Impact of Anthropogenic Activities on Genetic Diversity of *Celtis zenkeri* Engl in South-West Nigeria

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Abstract: *Celtis zenkeri* is a valuable tropical tree species for industrial and domestic purposes. For several years, exploitation of this species has not been sustainable and no reforestation or cultivation effort has been put in place both by governmental and non-governmental agencies. Anthropogenic activities have resulted in deforestation and subsequent reduction in the population size of important timber species. The research aim was to determine the impact of anthropogenic activities on genetic diversity of *Celtis zenkeri* in South-West Nigeria. Extensive sampling of *Celtis zenkeri* was carried out in four natural forest ecosystems. Two pristine forests (SNR, Akure forest and Osun Osogbo sacred grove) and two degraded forests (OA3 and Eda forest reserve). Young leaves were collected from each tree found in each site and preserved with silica gel for molecular analysis. In all, 130 accessions were sampled and were analyzed with five chloroplast microsatellite markers. In the result, allelic richness, which is a measure of genetic diversity and an indicator of a population's long-term potential for adaptability and persistence, was significantly higher in the two pristine forests than the two degraded forests. Statistically, a significant difference was not recorded for the observed heterozygosities (H_o) in the population of *Celtis zenkeri* in the pristine forests (Queen's plot and Osun-Osogbo Sacred Grove) and a degraded forest (OA3). The formal indicates the possibility of conserving genetic diversity using traditional conservation approaches such as taboos and restrictions. Though OA3 is a degraded forest, the impact of the anthropogenic activities may not have been severe when compared with Eda forest reserve. At present, the scale of human activities in Eda forest reserve and OA3 could pose a serious threat to the future viability of the species if unchecked. Therefore, *ex-situ* conservation and sustainable harvesting of *Celtis zenkeri* are recommended.

Keywords: Genetic Diversity, *Celtis zenkeri*, Anthropogenic Activities, Sustainable Harvesting

1. Introduction

Nigeria's forest covers about one-third of the country's land area (983,213 km^2), 10% was successfully put under reservation and a total of 1,160 constituted forest reserves, covering a land area of about 1,075 km^2. Today, most of these reserves only exist on paper [1]. Asides the use of many forest tree species for furniture and construction purposes, their leaves, barks, roots, seeds and flowers serve as health care [2]. Increase rate of human activities on primary forests tend to result in illegal felling of trees and change in land use pattern contributing to forest degradation. Therefore, illegal wood harvesting, change in forested land through encroachment by farmers establishing tree crop plantations are continue to threatening the of most important tree species [3].

Celtis (Ulmaceae) comprises hundred species and is widespread in all tropical and subtropical forests [4]. Eleven of these species are found in tropical Africa and the most widely used in South-West Nigeria is *Celtis zenkeri*. Their wood serves several construction purposes, especially in building, bridges, poles, pestles and tool handles [5]. The tree produces large quantities of litter which decays readily after fall and serves as good green manure [6]. Various parts of *Celtis zenkeri* are used in traditional medicine [7].

The natural population of *C. zenkeri* in the study area has since been dwindling due to unsustainable harvesting and unchecked anthropogenic activities. According to Holdsworth and Uhl (1997), reduction in population densities face further threats from habitat destruction following logging activities [8]. Forest loss and fragmentation, besides their direct effects, may also cause a reduction in gene flow between isolated subpopulations and mutation, inbreeding, genetic drift, disease infestation and virus attack which are associated with a small population size may arise [9]. According to Ellstrand and Elam (1993), populations that remain small for many generations suffer a loss of allelic diversity and random genetic drift [10]. Therefore, conservation efforts emphasizing the diversity within and between degraded forest and strict natural reserve as an additional strategy to safeguard genetic diversity in key areas of tree breeding and forest genetics becomes necessary. Hence, the need for this study.

2. Methodology

2.1. Study Area

The Queen's Plot, Akure Forest Reserve is a strict nature reserve located between longitude 5°1'48" E to 5°3'42" E and latitude 7°13'47" N to 7°17'45.6" N in Ondo State. The reserve covers about 66km^2 of land with a section designated as Strict Nature Reserve since 1954 and other sections set aside for artificial regeneration in the degraded part of the forest. The area is gently undulating and lies on a general altitude of 229m above sea level [11]. According to brief geological description of the forest reserve by Ola Adams and Hall (1987), the underlying rock is crystalline, mainly gneissose and referable to the basement complex [12]. The climate is humid tropical with seasonal variation. The mean annual rainfall is about 4000mm. Temperature ranges from 20.6°C to 33.5°C. The monthly mean temperature is about 27°C, a condition that is conducive to the development of tropical rainforest.

Eda forest reserve was placed under protection since 1941 with the aim of environmental protection and conservation of biological diversity. The reserve lies within lat. 7°41'3"N to 7°47'5"N and long. 5°36'1"E to 5°37'6"E and altitudinal range of 497 to 560m above sea level. The forest covers an area of 826 ha with two division namely: Plantation compartment (318ha) (Eda I), and Natural forest compartment (508ha). The forest reserve is characterized with an undulating terrain, gently sloped and has ultisol and oxisol soil types. The bedrock formation is underlain with basement complex and contains undifferentiated igneous rocks, laterites and white sand. Two seasonal changes were reported for this reserve namely: the wet season which occurs between April to October and the dry season which occurs between November to March and the mean annual temperature ranges from 21°C - 28°C, and mean annual rainfall is 1800 mm, and the relative humidity falls between 56% to 85%.

Oluwa forest reserve (OA3 stand), is located in ore, Ondo state. It lies within latitude 6° 55'N to 3°45'E and 7°20'N to 4°32'E covering an area of 678.06km^2. The reserve is managed by the Agricultural Development Program (ADP), Ondo State Ministry of Environment. The rainfall season for this reserve occurs between March to November, and the dry season occurs between December to February. The annual rainfall ranges from 1400 to 2000mm. The average annual temperature is 28°C with relative humidity of 70%. Soils are predominantly ferruginous tropical, typical of the variety found in intensively weathered areas of basement complex formations in the rainforest zone of South-western Nigeria. However, regrowth, forest thickets, and fallow regrowth are been adopted for the depleted primary forest reserve areas for regeneration at varying stages of development [13].

Osun-Osogbo sacred grove is located outside Osogbo town along the bank of Osun River in Osogbo Local Government Area of Osun State and dedicated to Osun goddess. It lies within latitude 6° 50′N to 7° 45′N and longitude 7° 05′E to 5° 30′E with an elevation about 350 m above sea level. Since 2005, Osun-Osogbo is regarded as UNESCO World Heritage site and Nigerian national monument. In honor of Osun goddess and other deities, the Grove is dotted with 40 sanctuaries and shrines, two palaces and several sculptures and artworks. Osun Osogbo Sacred Grove as a landmass of 75hectares encircled with a buffer zone of 47hectares [14]. It is bounded in the east by Osun State Agricultural Farm Settlements; in the North by Laro and Timehin Grammar Schools; and in the South by the entrance of Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital [15].

2.2. Method of Data Collection

Leaf samples were collected from the four (4) natural population of *Celtis zenkeri*. The coordinates of each living tree were recorded with a global positioning system (GPS). The leaves of *Celtis zenkeri* collected were cleaned and stored in a polyethylene bag containing silica gel. The forests used, sample size and GPS coordinate of each forest is presented in Table 1.

Table 1. List of forest reserves used, sampled size and their geographical locations.

S/N	Forest Reserves	Sampled Size	Coordinate Points	
			Latitude	Longitude
1	Queen's Plot, Akure Forest Reserve	55	7.26850	5.03554
2	Osun Osogbo Sacred Grove	38	7.75624	4.53344
3	OA3 Forest Reserve	24	6.84441	4.78953
4	Eda Forest Reserve	13	7.73643	5.62366

2.3. DNA Extraction

The sampled leaves were grounded to a fine powder with mortar and pestle. Fine powder 0.05g was measured and was put in 1.5mL micro tubes containing 400μL of the 2% Cetyltrimethylammonium bromide (CTAB) extraction buffer with the following modifications [20mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2% CTAB, plus 1% b-mercaptoethanol added just before use]. The micro tubes was vortexed for 10 seconds and incubated at 60°C for 30 min. a 60μl of chloroform-isoamylalcohol (24:1) was added to the solution, then vortexed for 10s and centrifuged at 10,000rpm for 3 minutes. The supernatant was transferred to a fresh tube (480μl of chloroform was added to 20μl of isoamylalcohol). Cold isopropanol was added to the supernatant at 0.7 of the total volume of collected supernatant. The samples was gently mixed by inversion and centrifuged at 10,000 rpm for 3 minutes. The DNA pellet adhered to the tube was then visualized (the volume of the supernatant collected was multiplied by 0.7 to know the volume of isopropanol to added). The liquid phase was gently released and DNA was washed twice with 500μl 70% ethanol (70% ethanol was prepared by adding 30ml of distilled water to 70ml of concentrated ethanol). The tubes was inverted to allow drying of pellet for 12hours upon filter paper at room temperature. 100μl TE buffer solution plus 5μl RNAse was added to the pellet and incubated at 37°C for 1 hour.

2.4. Polymerase Chain Reaction and Gel Electrophoresis

Polymerase Chain Reaction (PCR) was conducted at a total volume of 12.5μl, containing 6.25μl *Taq* polymerase 2X master mix with standard buffer, 1μl of DNA template, 0.5μl Forward Primer, 0.5μl Reverse Primer, 4.25μl of nuclease free water. The template DNA was denatured at 94°C for 5 min and then subjected to 35 cycles of 94°C for 30 s, 52 to 56°C for 50 s, and 72°C for 2 min. The final cycle included an extension at 72°C for 10 min. Amplified PCR products were separated by electrophoresis using 2% agarose gel in TBE buffer stained with 5μl ethidium bromide and visualized under UV light. Fragment sizes were estimated using 50–1000 bp Bench Top PCR ladder - DNA sizing marker.

2.5. Method of Data Analysis

Five (Table 2) out of seven primers used in this study showed amplicons and therefore selected for further analysis. Scored bands were analyzed with Cervus version 3.0.7. For each of the loci, number of alleles (A) per locus, number of individuals at the locus, observed (H_o) and expected (H_e) heterozygosities, polymorphic information content (PIC) and allelic richness (A_r) were estimated.

Table 2. *Molecular markers used in the study.*

S/N	Name	Sequence 5 - 3'	Bases
1	TRNH–PSBA-F	CGCGCATGGTGGATTCACAATCC	23
2	TRNH–PSBA-R	GTTATGCATGAACGTAATGCTC	22
3	TRNL–TRNF-F	GGTTCAAGTCCCTCTATCCC	20
4	TRNL–TRNF-R	ATTTGAACTGGTGACACGAG	20
5	CCMP1 F	CAGGTAAACTTCTCAACGGA	20
6	CCMP1 R	CCGAAGTCAAAAGAGCGATT	20
7	CCMP6 F	CGATGCATATGTAGAAAGCC	20
8	CCMP6 R	CATTACGTGCGACTATCTCC	20
9	CCMP10 F	TTTTTTTTTAGTGAACGTGTCA	22
10	CCMP10 R	TTCGTCGDCGTAGTAAATAG	20

Sources: TRNH–PSBA [16], TRNL–TRNF [17], CCMP1, CCMP6 and CCMP10 [18].

3. Result

The marker information for the studied species in each forest is presented in Table 3. The comparison of genetic diversity of *Celtis zenkeri* among the four natural populations is presented in Table 4. The number of individuals sampled in each population varied from 55 in Queen's Plot, Akure Forest Reserve to 13 in Eda Forest Reserve. The few numbers of *Celtis zenkeri* found in Eda and OA3 could be attributed to severe anthropogenic activities. Allelic richness of *Celtis zenkeri* was significantly higher in Queen's Plot. This was followed by the allelic richness of *Celtis zenkeri* in Osun Osogbo Sacred Grove, OA3 and Eda Forest Reserve respectively. The observed heterozygosity was statistically the same for C*eltis zenkeri* in Queen's Plot (0.09), Osun Osogbo Sacred Grove (0.13) and OA3 (0.09) respectively. However, no heterozygosity (0.00) was observed in the population of C*eltis zenkeri* in Eda Forest Reserve.

The expected heterozygosity obtained for *Celtis zenkeri* in Queen's Plot, Akure Forest Reserve (0.78) was not significantly higher than the expected heterozygosity for *Celtis zenkeri* in OA3 (0.78). Osun-Osogbo Sacred Grove had the second highest expected heterozygosity (0.74) and Eda forest reserve had the least expected heterozygosity (0.71) as presented in Table 4. In each forest, observed heterozygosity was comparatively lower than the expected heterozygosity. This indicates a serious genetic erosion in the population of *Celtis zenkeri* in South West Nigeria.

Table 3. Marker information for studied species in each forest.

Locus	Queen's Plot			Osun Osogbo			OA3			Eda Forest Reserve		
	K	N	PIC	K	N	PIC	K	N	PIC	K	N	PIC
TRNH-PSBA	6	28	0.76	5	25	0.66	4	11	0.66	4	6	0.62
TRNL-TRNF	7	30	0.76	6	31	0.73	5	13	0.73	4	6	0.62
CCMP1	5	28	0.73	5	26	0.68	5	13	0.71	4	6	0.62
CCMP6	6	29	0.72	6	26	0.67	5	12	0.70	2	4	0.38
CCMP10	7	24	0.70	6	26	0.67	6	11	0.71	4	6	0.67
Mean	6.2			5.6			5.0			3.6		

K = Number of alleles per locus, N = Number of individual, PIC = Polymorphic Information Content.

Table 4. Comparison of Genetic Diversity of Celtis zenkeri in all the populations assayed.

Population	Sample size	Allelic richness (A_r)	Observed Heterozygosity (H_0)	Expected Heterozygosity (H_e)
Queen's Plot, Akure Forest Reserve	55	6.20[a]	0.09[a]	0.78[a]
Osun-Osogbo Sacred Grove	38	5.60[ab]	0.13[a]	0.74[ab]
OA3 Forest	24	5.00[b]	0.09[a]	0.78[a]
Eda Forest	13	3.60[c]	0.00[b]	0.71[b]

Different alphabet means there is significant different between the variables along the column.

4. Discussion

The natural population of *Celtis zenkeri* is decreasing rapidly due to industrial demand for the wood and space for agriculture which competes for the same pieces of land upon which the forest stands [3]. Farmers naturally open new land areas and plow the land for new farming seasons [19]. The latter implication of this is that when the existing *Celtis zenkeri* is exploited either through logging or clearance for agriculture, the species population will gradually decrease. This explained the sampled size of *Celtis zenkeri* in OA3 and Eda forest reserves.

Generally, allelic richness and observed heterozygosity were significantly low in the degraded forests. This could be due to severe human activities in these forest reserves. This further affirmed that clearance for agriculture and logging as a major anthropogenic activity capable of reducing the population size and genetic diversity of important tree species. Statistically, there was no significant difference recorded for the observed heterozygosities (H_o) in the population of *Celtis zenkeri* in both pristine forests (Queen's plot and Osun-Osogbo Sacred Grove) and a degraded forest (OA3). The formal indicates that traditional conservation method, such as the use of taboos, could be a veritable tool for genetic diversity conservation of an important tree species. Though OA3 is a degraded forest, the impact of the anthropogenic activities may not have been serious when compared with Eda Forest Reserve. According to Davies *et al.,* (2015) with long and overlapping generation times and high levels of outcrossing and dispersal rates, trees might not respond immediately to logging and fragmentation in terms of loss of genetic diversity [20]. According to Kramer *et al.,* (2008), for post-fragmentation generations to show lower quantitative genetic variation, loss of genetic diversity or clear inbreeding effects in a generation or two, the forest would have to be very small and in patches [21]. It is clear that before genetic consequences for degraded forest could be observed, populations will be relatively small plus the adult trees would have been removed.

Eda Forest Reserve, a degraded forest reserve, recorded the lowest genetic diversity in this study. This reerve was placed under protection since 1941 with the aim of environmental protection and conservation of biological diversity.. The natural forest that was originally stocked with numerous economic tree species has recently been exposed to unsustainable timber harvesting [22]. This forest reserve is presently undergoing a lot of changes due to anthropogenic activities, especially logging and conversion of forest land to cocoa and plantain farms through encroachment [23]. Increase rate of human activities on primary forests has a result of illegal felling of trees and change in land use pattern contributed immensely to forest degradation [24]. The report of Olajuyigbe and Adaja (2014) recorded rapid population growth, clearance for agriculture, domestic use of wood and selective logging as major drivers of deforestation. These activities have also been noted in Eda Forest Reserve [25]. Obviously, the expansion of anthropogenic disturbances in primary forest areas is increasingly devastating. Activities such as logging, establishment of cocoa and plantain plantations have completely placed an immense pressure on the species diversity of Eda Forest Reserve and brought the genetic diversity of *Celtis zenkeri* to zero.

5. Conclusion

The population of *Celtis zenkeri* in Queen's Plot, Akure variation. Anthropogenic forces have in no small way brought about a drastic reduction in the population of this species and subsequently lost of genetic diversity in the Eda forest reserve. It should be noted that the distribution of genetic diversity within species has significant effect on survival and evolution of the species in changing environmental conditions. The scale of human activities in Eda Forest Reserve and OA3 could pose a serious threat to the future viability of this species if unchecked. Sustainable management practices and effective conservation for continuous cover forest for *Celtis zenkeri* are therefore recommended.

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