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Assessment of Genetic Diversity among Newly Selected Roselle (*Hibiscus sabdariffa* Linn.) Genotypes in Nigeria Using Rapd-Pcr Molecular Analysis

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Abstract In order to assess the genetic diversity among Nigerian Roselle (*Hibiscus sabdariffa* Linn.), certain genetic and morphological markers were used to group the various accessions collected into distinct genotypes. Molecular characterisation was then conducted on the twenty newly selected genotypes (NGR-HS-001, NGR-HS-002, NGR-HS-003, NGR-HS-004, NGR-HS-005, NGR-HS-006, NGR-HS-007, NGR-HS-008, NGR-HS-009, NGR-HS-010, NGR-HS-011, NGR-HS-012, NGR-HS-013, NGR-HS-014, NGR-HS-015, NGR-HS-016, NGR-HS-017, NGR-HS-018, NGR-HS-019, and NGR-HS-020) of the Roselle (*H. sabdariffa* Linn.), using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) based techniques. The molecular characterisation was carried out at the Biotechnology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan. Five RAPD primers i.e. OPH05, OPB17, OPT17, OPB11 and OPB04 were able to amplify 30 reproducible fragments of which 100 % were polymorphic, indicating that Nigerian Roselle accessions are genetically heterogeneous; it also showed that RAPD analysis is an effective molecular marker in detecting genetic diversity among different Roselle genotypes in Nigeria. All the primers gave a high genetic diversity and high polymorphic information contents, however, OPT17 gave the highest genetic diversity and polymorphic information content (0.79 and 0.78 respectively). The Dice Dissimilarity index for the calculation of distances among the roselle accessions revealed an interesting phenomenon because a clear divergence among the *H. sabdariffa* accessions was found. The dissimilarity index ranged from 0-1, which revealed a wide range of genetic identity. Dissimilarity coefficient of accession NGR-HS-017 was the highest (1.00); this accession was totally different or genetically distance from all the other accessions. In addition, a high dissimilarity index (1.00) was also obtained among some of the accessions; NGR-HS-018 was highly dissimilar from NGR-HS-002, NGR-HS-012 and NGR-HS-016. However, a quite number of the accessions showed moderately low to zero dissimilarity indices. It is therefore concluded that RAPD-PCR analysis provided very valuable means for determining relationships among *H. sabdariffa* accessions. Wide genetic variability observed among the Roselle accessions could be exploited in different breeding programs of the crop. Therefore, other DNA markers like SSR and RFLP should be used to ascertain these levels of relationships.

Keywords: RAPD-PCR, genetic variability, dissimilarity coefficient, genetically heterogeneous

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1. Introduction

Molecular markers referred to any specific Deoxyribonucleic Acid (DNA) segment whose base sequence is polymorphic in different organisms [1]. Such markers can be visualised by hybridization-based techniques such as Polymerase Chain Reaction (PCR) or by Restriction Fragment Length Polymorphism (RFLP) based methods. Molecular markers present numerous advantages over conventional phenotype based alternatives. DNA markers have proven to be efficient tools for the molecular characterisation of the plant species [2]. Polymerase Chain Reaction (PCR), molecular markers, Inter Simple Sequence Repeat (ISSR), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), etc., are useful in various application of plant breeding. Among them, RAPD and ISSR are the arbitrary markers in which only one primer is used [3]. Different types of molecular markers have been used in detecting differences between individuals [4].

The Random Amplified Polymorphic DNA (RAPD) marker is based on the amplification by PCR of random DNA segments, using single primers of arbitrary

nucleotide sequence. The amplified DNA fragments, referred to as RAPD markers, were shown to be highly useful in the construction of genetic maps. With RAPD method the resulted polymorphisms are detected by electrophoresis as different DNA fragments. The different DNA fragments are generated once the primers used usually anneal with multiple sites in different regions of the genome, producing multiple amplified products that often contain repetitive DNA sequences [5]. DNA fingerprinting techniques such as RAPD permits to identify taxa and to determine the phylogenetic relationships for systematic analysis [6].

Reproducible RAPD markers have been used to study genetic variability and evolutionary basis among the Malvaceae family, most especially the genus *Gossypium* [3]. It has also proven to be an efficient molecular technique for genetic fingerprinting of the cotton cultivars [7]. There are few studies on Roselle at the molecular level, Hussein *et al*. [8] noted that different primers showed distinct difference in the Roselle genotypes studied. In addition, Omalsaad *et al*. [9] used PCR based RAPD analysis to study genetic relationship among roselle and kenaf accessions from different parts of the world, including Nigeria. They found out that RAPD analysis is very efficient in showing similarities and differences among the accessions; this was because of high level of polymorphism detected by the DNA marker. Also, Prasad [10], successfully used RAPD molecular marker to characterize and determine the genetic diversity among *Hibiscus* species. He opined that, RAPD technique can be said to be reliable and promising for the characterisation of the *Hibiscus* germplasm and therefore Sequence Characterised Amplified Regions (SCAR) primers can be easily designed for many of these *Hibiscus* varieties and species. He concluded that RAPD markers have the potential for identification and characterisation of genetic variation within the varieties in a species.

Roselle (*Hibiscus sabdariffa* L.), an important member of the family Malvaceae, is a crop of great economic importance in Sub-Saharan Africa. In Nigeria, both the leaves and calyxes are consumed by the people [11]; they are rich sources of anti-oxidants, riboflavin, ascorbic acid, niacin, and carotene that are nutritionally important as well as amino acids and mineral salts [12]. The annual production of the crop worldwide is said to be very low; this is as a result of lack of improved varieties available to the Roselle farmers. In addition, the knowledge about the genetic diversity of the crop is scanty, and the data collected from the peasant Roselle farmers as regards that, are unreliable. For these reasons, the crop has a great economic potential in Nigerian Crop Improvement Programmes (NCIP), which is aimed at the development of varieties with a high content of important phytochemicals for nutritional and medicinal purposes. These are of great economic value for income generation to Nigerian farmers in particular and Nigerian government in general.

The knowledge of genetic diversity of this crop is very important in Nigeria, as it is the key factor towards the improvement of the crop. The advent of different molecular techniques has led plant breeders to estimate genetic diversity on the basis of data generated by different molecular markers, which provide a means of rapid analysis of germplasm which often corroborate phenotypic data. In addition, molecular marker-based genetic diversity is necessary for genetic mapping and marker-assisted selection in breeding. As imperative as the genetic improvement of Roselle may be, it can only be achieved when predicated on a good knowledge of baseline information regarding the existing germ-lines in the country; of course, such information is presently lacking in Nigeria. Therefore, this study is germane in addressing the current challenges facing the crop in other to bring about the improvement of the crop in Nigeria in the future. The aim of this study is to evaluate the genetic diversity existing among Roselle (*Hibiscus sabdariffa* Linn.) Germplasm in Nigeria, using molecular characterisation.

2. Materials and Methods

2.1. Molecular Characterisation using RAPD Molecular Markers

The different accessions of *Hibiscus sabdariffa* collected were subjected to DNA extraction using a modified procedure of [13]. This was done at the Genetic Engineering Laboratory, International Institute of Tropical Agriculture, Ibadan. For the DNA analysis and extraction procedures, approximately 1.5g of leaf tissue from each sample was ground in liquid nitrogen inside a 1.5 ml Eppendorf tube to which 700 µl of pre-heated plant extraction buffer was added. The tubes were incubated at 65°C for 20 minutes with regular mixing by occasionally inverting the tubes to homogenise the samples. The tubes were removed, allowed to cool for 2 minutes and 500 µl of ice-cold 5 M Potassium acetate added and incubated on ice for 20 minutes to precipitate protein.

They were centrifuged at 12000 rpm for 10 mins and the supernatants were transferred into freshly labelled tubes. Seven hundred (700) µl of Chloroform-Isoamyalcohol mixture in the ratio 24:1 was added and mixed gently to further precipitate protein and lipids. The tubes were centrifuged at 12000 rpm for 10 mins and the supernatants transferred into new tubes. Five hundred (500) µl of ice-cold Isopropanol was added, mixed gently by inverting the tube and incubated in -80^{o}C for 15mins to precipitate the DNA. Tubes were centrifuge at 12000 rpm for 10 mins and the supernatants were decanted to the last drop leaving the DNA pellet at the bottom of the tube. One hundred (100) µl of 70 % ethanol was added to wash the DNA pellet. The tubes were centrifuged and the ethanol decanted. The pellets were air dried until no trace of ethanol was found in the tubes. Ultra-pure water (60µl) was added to re-suspend the DNA to which 2 µl of RNase was added. This was incubated in 37°C for 30 minutes. The DNA quality was checked on 0.8 % Agarose and Nanodrop Spectrophotometer ND-1000 was used to quantify the DNA. The DNAs were stored in -20°C.

2.2. PCR Amplification using RAPD Primers

PCR amplification was performed in a 25µl reaction volume which contained 2.0 µl of template DNA, 2.5 µl of 10X buffer, 1.2 µl 50 mM $MgCl_2$, 1 µl of DMSO, 2.0 µl of 2.5 mM dNTPs, 0.2 µl of Taq polymerase and 10 µl of RAPD primer. The PCR cycle was carried out in a 9700 Applied Biosystems Thermal Cycler as follows: an initial denaturation at 94°C for 3 minutes followed by 45 cycles of 94°C for 20 seconds, 37°C annealing for 40 seconds

and 72°C for 1 min and a final step of 72°C for 7 min extension. The product was stored at 4°C and loaded on 2 % Agarose gel, stained with Ethidium Bromide and it was run at 80 Volts for 4 hrs. The molecular fragments were estimated using 50 base pair (bp) standard size marker. Photographs of the probes were taken under the UV light.

2.3. Data Analyses

For the DNA scoring and analyses, various bands shown on the gel were scored for both the control and other treatments. Presence of an allele was scored one (1) while absence earned zero (0). The data obtained were subjected to analyses using Darwin 5.0 software by [14]. Dice Dissimilarity index was used to calculate dissimilarity values between the germ-lines. Cluster diagram was drawn using Unweighted Pair Group Method Average (UPGMA). Estimates of genetic and environmental parameters were used to evaluate the breeding chances of these traits.

3. Results

3.1. DNA Fingerprint using RAPD Amplification

In the present study, five RAPD primers i.e. OPH05, OPB17, OPT17, OPB11 and OPB04 with their respective DNA sequences **(-5[1] - GGG TTT GGC A-3[1], - 5[1] -AGG GAA CGA G - 3[1], - 5[1]-CCA ACG-TCG T - 3[1], -5[1] - GTA GAC CCG T- 3[1] and 5[1] - GGA CTG GAG T - 3[1]**) were used to differentiate among the twenty roselle accessions; their polymorphism for each of the primers are presented in Table 1. The RAPD primers amplified 30 reproducible fragments of which 100 % were polymorphic. The data matrix of the RAPD-PCR for the twenty roselle accessions which indicates the behaviours of each of the primers and their replicates is shown in Table 2. It clearly indicates how the primers produced the polymorphic bands mentioned earlier.

Table 1. Polymorphic bands produced by the RAPD primers in the Roselle accessions

Primer	Monomorphic band	Polymorphic bands	Total bands	% polymorphism
OPH05	0	4	4	100
OPB17	0	12	12	100
OPT17	0	6	6	100
OPB11	0	4	4	100
OPB04	0	4	4	100

Table 2. Data Matrix of RAPD-PCR for the twenty *H. sabdariffa* accessions studied

DNA Marker	Accessions																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
OPH05																				
AF01	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AF02	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
AF03	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
AF04	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1
Total	**0**	**1**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**2**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**1**
OPB17																				
AF05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
AF06	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
AF07	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
AF08	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
AF09	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
AF10	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
AF11	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AF12	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
AF13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
AF14	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
AF15	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
AF16	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Total	**2**	**0**	**1**	**0**	**1**	**0**	**1**	**3**	**3**	**3**	**1**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**2**	**3**
OPT17																				
AF17	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1
AF18	0	0	0	0	0	1	1	1	1	0	0	1	0	0	1	0	0	0	0	0
AF19	0	1	0	0	0	1	1	1	1	0	0	1	1	0	0	0	1	1	0	0
AF20	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	1	1
AF21	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
AF22	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Total	**1**	**2**	**0**	**0**	**0**	**3**	**3**	**3**	**4**	**1**	**1**	**2**	**1**	**0**	**1**	**0**	**3**	**3**	**2**	**2**
OPB11																				
AF23	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
AF24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
AF25	0	0	1	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0
AF26	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Total	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**2**	**1**	**1**	**1**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**
OPB04																				
AF27	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	**1**
AF28	0	0	0	0	0	0	0	0	0	0	**1**	0	0	**1**	0	0	0	0	0	0
AF29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**0**
AF30	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	**1**	**1**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**1**	**1**

The result showed that OPH05 produced a total number of 4 bands of which all are polymorphic while OPB17 produced a total number of 12 bands, which are all polymorphic too. Similarly, OPT17 produced a total of 6 polymorphic bands and there was no monomorphic band obtained. In the same vein, OPB11 and OPB04 both produced 4 reproducible bands each, all which are polymorphic. This showed that for polymorphism of all the twenty roselle accessions under study, all the primers yielded a maximum polymorphism of 100%. All the primers gave reproducible results but the best primer used to differentiate was primer OPT17. In addition, OPT17 also gave the highest genetic diversity and polymorphic information content (0.79 and 0.78 respectively), Table 3.

Table 3. Major allelic frequency, Allele number, Gene diversity and Polymorphic Information Content detected by each of the DNA primers

DNA Markers	Major Allele Frequency	Sample Size	No. of observation	Allele Number	Availability	Gene Diversity	Polymorphic Information Content
OPH05	0.60	20.00	20.00	5.00	1.00	0.59	0.54
OPB17	0.50	20.00	20.00	9.00	1.00	0.72	0.70
OPT17	0.40	20.00	20.00	10.00	1.00	0.80	0.78
OPB11	0.70	20.00	20.00	5.00	1.00	0.48	0.45
OPB04	0.70	20.00	20.00	5.00	1.00	0.49	0.46
Mean	0.58	20.00	20.00	6.80	1.00	0.61	0.59

The Dice Dissimilarity index for the calculation of distances among the roselle accessions revealed an interesting phenomenon. A clear divergence among the *H. sabdariffa* accessions was found. The dissimilarity index ranged from 0-1, which revealed a wide range of genetic identity (Table 4). Dissimilarity coefficient of accession NGR-HS-017 was the highest (1.00); this accession was totally different or genetically distance from all the other accessions. In addition, a high dissimilarity index (1.00) was also obtained among some of the accessions; NGR-HS-018 was highly dissimilar from NGR-HS-002, NGR-HS-012 and NGR-HS-016 (Table 4). However, a quite number of the accessions showed moderately low to zero dissimilarity indices. NGR-HS-001 and NGR-HS-003 showed zero dissimilarity index, showing how closely related they are at molecular level. Also, NGR-HS-013 and NGR-HS-014 (0.40 dissimilarity index); NGR-HS-001 and NGR-HS-002 (0.40 dissimilarity index); NGR-HS-003 and NGR-HS-005 (0.40 dissimilarity index); while NGR-HS-001 showed a very low (0.20) dissimilarity index with NGR-HS-006 and NGR-HS-007. Similarly, NGR-HS-008 and NGR-HS-019 as well as NGR-HS-010 and NGR-HS-012 showed a very low (0.2) dissimilarity index. On the other hand, NGR-HS-008 showed a relatively high (0.8) dissimilarity index with most of accession; also NGR-HS-018 showed a relatively high dissimilarity index with most of the accessions (Table 4). The similarity matrix was obtained after multivariant analysis as shown in Table 4; this was used to prepare an un-weighted pair group method of arithmetic mean (UPGMA) dendrogram and has been presented in Figure 1. This dissimilarity matrix indicated segregation among the different accessions of *H. sabdariffa*.

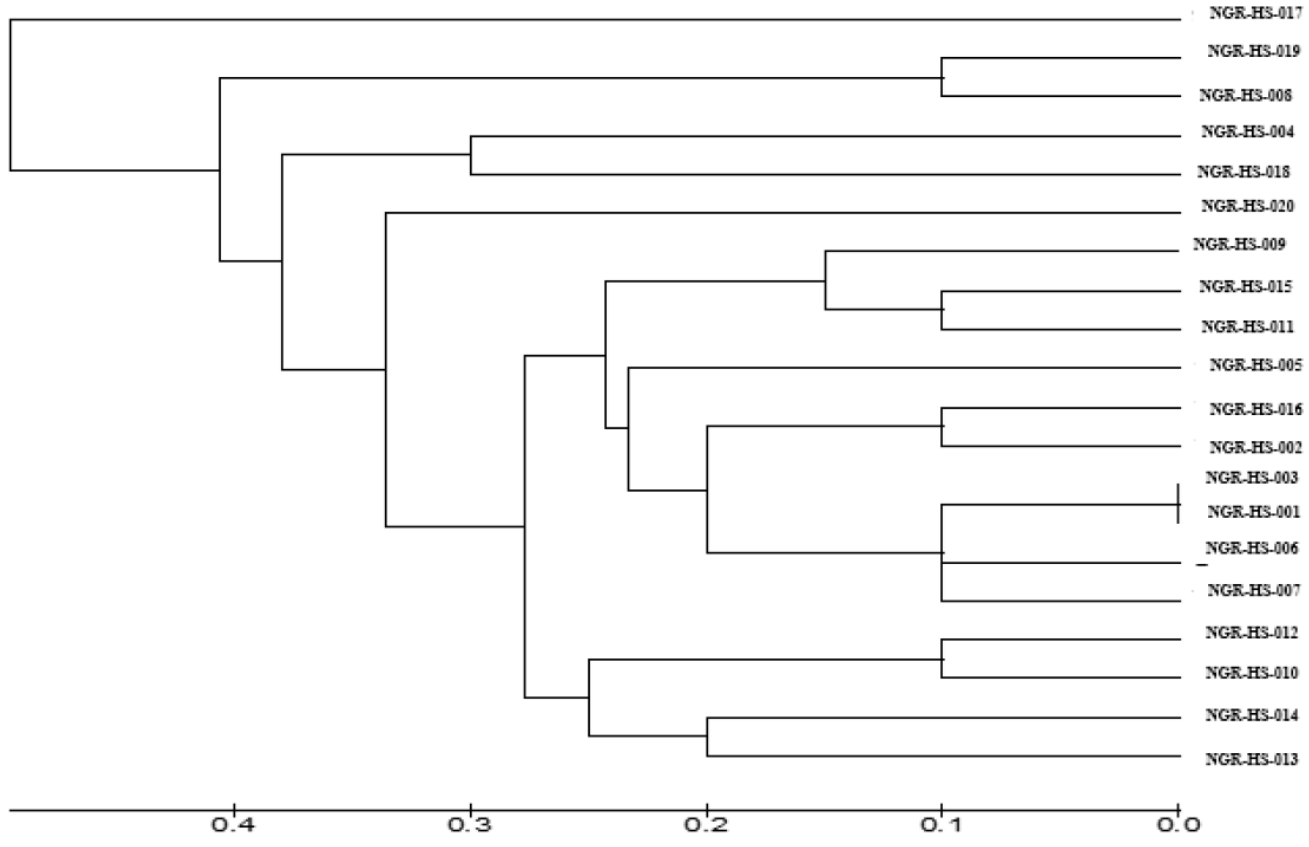

Figure 1. Dendrogram based on UPGMA analysis of genetic dissimilarity of Roselle accessions, showing relationships among them

Table 4. Dice Dissimilarity index among the twenty Roselle accessions

OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	**0.00**	0.40	0.00	0.60	0.40	0.20	0.20	0.80	0.40	0.40	0.40	0.60	0.60	0.40	0.40	0.40	1.00	0.80	1.00	0.60
2	0.40	**0.00**	0.40	0.80	0.60	0.40	0.40	0.80	0.60	0.60	0.60	0.60	0.80	0.60	0.60	0.20	1.00	1.00	1.00	0.80
3	0.00	0.40	**0.00**	0.60	0.40	0.20	0.20	0.80	0.40	0.40	0.40	0.60	0.60	0.40	0.40	0.40	1.00	0.80	1.00	0.60
4	0.60	0.80	0.60	**0.00**	0.60	0.60	0.60	1.00	0.80	0.60	0.80	0.80	0.60	0.60	0.80	0.80	1.00	0.60	1.00	0.80
5	0.40	0.60	0.40	0.60	**0.00**	0.40	0.40	0.80	0.60	0.40	0.60	0.60	0.60	0.60	0.60	0.60	1.00	0.80	1.00	0.60
6	0.20	0.40	0.20	0.60	0.40	**0.00**	0.20	0.80	0.40	0.40	0.40	0.60	0.60	0.40	0.40	0.40	1.00	0.80	1.00	0.60
7	0.20	0.40	0.20	0.60	0.40	0.20	**0.00**	0.80	0.40	0.40	0.40	0.60	0.60	0.40	0.40	0.40	1.00	0.80	1.00	0.60
8	0.80	0.80	0.80	1.00	0.80	0.80	0.80	**0.00**	0.80	0.60	0.60	0.60	0.80	0.80	0.40	0.60	1.00	0.80	0.20	0.80
9	0.40	0.60	0.40	0.80	0.60	0.40	0.40	0.80	**0.00**	0.60	0.20	0.80	0.80	0.60	0.40	0.60	1.00	0.80	1.00	0.60
10	0.40	0.60	0.40	0.60	0.40	0.40	0.40	0.60	0.60	**0.00**	0.40	0.20	0.40	0.40	0.40	0.60	1.00	0.80	0.80	0.60
11	0.40	0.60	0.40	0.80	0.60	0.40	0.40	0.60	0.20	0.40	**0.00**	0.60	0.60	0.40	0.20	0.60	1.00	0.80	0.80	0.60
12	0.60	0.60	0.60	0.80	0.60	0.60	0.60	0.60	0.80	0.20	0.60	**0.00**	0.60	0.60	0.60	0.60	1.00	1.00	0.80	0.80
13	0.60	0.80	0.60	0.60	0.60	0.60	0.60	0.80	0.80	0.40	0.60	0.60	**0.00**	0.40	0.60	0.80	1.00	0.80	0.80	0.80
14	0.40	0.60	0.40	0.60	0.60	0.40	0.40	0.80	0.60	0.40	0.40	0.60	0.40	**0.00**	0.40	0.60	1.00	0.80	0.60	0.80
15	0.40	0.60	0.40	0.80	0.60	0.40	0.40	0.40	0.40	0.40	0.20	0.60	0.60	0.40	**0.00**	0.60	1.00	0.60	0.60	0.60
16	0.40	0.20	0.40	0.80	0.60	0.40	0.40	0.60	0.60	0.60	0.60	0.60	0.80	0.60	0.60	**0.00**	1.00	1.00	0.80	0.80
17	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	**0.00**	0.80	1.00	1.00
18	0.80	1.00	0.80	0.60	0.80	0.80	0.80	0.80	0.80	0.80	0.80	1.00	0.80	0.80	0.60	1.00	1.00	**0.00**	0.80	0.80
19	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.20	1.00	0.80	0.80	0.80	0.80	0.60	0.60	0.80	1.00	0.80	**0.00**	1.00
20	0.60	0.80	0.60	0.80	0.60	0.60	0.60	0.80	0.60	0.60	0.60	0.80	0.80	0.80	0.60	0.80	1.00	0.80	1.00	**0.00**

The dendrogram showed five major clusters; the first major cluster contains only accession NGR-HS-017 (a green roselle type with large lobed leaves and green calyces). The second major cluster contains NGR-HS-019 (a green roselle type with lobed leaves and green calyces) and NGR-HS-008 (a deep red roselle type with lobed leaves and deep red calyces). This was followed by the third cluster, containing NGR-HS-004 (a deep red roselle type with large lobed leaves and deep red calyces) and NGR-HS-018 (a green roselle with large lobed leaves and green calyces). The fourth cluster contains NGR-HS-020 (a green roselle type with tiny lobed leaves and green calyces). The fifth and last cluster was broadly divided into two large groups; the first group was further divided into three sub-groups containing NGR-HS-009, NGR-HS-011 and NGR-HS-15 for the first subgroup. The second sub-group contains only NGR-HS-005 only, while the last sub-group contains NGR-HS-016, NGR-HS-002, NGR-HS-003, NGR-HS-001, NGR-HS-006 and NGR-HS-007. The second large group of the fifth cluster is further divided into two sub-groups. The first sub-group contains NGR-HS-012 and NGR-HS-010, while the second sub-group contains NGR-HS-014 and NGR-HS-013.

4. Discussion

4.1. Genetic Diversity of the Roselle Accessions using RAPD Markers

Genetic diversity and genetic relationship have been widely studied using RAPD markers in a number of plant species. For example, [9] in *Hibiscus* spp; [15] in *Olea europaea*; [16] in *Allium sativum*.

The high level of polymorphism reported demonstrated that Nigerian Roselle accessions are genetically heterogenous, confirming that they are highly variable species which supported the grouping of the different roselle types based on some morphometric parameters. The result is slightly different from that of [17], who reported a very low genetic diversity (0.02-0.09 dissimilarities) among ninety-four roselle accessions from Thailand. [9], had earlier reported that there exist a wide range of genetic identity among roselle accessions and concluded that roselle had relatively low genetic variation. This present results disagrees with that general conclusion by [9]; this might be due partly to the higher number of accessions studied in this present work. The presence of genetic similarities among some of the accessions as shown from the result might be recorded for high rates of gene flow, genetic exchange and the presence of natural selection on the species. Similar conclusion had been drawn by [18]. However, this present work is in perfect agreement with the work of [19] and [20] on *H. sabdariffa*.

It is also evident from the present results that RAPD molecular marker was able to detect small genetic differences among the Nigerian Roselle accessions. Similar reports on the ability of RAPD in detecting genetic diversity in crops have been reported by [9] and [19]. The clustering together might have been recorded for their similarities in leaf shapes and some other morphological parameters; Ahmed [20] had also reported that some green roselle types and coloured roselle types have been clustered together when he used both RAPD and ISSR molecular markers to study the genetic diversity in the crop. Natural hybridization and selection between genetically related accessions like NGR-HS-001 and NGR-HS-016 could have led to the evolution of NGR-HS-002 which is genetically related to both accessions. Similarly, study of the diversity among the accessions using only the morphological characteristics is not reliable. This is because most of the quantitative traits are influenced by environmental factors, thus, they vary a lot between species [21]. Therefore, data obtained from this

research demonstrated that morphological analysis followed with RAPD molecular marker analysis are very valuable for determining relationships among *H. sabdariffa* accessions similar assertions have been made by [9] and [21]. It is therefore concluded that RAPD-PCR analysis provided very valuable means for determining relationships among *H. sabdariffa* accessions. Wide genetic variability observed among the Roselle accessions could be exploited in different breeding programs of the crop. Therefore, other DNA markers like SSR and RFLP should be used to ascertain these levels of relationships.

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