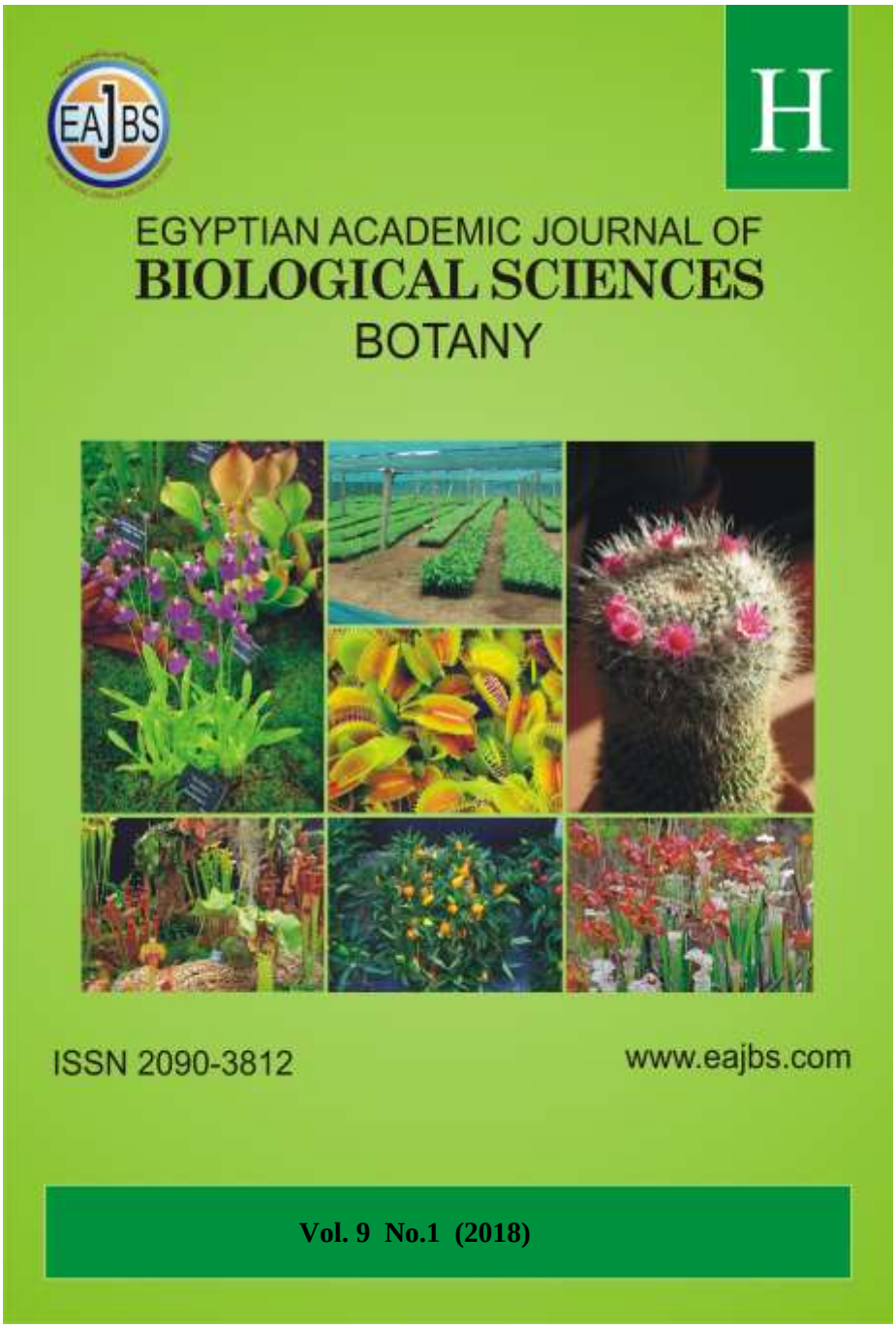


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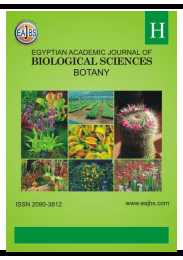

# Genetic Diversity of *Vernonia* as Revealed By Random Amplified Polymorphic DNA (RAPD) Markers

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**ABSTRACT**

*Vernonia* Schreb. is a genus in the family Asteraceae. It has over 1000 species which may be trees, shrubs, woody climbers or herbs. Some of the species are economically important as sources of food and herbal medicine as well as for industrial purposes. This study was carried out to assess the genetic diversity of *Vernonia* species in Nigeria. Molecular characterization was carried out on a total of 50 *Vernonia* samples consisting of 34 samples (13 species) from field collections and 16 samples (10 species) from Ife Herbarium collections making a total of 23 species using 4 RAPD primers. Analysis of the molecular data was done using NTsys version 2.02j computer programme. Results from molecular studies showed amplification through polymerase chain reaction (PCR) with 4 RAPD markers namely OPA-02, OPD-14, OPC-10 and OPE-01. Analysis of the molecular data grouped the samples into 4 Groups with only one species ungrouped. Group I had 17 species, II had 3 species, III had 6 species while IV had 2 species. *V. amygdalina* was represented in all 4 Groups, *V. glabra* and *V. guineense* were present in Groups 1 and 3, *V. tenoreana* and *V. purpurea* were in Groups 1 and 2 respectively, while *V. colorata* remained ungrouped. All 50 *Vernonia* samples used in the molecular study clustered at 64% and analyzed at a similarity coefficient of 72%. This analysis revealed that RADP markers are useful tools in assessing the level of genetic diversity in *Vernonia*.

## INTRODUCTION

*Vernonia* Schreber is a genus of about 1000 species of trees, shrubs, woody climbers or herbs in the family Asteraceae (Jones, 1981; Gilbert, 1986; Dematteis and Pire; 2008, Kemka-Evans and Okoli, 2013; Martucci *et al*., 2014). Some species such as *Vernonia altissimo* Nutt.*Vernonia cinerascens* Sch.Bip. *Vernonia galamensis* L and *Vernonia noveboracensis* (L.) Michx.are known as Ironweed, while some species are edible and of economic value. The plants are characterized by intense purple flowers and compound inflorescences that have the appearance of a single 'composite' flower. The genus was named after an English botanist William Vernom. Common species include *V. biafrae* Olive & Hiern, *V. colorata* (Wild) Drake, *V. cinerea* (Linn.) Less. *V. nigritiana*

Hook F., *V. oocephala* Balc, *V. purpurea* Sch. Dip. and *V. tenoreana* Olive. Of these, the most popular species is *V. amygdalina* Del. which apart from being used as soups, is also used locally in traditional medicine in South-Eastern Nigeria where it is known as 'Olugbu' or 'Onugbu' among the Igbo -speaking people of Eastern Nigeria. It is called 'Ewuro' among the Yoruba-speaking people of South-Western Nigeria and 'Shuwaka' among the Hausa-speaking people of Northern Nigeria (Nwakanma *et al*., 2011a).The leaves are simple or rarely compound; they may be alternate or opposite in arrangement.

The species within this genus present a great variability in habit and morphology, leading to diverse criteria of taxonomic delimitation (Dematteis and Pire, 2008; Angulo and Dematteis, 2009; Martucci *et al*., 2014). Ayodele (1997) reported that species of the genus *Vernonia* display different plant forms (i.e. growth habits) namely arborescent, shrubby and herbaceous forms. There are annuals, herbaceous or woody perennials and scramblers. There are also weedy climbers or stragglers (Hutchinson and Dalziel, 1963; Faust, 1972; Olorode, 1984; Burkill, 1985,; Mih *et al*., 2008a). The various growth forms have different types of inflorescence. However, some types of the inflorescence cut across a number of growth forms (Ayodele, 1994).

*V. amygdalina, V. calvoana, V. colorata* and some other species are commonly grown as vegetables in Benin, Cameroun, Nigeria, Gabon, DR Congo and to a lesser extent in their neighbouring countries for local consumption or are used as hedge-rows in farms and in home gardens (Burkill, 1985). Mih *et al*., (2008 a, b) reported that *V. guineensis*, is also in the list of plants eaten as leaf vegetables along with those listed above here. *V. amygdalina* has been reported to be non-hepatotoxic (Ojiakor and Nwanjo, 2006). The leaves of *V. amygdalina* have also been reported to be pesticidal to the larvae of *Callosobruchus maculatus* (Coleoptera; Bruchidae) and *Sitophilus zeamais* (Coleoptera; Curculioniadae) which are serious pests of economic crops (Kabeh and Jalingo, 2007), while constituents of essential oil from *V. amygdalina* has been documented as weevil protectants (Asawalan and Hassanali, 2006 Random amplified polymorphic DNA (RAPD) analysis is widely used for studying the taxonomy of various genera and species for differentiation of intra-species variation and to study the genetic diversity of various cultivars and lines (Williams *et al.,* 1990). RAPD markers offer the quick screening of different regions of the genome for genetic polymorphism and are applied widely, particularly in plant molecular biology for the detection of genetic variation, construction of linkage maps in plants and animals and in the bulk segregant analysis for identifying markers linked to target genes (Huang *et al*., 2003). Genetic diversity studies have been reported for other plants such as vegetable *Solanum* (Sifau, 2013), jute plant – *Corchorus olitorius* (Ogunkanmi *et al.,* 2010), stoke asters – *Stokesia laevis* (Gettys and Werner, 2001) and *Amaranthus* species (Tony-Odigie *et al.,* 2012).

## MATERIALS AND METHODS

### Plant Materials of *Vernonia*

A total of 50 *Vernonia* samples representing a total of 23 species were used for molecular studies. Of these, 34 samples were selected from field trip collections (on the basis of suitability after sample drying because not all the samples dried well – some grew moulds) from the five (5) agro-ecological zones of Nigeria while the other 16 samples were obtained from Ife Herbarium, Obafemi Awolowo University (OAU) Ile Ife, Osun State (Table 1).

Table 1: List of 50 *Vernonia* samples used for the molecular characterization of the species

| S/N | SAMPLE ID CODE | NAME OF SAMPLE | AGRO-ECOLOGICAL DESCRIPTION | LAND USE STATUS | ORIGIN OF SAMPLE |
|---|---|---|---|---|---|
| 1 | T01 | *V. amygdalina* | Mid Altitude | Domestic bush | Lekitaba, Taraba State |
| 2 | T02 | *V. amygdalina* | Mid Altitude | Domestic bush | Lekitaba,Taraba State |
| 3 | T04 | *V. glabra* | Mid Altitude | Domestic bush | Lekitaba,Taraba State |
| 4 | T05 | *V. glabra* | Mid Altitude | Domestic bush | Lekitaba,Taraba State |
| 5 | T06 | *V.glabra* | Mid Altitude | Domestic bush | Lekitaba,Taraba State |
| 6 | T07 | *V. tenoreana* | Mid Altitude | Domestic bush | Lekitaba,Taraba State |
| 7 | T08 | *V. tenoreana* | Mid Altitude | Domestic bush | Lekitaba,Taraba State |
| 8 | T15 | *V. purpurea* | Mid Altitude | Cultivated farm | Lekitaba,Taraba State |
| 9 | T17 | *V. bamendae* | Mid Altitude | Bush near farm | Lekitaba,Taraba State |
| 10 | T21 | *V. migeodii* | Mid Altitude | Open stony ground | Tunga, Taraba State |
| 11 | T22 | *V. smithiana* | Mid Altitude | Open grassland | Tunga, Taraba State |
| 12 | T23 | *V. oocephala* | Mid Altitude | Open grassland | Tunga, Taraba State |
| 13 | T24 | *V. guineense* | Mid Altitude | Open grassland | Tunga, Taraba State |
| 14 | T28 | *V. amygdalina* | Mid Altitude | Cultivated garden | Tunga, Taraba State |
| 15 | T29 | *V. glabra* | Mid Altitude | Open grassland | Nguroje, Taraba State |
| 16 | T30 | *V. glabra* var. *occidentalis* | Mid Altitude | Open grassland | Nguroje, Taraba State |
| 17 | T31 | *V. calvoana* | Mid Altitude | Abandoned land | Nguroje, Taraba State |
| 18 | T32 | *V. myriantha* | Mid Altitude | Abandoned land land | Nguroje, Taraba State |
| 19 | B02 | *V. amygdalina* | Sudan Savanna | Cultivated garden | Birnin Kebbi, Kebbi State |
| 20 | B05 | *V. amygdalina* | Sudan Ssavannah | Cultivated garden | Birnin Kebbi, Kebbi State |
| 21 | Z02 | *V. amygdalina* | Sudan Savannah | Domestic garden | Guzau, Zamfara State |
| 22 | C02 | *V. amygdalina* | Humid Forest | Cultivated garden | Calabar, Cross River State |
| 23 | C03 | *V. amygdalina* | Humid Forest | Cultivated gaarden | Calabar, Cross River State |
| 24 **or** 51 | IFE 25 | *V. purpurea* | Indeterminable | Herbarium collection | IFE Herbarium |
| 25 | K06 | *V. camporum* | Southern Guinea Savanna | Domestic bush | Kontangora, Niger State |
| 26 | K07 | *V. camporum* | Southern Guinea Savanna | Domestic bush | Kontangora, Niger State |
| 27 | K02 | *V. amygdalina* | Southern Guinea Savanna | Domestic garden | Kontangora, Niger State |
| 28 | L01 | *V. amygdalina* | Derived Savanna | Domestic garden | Lafia, Nassarawa State |
| 29 | L04 | *V. amygdalina* | Derived Savanna | Domestic garden | Lafia, Nassarawa State |
| 30 | U05 | *V. amygdalina* | Humid Forest | Domestic garden | Umuahia, Abia State |
| 31 | U09 | *V. amygdalina* | Humid Forest | Domestic garden | Umuahia, Abia State |
| 32 | U10 | *V. amygdalina* | Humid Forest | Domestic garden | Umuahia, Abia State |
| 33 | IKO 1 | *V. amygdalina* | Humid Forest | Domestic garden | Ikorodu, Lagos State |
| 34 | IKO 3 | *V. amygdalina* | Humid Forest | Domestic garden | Ikorodu, Lagos State |
| 35 | IKO 5 | *V. amygdalina* | Humid Forest | Domestic garden | Ikorodu, Lagos State |
| 36 | IFE 01 | *V. ambigua* | Indeterminable | Herbarium collection | IFE Herbarium |
| 37 | IFE 04 | *V. cinerea* | Indeterminable | Herbarium collection | IFE Herbarium |
| 38 | IFE06 | *V.colorata* | Indeterminable | Herbarium collection | IFE Herbarium |
| 39 | IFE 08 | *V. conferta* | Indeterminable | Herbarium collection | IFE Herbarium |
| 40 | IFE 09 | *V. pauciflora* | Indeterminable | Herbarium collection | IFE Herbarium |
| 41 | IFE 10 | *V. galamensis* | Indeterminable | Herbarium collection | IFE Herbarium |
| 42 | IFE14 | *V. glabberima* | Indeterminable | Herbarium collection | IFE Herbarium |
| 43 | IFE 15 | *V. guineense* | Indeterminable | Herbarium collection | IFE Herbarium |
| 44 | IFE 16 | *V. migeodii* | Indeterminable | Herbarium collection | IFE Herbarium |
| 45 | IFE 18 | *V. nestor* | Indeterminable | Herbarium collection | IFE Herbarium |
| 46 | IFE 19 | *V.nigrithiana* | Indeterminable | Herbarium collection | IFE Herbarium |
| 47 | IFE 21 | *V. tenoreana* | Indeterminable | Herbarium collection | IFE Herbarium |
| 48 | IFE 26 | *V. smithiana* | Indeterminable | Herbarium collection | IFE Herbarium |
| 49 | IFE 23 | *V. purpurea* | Indeterminable | Herbarium collection | IFE Herbarium |
| 50 | IFE 22 | *V. perottetii* | Indeterminable | Herbarium collection | IFE Herbarium |

Key: Ife Herbarium is in the Department of Botany, Obafemi Awolowo University, Ile Ife

**DNA Extraction:**

Total genomic DNA was extracted from 50 dried leaf samples of 23 species of *Vernonia* (using the modified minipreparation protocol described by Dellaporta *et al.*, (1983) as follows: Approximately 1g of dried plant herbarium sample was ground to very fine powder in cryo vials using a mini bead beater (Biospec, USA). The powder was transferred to a 30ml tube and 1 ml of extraction buffer was added to it. 50 ml of 20% SDS was then added and mixed thoroughly by shaking. The tubes were then incubated at 65 $^{o}$C for 30 mins. After this, 250 µl of 5 M Potassium acetate was added and incubated on ice for 20 mins. The contents of the tubes were centrifuged at 14,500 rpm for 10 minutes. The supernatant was transferred into fresh tubes containing 500 µl of isopropanol. The tubes were mixed (by turning them up and down) and incubated at -20$^{o}$C for 30 minutes. Then, the tubes were spun at 14,500 rpm for 15 mins. The supernatant was poured out and the tubes left to drain by inverting them on a paper towel. The pellets were re-dissolved in 0.7 ml of 10 mM EDTA, pH 8, transferred to Eppendorf tubes and centrifuged at 14,500 rpm for 10 minutes to pellet the insoluble debris which were washed with 500 µl of 70% Ethanol.

**Checking the Quality of Extracted DNA by Electrophoresis:**

The quality and purity of extracted DNA were checked with 1% Agarose gel. A 1% agarose gel was prepared by mixing 1.5g agarose with 150ml 1X TBE Buffer. It was melted in microwave for 2 mins until all agarose was dissolved. Once it is cooled to about 56$^{o}$C, 5µl of Ethidium Bromide was added (in the fume chamber) and swirled to mix properly. The gel was poured into the tray containing combs and left to stand for at least 30 mins to set before loading samples. Combs were carefully removed, and the gel was placed into an electrophoresis tank containing TBE buffer. Using a 1-20 µl pipette range, 5 µl of DNA was mixed with one drop (2µl) of loading dye and the mixture was loaded into the well on the gel. This was run at 100 MA for 1 hr. Thereafter, the gel was viewed (with the use of eye protection) under the ultraviolet transilluminator attached to a computer system and photographs of the gel were taken with the aid of gel documentation unit (Uvitech) and saved.

**Spectrophotometric Analysis:**

The spectrophotometer, Eppendorf BioPhotometer plus (UV/Vis Photometer), was first standardized (blanked) with 100 µl of distilled water before proceeding with the DNA samples. Then, for each sample, 5 µl of DNA was added to 95 µl of distilled water in a cuvette then placed in the cuvette compartment of the spectrophotometer. Both the measurement and calculation of results were performed at the press of the sample button on the equipment. The large LCD screen displayed the sample concentrations, absorption values, OD260/OD280 and OD260/OD230 ratios, and sample dilution at a glance. These were recorded for each sample.

**Polymerase Chain Reaction (PCR):**

It involved initiation, denaturation, annealing, elongation and the final extension of the DNA fragment over varying temperatures of 94 $^{o}$C, 94 $^{o}$C and 48 $^{o}$C or 50 $^{o}$C, 72 $^{o}$C and 72 $^{o}$C respectively. The reaction mixture was held at 4 $^{o}$C. All PCR reactions were carried out in 20 µl reaction mixtures of master mixes [containing 2 µl total genomic DNA, 1 µl of 200 pM a given primer (OPA 02, OPC 10, OPD 14 and OPE 01), 1 µl of 1 mM dNTPs (deoxyribonucleotide triphosphates); each of 1x PCR solution – 200 µM dATP (2' deoxyadenosine 5' triphosphate), 200 µM dCTP (2' deoxycytidine 5' triphosphate) 200µM dGTP (2' deoxyguanosine 5' triphosphate) and 200 µM dTTP (2'deoxythymidine 5' triphosphate), 5x Reaction Buffer B (0.4M Tris-HCl, 0.1 M $(NH_4)_2SO_4$, 0.1% w/v Tween-20(Polyoxyethylene Sorbitan monolaurate with 20 ethylene oxide units); 7.5 mM $MgCl_2$] (1x PCR solution – 1.5 mM $MgCl_2$) and ultra-pure water., Blue dye (Migration equivalent

to 3.5-4.5 kb DNA fragment), Yellow dye (Migration rate in excess of primers in 1% agarose gel <35-45 bp), compound that increases sample density for direct loading and double distilled water was loaded into Eppendorf Thermocycler machine from USA for DNA amplification.

Polymerase Chain Reaction (PCR) is a technique in molecular biology by which a small fragment of deoxyribonucleic acid (DNA) can be rapidly cloned, or duplicated, to produce multiple DNA copies. PCR can be used to identify individuals from minute amounts of tissue or blood, to diagnose genetic diseases and to research evolution (Adekoya, 2009). PCR proceeds in a series of cycles, or rounds. Each successive round doubles the amount of DNA and thus more than 1 billion copies of a single DNA fragment can be made in just a few hours. There are three phases in a polymerase chain reaction. In the first phase, called denaturation, the template, or piece of original DNA is heated to a temperature of from 90$^{o}$C to 95$^{o}$ C (194$^{o}$ to 203$^{o}$ F) for 30 seconds, which causes the individual strands to separate. In the second phase, called annealing, the temperature of the mixture is lowered to 55$^{o}$ C (131$^{o}$ F) over a 20-second period, allowing the oligonucleotide primers to bind to the separated DNA. In the third phase, called polymerization, the temperature of the mixture is raised to 75$^{o}$ C (167$^{o}$ F), a temperature at which the polymerase can copy the DNA molecule rapidly. These three phases are carried out in a vial and make up one complete cycle, which takes less than two minutes to complete. Theoretically, the PCR cycle can be repeated indefinitely, but the polymerase, nucleotides and primers are usually renewed after 30 cycles. Thirty PCR cycles can produce 1 billion DNA copies in less than three hours (Adekoya, 2009).

Amplification conditions for this research were an initial denaturation step of 3mins at 94 $^{o}$C followed by 45 cycles each consisting of a denaturation step of 1 min at 94 $^{o}$C,the annealing step of 1 min at 37$^{o}$C and an extension step of 1 min at 72$^{o}$C. The last cycle was followed by 5 mins extension at 72 $^{o}$C. The amplified products were subjected to electrophoresis on 2% agarose gels and visualized by staining with Ethidium Bromide under ultraviolet (UV) light and photographed using gel documentation system Uvitech.

A total of 4 Random Amplified Polymorphic DNA (RAPD) primers were used in the study to detect polymorphisms between individual specimens of the same species and between specimens from different species and locations. The sequence and information of the primers used are presented in Table 2.

Table 2: Primers and sequences of RAPD Primers used in the study

| S/NO. | NAME OF PRIMER | SEQUENCE (5' to 3') |
|---|---|---|
| 1 | OPA 02 | TGCCGAGCTG |
| 2 | OPC 10 | TGTCTGGGTG |
| 3 | OPD 14 | CTTCCCCAAG |
| 4 | OPE 01 | CCCAAGGTCC |

**Agarose Gel Electrophoresis for the Amplification of PCR Products:**

Gel preparation – 2% agarose was dissolved by heating in the microwave oven for 45 minutes in an appropriate volume of Tris-Borate EDTA (TBE) buffer 0.5X TBE (950 ml of $dH_2O$ with 50 ml of 10X TBE. 5 µl of Ethidium Bromide was added (in the fume chamber) and swirled to mix properly. After the agarose was cooled to 56 $^{o}$C, it was poured into the gel tray that was prefixed with a comb to create wells in the gel in which the DNA was run. The gel was immersed into the electrophoresis tank containing Tris-Borate EDTA (TBE) buffer. The comb in the gel was removed to expose the wells formed.

**Loading of Samples and Running the Gel:**

A drop (2 µl) of loading buffer (0.25%) bromophenol blue, 0.25% Xylene cyanol FF and 30% glycerol in water was placed on a parafilm and 8-10 µl amplified DNA mixed with it. The mixture was introduced into the well in the gel using a 1-10 µl range pipette. A standard DNA marker (1kb DNA ladder from Solis BioDyne) was treated in a similar manner. The gel was run within 80 – 100 V range for 2 h. (Figures 1 and 2).

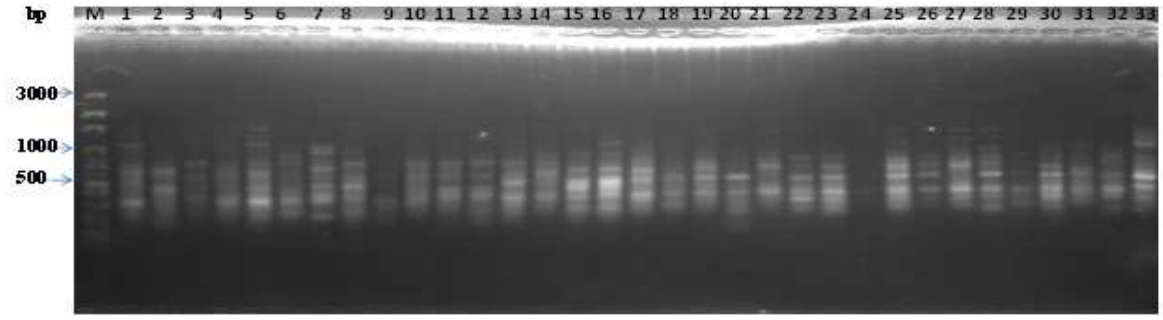
**Gel Scoring and Data Analysis:**

Fragments that were clearly resolved on the gel were scored as 1 or 0 i.e. present or absent respectively on all the plates for all the species from various locations. The bands that could not be confidently scored were regarded as missing data. Pair-wise distance (similarity) matrices were computed using sequential, hierarchical and nested (SAHN) clustering option of the NTSYS-pc 2.02j) software package (Rohlf, 1993). The program generated dendrograms, which grouped the test lines on the basis of Nei genetic distances (Nei, 1972) using unweighted pair group method with arithmetic average (UPGMA) cluster analysis (Sneath and Sokal, 1973).

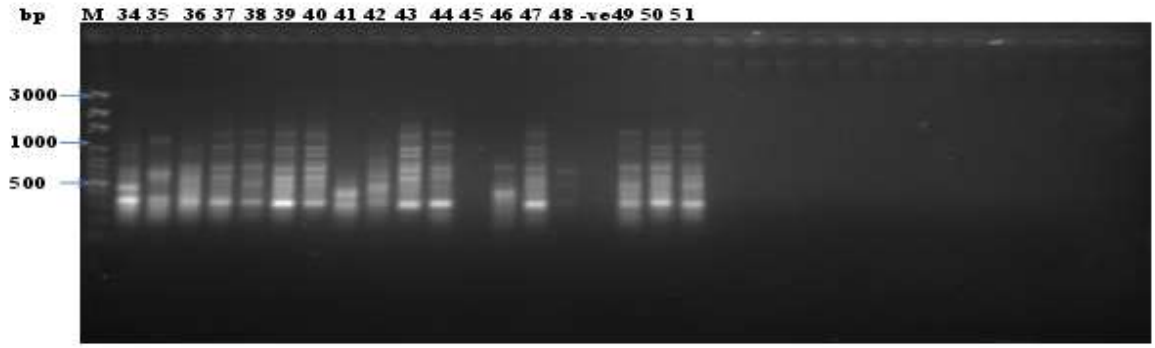
## RESULTS AND DISCUSSION

**Results from Molecular Studies:**

The results from molecular studies are presented in Figures 1-3 below:

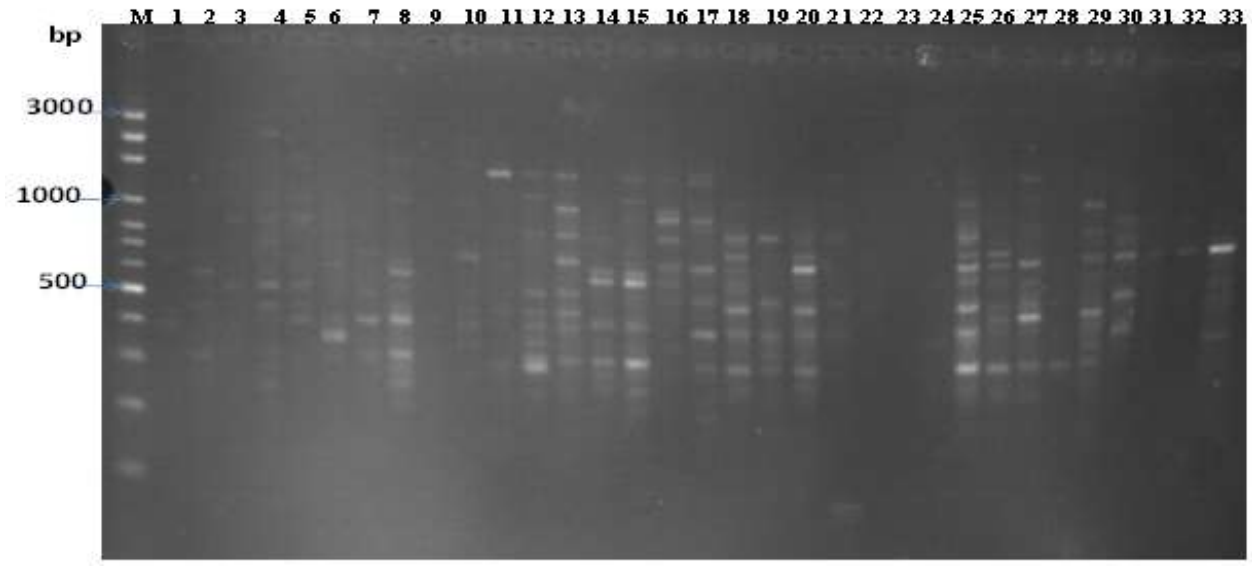



a




b

**Fig.1 (a) and (b):** PCR gel obtained with RAPD primer OPA 02 on the 50 *Vernonia* samples used for molecular studies among the species. Bands appear on each sample's well lane run on a 2% agarose gel. M= 100 base-pair (bp) ladder. (a) contains samples 1-33 while (b) has 34-51.




a


b

**Fig. 2 (a and b):** PCR gel obtained with RAPD primer OPD 14 on the 50 *Vernonia* samples used for molecular studies among the species. M = 100 base pair (bp) DNA ladder. (a) contains samples 1-33 while (b) contains 34-51. Bands on each well lane show amplification with the primer.

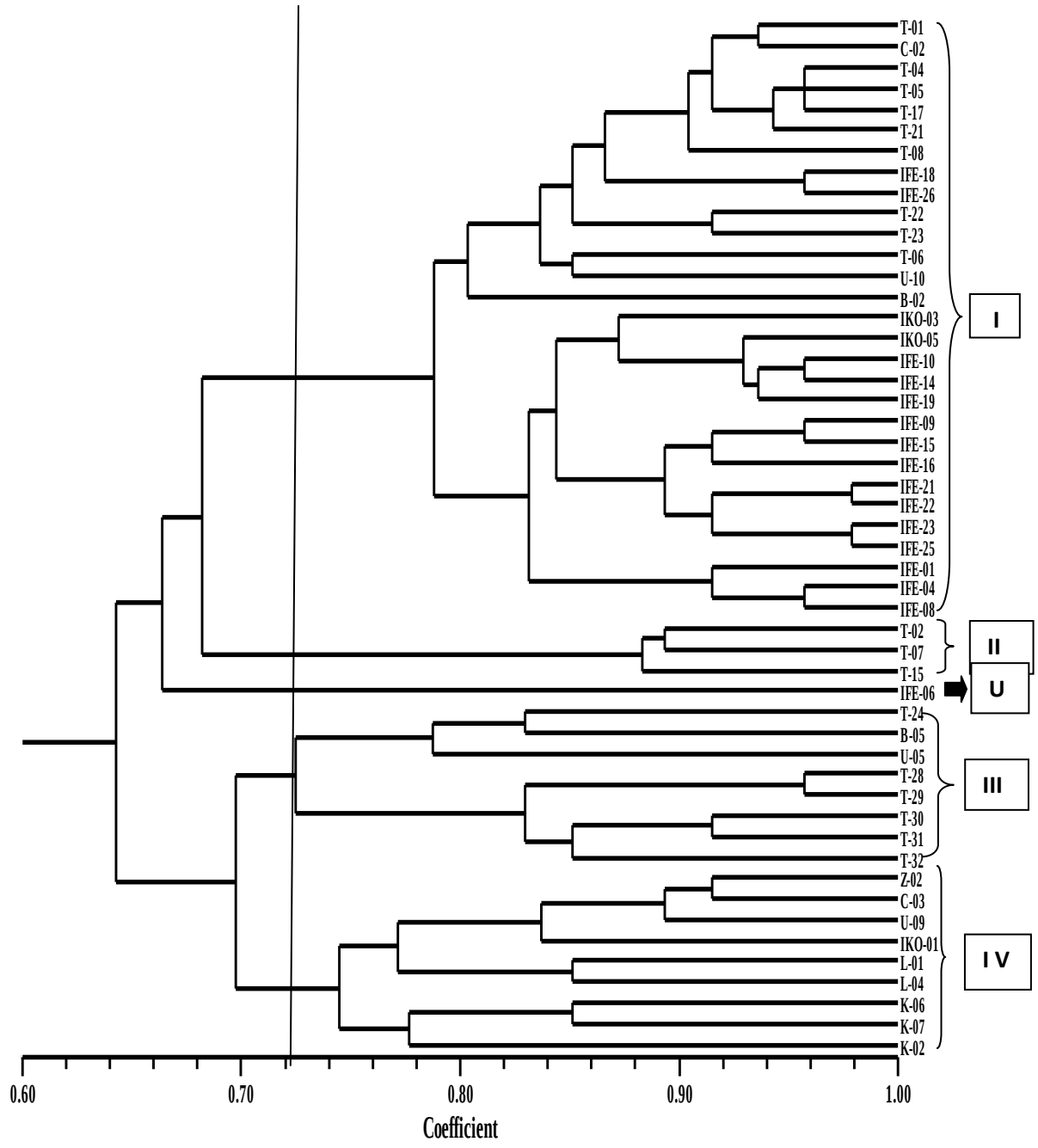



**Fig. 3:** Dendrogram generated from molecular data using NTsys version 2.02j computer programme showing Relationships among the 50 *Vernonia* samples consisting of 23 species which grouped samples into Groups(I-IV) while 1 sample remained ungrouped (U) at a coefficient of 0.72 (or 72%).

## DISCUSSION

Analysis of molecular data (represented by Figures 1-2 using NTsys version 2.02j computer programme revealed that all 23 *Vernonia* species used (from 50 samples in the study) calculated a mean genetic similarity coefficient of 64% using RAPD markers (Fig. 3). This means that there was about 36% genetic variation among all the *Vernonia* species

studied indicating high polymorphism. However, at a similarity coefficient of 72%, the molecular analysis resolved the species tested into 4 Groups with only one species (*V.colorata*) ungrouped. The groups reflect taxa affinities. Group 1 contained 29 samples made up of 17 species (*V. amygdalina, V. bamendae, V. cinerea, V. conferta, V. galamensis, V. glabra, V. glabberima, V. guineense, V. migeodii, V. nestor, V. nigritiana, V. oocephala, V. pauciflora, V. perottetii, V. purpurea V. smithiana and V. tenoreana*). Group II had 3 samples consisting of 3 species (*V. amygdalina, V. purpurea* and *V tenoreana*). Group III incorporated 8 samples with 6 species (*V. amygdalina, V calvoana, V. glabra, V glabra* var. *occidentalis and V. guineense* and *V. myriantha*) while Group IV admitted 9 samples which had 2 species (*V. amygdalina* and *V. camporum*). Only *V. colorata* remained ungrouped. It is observed here that *V. amygdalina* was found in all 4 Groups in the molecular analysis. *V. glabra* was found in Groups I and III while *V. guineense* was represented in Groups I and III. On the other hand, *V. purpurea* and *V. tenoreana* were both represented in Groups I and I1. The consistent clustering between *V. amygdalina* and *V. camporum* seems to confirm the fact that these two species may have arisen from the same putative parents or may have diverged from each other in the course of their evolutionary adaptation in different agro-ecological zones of Nigeria. It is posited here that *V. amygdalina* and *V. camporum* may be more closely related (inter – specific similarity) than they are with all other species used in the study. Again the consistent representation of *V. amygdalina* in all 4 clusters (Groups) shows the huge intra-specific relationships that exist within the species. This may be due to ecotypic variation or epistatic gene interactions which may require more investigation.

The consistent presence of *V. amygdalina* in all 4 Groups in this study is also suggestive; *V. amygdalina* is successful because it has wide morphological amplitude - it is able to occupy all ecosystems in which virtually all the other species thrive. The possibilities are that it may be that all other species had arisen from *V. amygdalina* or that they had actually diverged from it depending on ecological requirement of different environments in which they found themselves after dispersal. The stronger possibility is that it could be a polyploidy of some order which may encompass the basic genomes of the other species. Ayodele (2008) reported that *V. amygdalina* has a chromosome number of 2n= 40. Other species such as *V. galamensis* has 2n=18 (Gilbert, 1986), *V. hymenolepis* has 2n=20, while *V.glabra* has 2n=18 and *V. calvoana* has 2n=20 chromosomes. *V. amygdalina* 2n = 40 (Gilbert, 1986; Ayodele 2008) or 2n = 36 (Jones, 1976; Kemka-Evans and Okoli, 2013) is a tetraploid. *V. cinerea* 2n = 18 is a diploid and *V. glabra* 2n = 18, *V. calvoana* 2n = 20 and *V. galamensis* 2n = 18, are also diploids. According to Jones (1976), there are several kinds of polyploidy number relationships in flowering plants. *Vernonia* in the old world has a dibasic chromosome number of n = 9 or n = 10 with polyploids of n = 18, 20 or 30 whereas in the new world, it has a basic chromosome number of n = 17 with polyploids of 34, 51, 58 or 68.

The molecular outcome presented above agrees with Baye and Becker (2005) who reported an overall genetic diversity for all traits of 0.76 using Shanon-Weaver diversity index (H") which means that there is high polymorphism among different *Vernonia* lines. Similar results had been reported by earlier workers for other plants; Ogunkanmi *et al*., (2010) reported 74% for *Corchorus olitorus* genotypes with RAPD primers while Tony-Odigie *et al*., (2012) reported a coefficient of 0.75 in *Amaranthus viridis* genotypes also tested with RAPD primers. Wang *et al.,* (1998) distinguished 8 accessions of *Opuntia* on the basis of phenotypic and molecular analysis. A clear separation was obtained among species and accessions of *Passiflora* by Crochemore *et al*., (2003) using RAPD markers. Also, Azeez *et al*., (2009) used RAPD technique to assess the genetic diversity among 30 accessions of *Santalum album*. Cluster analysis using UPGMA separated the accessions

into two major groups. Gettys and Werner (2001) reported that similarity indices suggest that cultivars of Stokes Aster are very closely related, with values for all Pairwise comparisons of Stokes Aster ranging from 0.92 to 0.68 using RAPD markers.

Molecular markers have been used extensively in genetic diversity analysis and germplasm organization in crops such as *Solanum* and its related species (Sifau,2013), Cowpea and its wild relatives (Ogunkanmi, 2005), as well as in the assessment of genetic relationships between species of Jute plants (Ogunkanmi *et al.,* 2010) and *Amaranthus* species (Tony-Odigie *et al.,*2012). Earlier studies by other researchers had considered such crops as *Arachis* (Lanham *et al*., 1992); *Brassica*s (Dos-Santos *et al.,* 1994); genetic estimation as predictors for hybrid performance (Bernado, 1994); genetic map construction for the localization of loci conditioning simply inherited traits (e.g. Pto locus for resistance to *Pseudomonas syringae* pathovar tomato (Pst) (Carland and Staskawawiz, 1993) as well as in QTL analysis (Edwards *et al*., 1987; Fatokun *et al.,* 1992; Ubi *et al.,* 2000).

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