Applied Tropical Agriculture
Volume 23, No. 1, 35-40, 2018.

Assessment of the Genetic Variation in *Chrysophyllum albidum* G. Don Using CP SSR Marker

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ABSTRACT

Chrysophyllum albidum is among the forest tree species that provides Non-Timber Forest Products (NTFPs) of enormous domestic significant to both rural and urban dwellers in West Africa, with great export potentials. The fruit pulp is widely consumed and thus play a significant role in food security. C. albidum, which is classified among the endangered tree species with a high probability of going into extinction in the nearest future, is endowed with a high degree of climatic adaptability. This indicate that there may exist a tremendous genetic variation that needs to be identified for immediate conservation. In this study, total genomic DNA was extracted from 0.020 g powder of silica gel dried leaves using the QIAGEN DNeasy plant Mini Kit according to the manufacturer's instruction. DNA was amplified by Polymerase Chain Reaction (PCR). The estimation of total gene diversity (Ht), gene diversity due to variation within population (Hs), gene diversity due to differences among populations (Gst) and gene flow (Nm) were 0.1330±0.041, 0.0472±0.002, 0.645, and 0.275 in that order. This reveals that genetic base of this important tree species has been seriously eroded. Therefore, urgent steps must be taken by individual, organizations and government to conserve the genetic base of this important tree species.*

Key words: *Chrysophyllum albidum*¸ conservation, genetic variation, microsatellites

INTRODUCTION

Chrysophyllum albidum G. Don, commonly known as "African Star Apple", is a native to many parts of tropical Africa. It is widely distributed in West, Central, and East Africa for its edible fruits and various ethno-medical uses (Keay, 1989; Kang, 1992; Amusa et al., 2003; Orwa et al., 2009). This fruit tree grows as a wild plant and belongs to the family Sapotaceae. It has up to 800 species and make up almost half of the order Ebernales (Ehiagbonare et al., 2008). Ecologically, the tree has an efficient nutrient cycling and the high rate of mineralization of the leaves improves the quality of the top soil (Adesina, 2005). *C. albidum* is among the forest tree species that provides Non-Timber Forest Products (NTFPs) of enormous domestic significant to both rural and urban dwellers in West Africa, with great export potentials. The fruit pulp is widely consumed and thus plays a significant role in food security (Onyekwelu and Stimm, 2011). According to Onyekwelu et al., (2011), *C. albidum* fruit serves as a delicacy and another source of food, income and rural employment through their collection and sale. Other parts of *C. albidum* (such as leaves, seeds, roots and bark) are also used for curing of different diseases such diabetes, ulcer, sterility and sexual weakness, blood pressure, asthma etc. (Houessou, 2012).

In Nigeria, *C. albidum* is enlisted among the endangered tree species (Formecu, 1999), with a high probability of going into extinction in the nearest future, which could affect the livelihood of millions, especially the rural dwellers that depends on them as a substitute for food, medicinal uses and source of household income (Onyekwelu and Stimm, 2011). *C. albidum* is capable of adapting under different climatic conditions. It exists in rainforest, as well as in the savanna where there is less rainfall and high temperature. The wide geographical and climatic distribution is an indicative of the fact that there exists a tremendous genetic variation, which needs to be identified and catalogued (Dhillon et al., 2007).

Understanding arrangements of genetic variation within plant species is of ultimate importance to the improvement of conservation strategies, both for defining appropriate units for in-situ conservation and for developing effective sample collection strategies for ex-situ conservation (Holsinger and Gottlieb, 1991; Hogbin and Peakall, 1999; Newton et al., 1999). According to KOSKELA et al. (2007), genetic variation is the fundamental constituent, which ensures survival and thus the stability of forest ecosystems as its quantity and quality determines the

potential of population that will adjust to the changing environmental condition. A large number of studies have been undertaken to evaluate the extent of genetic variation in some threatened plant species such as *Khaya* species (Hamrick et al., 1991; Hamrick and Godt, 1996; Gitzendammer and Soltis, 2000). This reflects the significance accorded to the maintenance of genetic variation in conservation programmes, as its loss may reduce the evolutionary viability of populations by decreasing their ability to adapt to changing environmental conditions (Hamrick et al., 1991; Ennos, 1996). More recently, molecular genetic markers based on variation in DNA became frequently applied tools to reveal genetic variation within species and also to clarify phylogenetic relations among more or less closely related species, as commonly encountered in the tropical rainforest.

Simple sequence repeats which are microsatellite markers are considered the method of choice for this study because of their co-dominant nature, high levels of polymorphism and reproducibility (Weising et al., 2005) and gives more information than dominant marker data for the estimation of population structure and genetic diversity (Mariette et al., 2005; Evanno et al., 2005). The use of Random Amplified Polymorphic DNA (RAPD) markers is not perfect for population genetics, with possible problems of bias (Lynch and Milligan, 1994) and reproducibility between laboratories because of sensitivity to minor changes in Polymerase Chain Reaction (PCR) conditions and difficulty in interpretation because of co-migration of brands of similar size (Weising et al., 2005). White *et. al.,* (2007) stated that it is known that the genetic information present in cpDNA is of great interest for phylogeny and population genetic studies mostly due to its way of transmission, typically uniparental. Therefore the chloroplast marker has higher levels of intra-population variation when compared to nuclear markers (Petit *et. al*., 2002). Hence, the use of chloroplast Simple Sequence Repeat (cpSSR) marker in this study.

METHODOLOGY

The study area

Osun State is an inland state, created in 1991 from old Oyo State, which is located in the South-Western part of Nigeria. It lies within latitude 7.0° and 9.0° N, and longitude 2.8° and 6.8° E. It is bounded in the north by Kwara State, in the east partly by Ekiti State and partly Ondo State, in the south by Ogun State, and in the west by Oyo State. The State is made up of thirty Local Government Areas (LGAs) (Sofoluwe, 2011). Osun State is located in the moderately hot, humid tropical climatic zone of south-western Nigeria. The state exhibits the typical tropical climate with prominent wet and dry seasons with fertile soil which encourage the production of crops and livestock. The rainy season generally occur between April and October while the dry season occurs between November and March. The mean annual temperature for the state varies between 21.1ºc and 31.1ºc. The mean temperatures are highest at the end of the harmattan, which is from the middle of January to the onset of the rains. Rainfall figures over the state vary from an average of 1000 mm in the derived savannah agro-ecology to 1200 mm in the rain forest at the onset of heavy rains to 1600 mm at its peak in the rain forest part of the state (Idowu *et. al.,* 2012).

Collection of Plant Materials

Two juvenile leaves were collected from fifteen accessions of *C. albidum* across Osun State. The leaves were cleaned and preserved with silica gel in sealed nylon and brought to the Biosafety Laboratory behind School of Postgraduate Studies, of the Federal University of Technology, Akure, Nigeria for DNA extraction. The GPS location of *C. albidum* population sampled in this study were recorded as shown in Figure 1 below.

DNA Extraction

Total genomic DNA was extracted from 0.020 g powder of silica gel dried leaves using the QIAGEN DNeasy plant Mini Kit according to the manufacturer's instruction (Qiagen Valencia, CA, USA). Also, the concentration of DNA extracted was determined using JENWAY 6305 Spectrophotometer and diluted to 10 ng/µl with ddH20 and stored at -20ºC for PCR amplifications.

PCR Amplifications and Gel Electrophoresis

DNA was amplified by Polymerase Chain Reaction (PCR), using five universal primer pairs of cpSSR markers. PCR reaction was performed in a volume of 25µl in a DNA thermal cycler (Eppendorf, mastercycler gradient). The reaction mixture contained 4µl of 40 ng DNA, 12.5µl of Go Taq Green master Mix, 2x (Promega), 1 µl of each primer and 6.5 µl of Nuclease-free water. The cycling parameters were: 1 cycle of 94ºC for 5mins; 40 cycles of 1 minute denaturing step at 94ºC, 1 minute annealing temperature at 55ºC and 1 minute extension at 72ºC, follow by 15 minutes at 72ºC (post-extension). The PCR products were store at 4ºC until analysis. Amplified PCR products were separated by electrophoresis using 2% agarose gel in 1x TBE (Tris base EDTA) buffer, stained with 5µl ethidium bromide and visualize under UV light. Fragment sizes were estimated using 50-1000 bp Bench Top PCR Markers (ladder – DNA sizing marker).

Data Analysis

For cpSSR markers, because of the non-recombining nature of the chloroplast genome, each chloroplast primer pair was considered as 'locus' to refer to a cpSSR site (defined by the terminal of a PCR primer pair) and length variants at a cpSSR site were treated as 'alleles'.

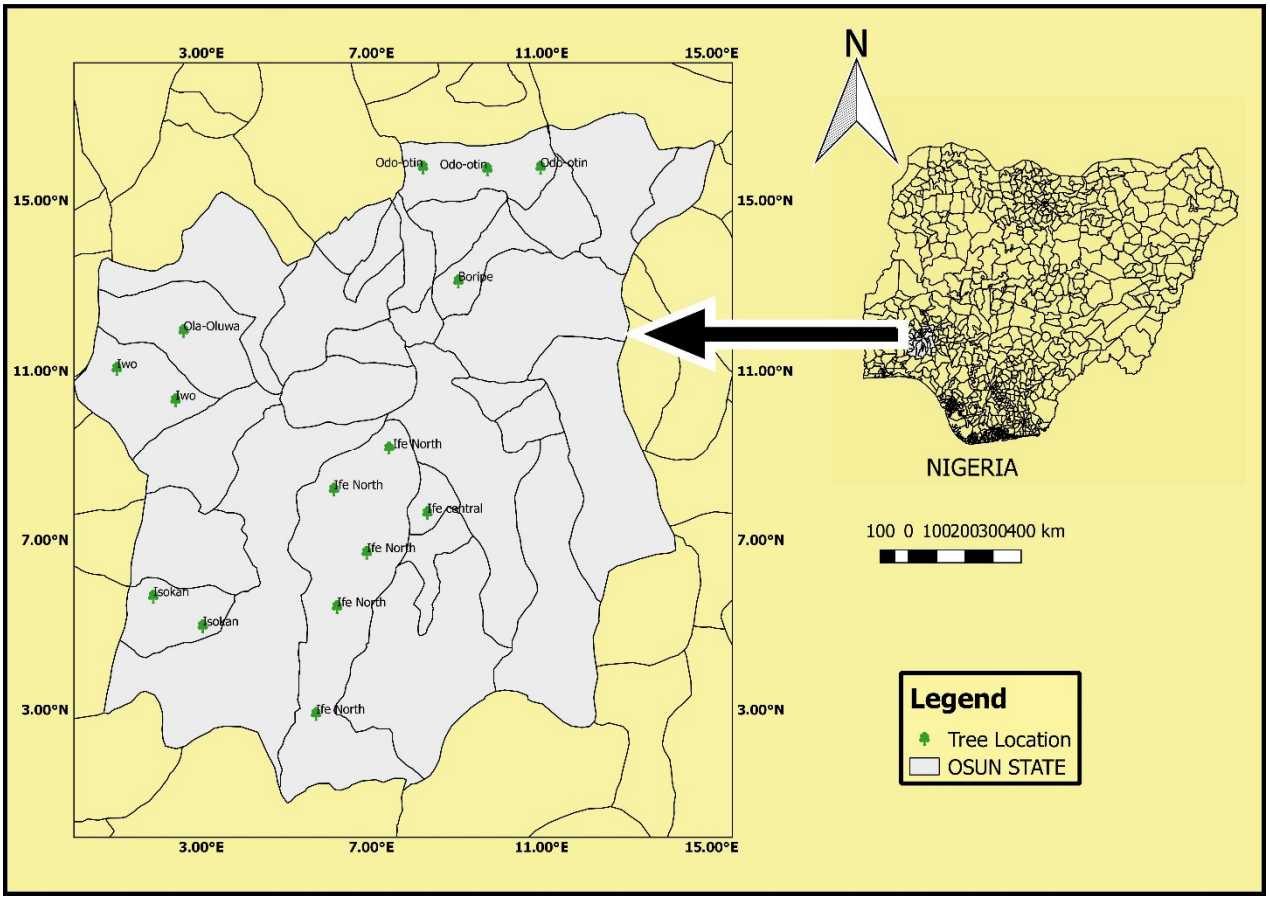

Figure 1: Map of Nigeria with the Study Area (Osun State) showing the tree locations

The cpSSR data was visually scored by the presence of specific alleles in each accession at a cpSSR locus. The scored cpSSR data was analyzed as a haploid co-dominant marker for the analysis of total allelic diversity using the POPGENE program version 1.32 (Yeh et al., 1999). The frequencies of the bands were used to calculate the total genetic diversity (h) (Nei, 1978), observed number of alleles (na), effective number of alleles (ne), and Shannon's information index (I).

RESULTS

The largest fragments amplified from the various cpSSR primers were found in the range of 100 bp to 127 bp while the smallest fragments were found in the range of 50 – 60 bp. Out of the total scored bands, 42.9% bands were polymorphism and 57.1% bands were monomorphic, revealing a low degree of polymorphism. The polymorphism information index (PIC, which is the primer's discriminating power) value ranged between 00.0 and 20.0. The highest polymorphic information index (PIC) value was recorded for ccmp2 (20.0) (Table 1). The marker index (MI) values ranged from 0.00 to 1,200. The highest value (1,200) was scored for ccmp2 and the lowest value (0.00) for ccmp3, ccmp4, ccmp5, and ccmp6. The observed number of alleles, effective number of alleles, Shannon's information index (I), and Nei's genetic diversity for all the accessions were put at 1.60±0.55, 1.22±0.33, 0.14±0.18, and 0.23±0.26 respectively (Table 2). The mean estimate of total gene diversity (H_t), gene diversity due to variation within population (H_s), gene diversity due to differences among populations (G_{st}) and gene flow (Nm*) were 0.1330±0.041, 0.0472±0.002, 0.645, and 0.275 respectively as presented in Table 3.

DISCUSSION

Plant species, especially the perennials such as trees, rely on the availability of genetic diversity for stability and survival under the ever-changing environments. Population with high level of genetic variation are valuable since they offer a diverse gene pool from which gene conservation and improvement programs can be made (Machua et al., 2007).

Table 1: Marker Attributes of Different Chloroplast Simple Sequence Repeat (cpSSR) Primers used in this Study

S/N	Primers	Seq 5' to 3'	Total Bands	Polymorphic bands	POL (%)	PIC	MI
1	ccmp2	GATCCCGGACGTAATCCTG	5	3	60	20	1200
2	ccmp5	TGTTCCAATATCTTCTTGTCATTT	1	0	0	0	0
3	ccmp6	CGATGCATATGTAGAAAGCC	1	0	0	0	0
Mean			2	1	20	6.66	400
Total			7	3	60	20	1200

Note: POL – Polymorphism Percentage, PIC – Polymorphic Information Content, MI – Marker Index

The reduction in the genetic diversity limits species ability to keep pace with the changing selection pressure and their roles in the ecological and evolutionary development of the biosphere (Machua et al., 2007; Runo et al., 2004). Therefore, maintenance of genetic diversity is important as the diversity carries forward both ecological adaptation and microevolution. The over exploitation of *C. albidum* threatens their genetic diversity and hence could limit their conservation and evolutionary development of the remaining populations.

Table 2: Genetic Variability across all the Accessions using cpSSR Markers

Markers	Sample Size	na*	ne*	h*	I*
ccmp2	15	2	1.8	0.44	0.64
ccmp5	15	2	1.14	0.12	0.25
ccmp6	15	1	1	0	0
Mean	15	1.67	1.31	0.19	0.29
St. Dev.		0.55	0.33	0.18	0.26

na = Observed number of alleles, ne* = Effective number of alleles, h* = Nei's gene diversity; and I* = Shannon's Information index*

Table 3: Nei's Analysis of Gene Diversity in Subdivided Populations

Locus	Sample Size	Ht	Hs	Gst	Nm*
ccmp2	15	0.48	0.11	0.77	0.1458
ccmp5	15	0.09	0.06	0.3	1.1667
ccmp6	15	0	0	****	****
Mean	15	0.19	0.06	0.54	0.2754
St. Dev.		0.04	0		

H_t = Total Gene Diversity, H_s = Gene Diversity due to Variation within Populations, G_{st} = Gene Diversity due to differences among Populations; and Nm = Gene flow*

The percentage of polymorphic loci (42.9%) detected in this study is indicative of how the genetic base of this species had been eroded. The mean estimate of G_{st} over all loci was 0.6, which can be interpreted as, 60% of the total genetic diversity in *C. albidum* species is due to differences among populations. Similarly, 40% of the diversity measured is among trees within populations. According to Hamrick and Godt (1989), species within small geographic ranges tends to maintain less genetic diversity than geographically widespread species. This result suggests that *C. albidum* might have undergone the random changes in genetic composition. These random changes might result in random genetic drift induced by drastic changes in population size (Aparajita and Rout, 2008). Drastic environmental changes may also result in the loss of adaptedness of a particular genotype. According to Jill *et al.,* (2011), adaptations are due to inherited genes. Apaprajita and Rout (2008) observed similar genetic pattern for *Albizia lucida* populations using ISSR makers. It revealed a relatively higher level of genetic diversity within populations of *Albizia lucida* and displayed substantial genetic differentiation among *Albizia lucida* population.

The very low genetic diversity of *C. albidum* recorded in this study has a number of implications for the management and conservation of this species. From a conservation point of view, *C. albidum* in the study area should be managed and maintained because the loss of any tree may lead to further loss of genetic variation for the species. Schaal et al., (1998) reported that genetic diversity is a prerequisite for future adaptive change and evolution and has a profound effect for species conservation.

CONCLUSION AND RECOMMENDATION

In this study, we discovered very low genetic variation in *C. albidum.* We also found that 40% of the total genetic diversity is due to differences within trees in a population. These findings have a number of implications for the management and conservation of this species. Therefore, in-situ conservation should urgently be put in place for preserving the remnant inherent diversity towards capturing the existing local adaptation. Domestication of *C. albidum*

in field plots is also proposed as means of conservation to ensure local people needs for income from the sale of tree products are continually met. This will also decrease pressure on the few natural populations and protect its genetic diversity.

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