}$	166-200	GU735075
Parpan 9	F: FAM-GGGGCTTGTGTCTCTCACTG R: ACTTTGAAGGCACGAGATGG	58.0	$(AC)_{12}$	204-262	GU735076
Parpan 11	F: HEX-ACGTAGGGAATAGGGCCATC R: CTACGTACGAGCCGACACTC	58.5	$(TG)_5CA(TG)_{16}$	94-214	GU735077
Parpan 13	F: TAMRA-CCTCCCTCGCTTCACAATC R: CACATGCAAATGAAAATGGTG	58.5	$(GT)_{17}TT(GT)_8$	86-194	GU735078
Parpan 14	F: HEX-ACATCAAAATGGTCGCTCAAC R: CAAATGTTCTTGTATGGAGCAAG	60.0	$(GT)_{20}$	76-116	GU735079
Parpan 15	F: HEX-TGGCCTCACTGCATACTGAC R: TGGGATGAACAAAACTGTGC	55.0	$(AC)_{24}$	104-152	GU735080
Parpan 21	F: HEX-TGCTTTGTGCGACTTGAATC R: CATTGTTCAGCATATAGGCATACAG	58.0	$(GT)_{15}$	153-185	GU735081

3. Results

3.1. PCR Products

Five (5) loci on the nine (9) investigated in the study showed a good amplification. These were the loci Parpan 3, Parpan 4, Parpan 9, Parpan 13 and Parpan 15. The figure 2 shows in a black white image the different PCR products compared with the size markers.

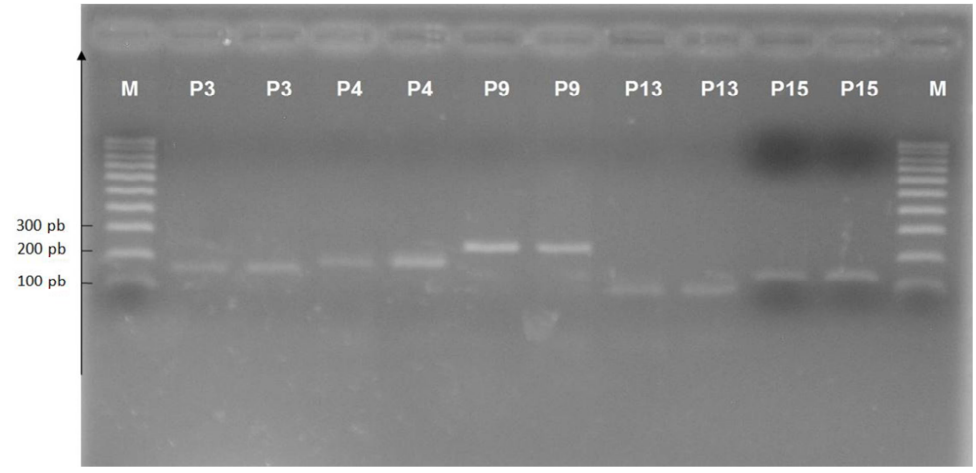

Figure 2. *Comparison of the different products of PCR with size markers (M); P = Parpan (black white image).*

3.2. Comparison of the Amplification of Microsatellites in Some Species of the Genus Parkia

The table 2 summarizes the comparison of the amplification of nine nuclear microsatellite in some species of the genus *Parkia*, *Parkia biglobosa* including. The sizes of the products of PCR in this study are similar to those obtained by Luettmann *et al.* (2010) [11]. According to these authors [11], all the nine nuclear microsatellites developed in *P. panurensis* showed a good amplification and then transferability for all the related *Parkia* species that they studied, i.e. *P. ingneiflora*, *P. multijuga*, *P. nitida*, *P. plathycephala*, *P. bahiae* and *P. pendula*. In our study, five (5) of the nine (55.56%) showed a good amplification *in Parkia biglobosa*. This could be explained by the genetic differentiation of *Parkia biglobosa* from the other taxa due to divergent evolutionary events during their life history. There is no hard and fast rule for predicting the transfer of a marker from one species to another, but the closer the species are, the more likely it is.

Table 2. *Comparison of the amplification of nine nuclear microsatellite in some species of the genus Parkia, Parkia biglobosa including (+ = good amplification with a band of a similar size to that of the original sequence; - = no amplification).*

Locus	*Parkia panurensis*[c]	*Parkia ingneiflora*[c]	*Parkia multijuga*[c]	*Parkia nitida*[c]	*Parkia plathycephala*[c]	*Parkia bahiae*[c]	*Parkia pendula*[c]	*Parkia biglobosa*[d]
Parpan 3	+	+	+	+	+	+	+	+
Parpan 4	+	+	+	+	+	+	+	+
Parpan 5	+	+	+	+	+	+	+	-
Parpan 9	+	+	+	+	+	+	+	+
Parpan 11	+	+	+	+	+	+	+	-
Parpan 13	+	+	+	+	+	+	+	+
Parpan 14	+	+	+	+	+	+	+	-
Parpan 15	+	+	+	+	+	+	+	+
Parpan 21	+	+	+	+	+	+	+	-

[c]Source: Luettmann et *al.* (2010) [11].
[d]Source: This study.

3.3. Synthesis of Microsatellite Loci that Can Be Used in Genetic Studies on Parkia biglobosa

The table 3 presents the characteristics of the microsatellite loci that can be used in genetic studies on *Parkia biglobosa*. These microsatellite loci have been identified in this study and by Lassen *et al.* (2014) [12]. Indeed, for *Parkia biglobosa*, Lassen *et al.* (2014) [12] developed eleven microsatellites loci from samples from Burkina Faso, using pyrosequencing. Ten of these eleven microsatellite markers have proven to be highly and easy polymorphic to genotype. These ten loci characterized by Lassen *et al.* (2014) [12] and the five loci identified in the present study will be useful for reproductive and population genetic studies on *Parkia biglobosa*.

Table 3. *Characteristics of the microsatellite loci that can be used in genetic studies on Parkia biglobosa.*

Locus	Primer sequences (5' - 3')	Annealing temperature (°C)	Repeat motif	Allele size range (bp)	GenBank accession no.	References
Parpan 3	F: FAM-CACGTTAATTCAATCAAAATGGTG R: TTTTGCCTTTTTCGGACTTG	56.5	$(GT)_{15}$	155-209	GU735073	Luettmann *et al.* (2010) [11] and this study
Parpan 4	F: TAMRA-TTGATGGGAGTGGGAAAAAG R: CAGGAGGTGGTCTCTTCAGG	54.0	$(GT)_{13}(GA)_{15}$	148-210	GU735074	
Parpan 9	F: FAM-GGGGCTTGTGTCTCTCACTG R: ACTTTGAAGGCACGAGATGG	58.0	$(AC)_{12}$	204-262	GU735076	
Parpan 13	F: TAMRA-CCTCCCTCGCTTCACAATC R: CACATGCAAATGAAAATGGTG	58.5	$(GT)_{17}TT(GT)_8$	86-194	GU735078	
Parpan 15	F: HEX-TGGCCTCACTGCATACTGAC R: TGGGATGAACAAAACTGTGC	55.0	$(AC)_{24}$	104-152	GU735080	Lassen *et al.* (2014) [12]
PbL02	F: CGAAATAAGAACTCGGACCAAA R: ATGCCGTGTTCTGTTTCACC	55.0	$(GA)_{17}$	180-205	KJ475533	
PbL03	F: TTCGATTCAATTCAACTTGCAG R: TCGGTGCTAGCAATATCAGC	55.0	$(GA)_{17}$	90-138	KJ475534	
PbL04	F: GAAGCCTTGGAATGAAGTTGA R: GAAAACGGAAGGCATGGTTA	55.0	$(CA)_{17}$	161-181	KJ475535	
PbL05	F: GAATCAGAGAAGCCCTTAGGTT R: GCCGCTTGTTTTCTTGTGA	55.0	$(AC)_{17}$	183-259	KJ475536	
PbL09	F: TGACGTATTTGAGTGTCTTTTACACA R: GCAGAAAATCACAAATGCAGA	55.0	$(AG)_{18}$	126-170	KJ475537	
PbL11	F: TCACGGCAATAGAACTCATCA R: ACGGAGCAGGATGAAGTCAG	55.0	$(CCT)_{18}$	170-206	KJ475538	
PbL12	F: ATCTAGGCATCCATGCAAATG R: CAGAAGTTGTTAGACAATTATGGTGA	55.0	$(TG)_{19}$	108-137	KJ475539	

Locus	Primer sequences (5' - 3')	Annealing temperature (°C)	Repeat motif	Allele size range (bp)	GenBank accession no.	References
PbL15	F: CCCCAAGAGCAACAACTCAT R: GGCAGTGTTCTATAGGAAATAGTCG	55.0	$(CT)_{19}$	120-140	KJ475540	
PbL21	F: TGTTGCTTTTGCTTTTGCTG R: CCCTCTGCAGAATTGAGTCC	55.0	$(CA)_{21}$	269-305	KJ475542	
PbL22	F: TTGCGAATAGGATATGGGTTG R: GAAGACGACAGGCTGCTACA	55.0	$(AC)_{22}$	164-218	KJ475543	

4. Discussion

The results of the present study confirm that a high potential of the transferability of the microsatellite markers exists among the closely related taxa in the genus *Parkia*. Similar results well reported by Ekué *et al.* (2009) [15] for *Litchi chinensis* on *Blighia sapida*, Ouinsavi *et al.* (2006) [16] for *Milicia excelsa* on *Milicia regia* and seem to be a common phenomenon in many other in many genera such as *Pithecellobium* [17], *Shorea* [18, 19], *Avicennia* [20], *Dioscorea* [21] and others species. This potential shown here suggests that considerable time and resources might be saved when primers are available from closely related species [17].

Ekué *et al.* (2009) [15] shown that the cross-transferability of lychee SSR markers to ackee observed in their study renders these markers useful for investigating the genetic diversity and structure of *Blighia sapida* populations. So, the five loci identified in the present study can be added to the ten loci characterized by Lassen *et al.* (2014) [12] for facilitate reproductive and population genetic studies on *Parkia biglobosa* and the genetic study of the populations of various species in the genus *Parkia*. Amusa *et al.* (2014) [22] concluded in their study titled: "genetic diversity of *Parkia biglobosa* from different agroecological zones of Nigeria using RAPD markers" that efforts should be made to use more specific molecular markers to ensure a better understanding of the diversity in *Parkia biglobosa* so as to enhance the selection of germplasm in conservation of the species as well as fostering a better understanding of the diversity within the species. A genetic diversity study of *Parkia biglobosa* from different agroecological zones of Benin using these different microsatellites is currently underway.

5. Conclusion

This study has identified five (5) microsatellites (loci Parpan 3, Parpan 4, Parpan 9, Parpan 13 and Parpan 15) that can be used with success on *Parkia biglobosa*. The identification of these loci is important for the evolution of research on *Parkia biglobosa*, especially in the genetic diversity analyses in which the most commonly used markers are the microsatellites. The results obtained in this study are then of a great importance for the identification, the rational exploitation and conservation of genetic resources of *Parkia biglobosa*. More in-depth studies can be conducted on this transferability. It would be interesting to test on this species other microsatellite markers already developed for other species belonging to the Mimosoïdeae, subfamily to which *Parkia biglobosa* belongs. The microsatellites markers identified in this study are an important tool for studies that are being carried out to characterize the diversity and genetic structure of natural populations of *Parkia biglobosa*.

Author's Contributions

KK conceived, designed the research and drafted the manuscript. KK, AAM and PS performed the experiments. AAM, PS and HA gave conceptual advice, read and improved the drafted manuscript. CAg, CAh and JCG supervised the work and improved the manuscript. All authors have read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

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