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Full Length Research

Assessment of population structure and genetic diversity study of landraces and elite rice varieties commonly grown in Nigeria

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ABSTRACT: A set of 21 rice genotypes including both elite varieties and landraces were characterised based on their population structure using genomic simple sequence repeat (gSSR) markers. A model-based method was used to delineate clusters of individuals based on their genotypes at multiple loci using a Bayesian approach. The population structure analysis separated the 21 genotypes into 3 sub-populations and the grouping were consistent with the ancestral heritage of most of the genotypes whose pedigree is known. Whereas, further clustering analysis based on unweighted pair group method with arithmetic mean (UPGMA), separated the 21 genotypes into 5 clusters. The reason for the disparity in the number clusters may be because of the different grouping methods. The knowledge obtained from both population structure and genetic diversity of the selected rice genotypes is an important contribution to future development of rice varieties for deferent agro-ecological zones in Nigeria.

Keywords: Elite varieties, pedigree, population structure, rice.

INTRODUCTION

Rice is the third most important food crop in the world based on production with a total of 770 million tons produced in 2017 and rivalled by wheat (771 million tons) and (maize 1.1 billion tons) (FAOSTAT, 2019). It may even be regarded as the second most important food, consumed by humans since only 14% of world maize production is consumed as food (FAOSTAT, 2019). The total harvested area of rice in 2017 was 167 million hectares and its average yield per hectare was 4.7 tons/ha (FAOSTAT, 2019). West Africa comprises of total land area of about 6 million km^2, and rice occupies about 8% of the total crop area, ranking fifth after millets (21%), sorghum (19%), maize (12%) and cassava (9%) (FAOSTAT, 2009). Where It is a primary source of human caloric intake, a staple food crop, grown by 10 to 15 million farmers and presently a commercial crop in Nigeria and Ghana (Hansda, 2018). Long-standing debate exist on the ancestry of the two cultivated *Oryza* species that is *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). For instance, Sweeney and McCouch (2007) reported that the contention on Asian rice with respect to its direct progenitor is whether it is either *Oryza rufipogon* a perennial species or/ and *Oryza nivara*. Nayar (1973) also proposed that African rice might have originated from Asian rice contrary to the initial proposition by Roschevicz (1931) that its ancestor is *Oryza Barthii* which was later supported by Porteres (1945, 1950, 1956, 1962, 1976) and Morishima et al., 1963). Furthermore, the identity of rice species which has been cultivated in the last 2000 years in Africa is uncertain, this is because four distinct species that is African rice (*Oryza glabberima*) or Asian rice (*Oryza sativa*), the wild species, *Oryza barthii* and *Oryza*

longistaminata occur widely in the region (Nayar 2010). According Oka et al. (1978) in two extensive field surveys; it was observed that over two-thirds of rice fields in West Africa comprise of arbitrary mixtures of two to four rice species. The reasons given for arbitrary mixture include difficulties in identification at early stages, insurance against unpredictable weather conditions and similarity in cooking quality in case of African and Asian rice (Oka and Chang, 1964; Oka et al., 1978). In the process of crop improvement, larger proportion of the traditional cultivars have been replaced by modern cultivars. In the 1990s, only about 15% of the global area was devoted to cultivation of rice landraces (Day Rubenstein et al., 2005)*, Oryza sativa* has steadily replaced *O. glaberrima* in West Africa since the mid-20th century (WARDA, 2003; Linares, 2002). Three types of rice are said to be cultivated in Nigeria namely; African rice, Asian rice and NERICA (Linares, 2002; Somado et al., 2008).

Population is a community of individuals which shares a common gene pool and the genetic structure of a population, determines its capacity to be improved or otherwise changed by selection (Hayward and Breese, 1993). This is because population evolved through the action of past selective forces on the genes controlling variability. Despite the successes in crop improvement through conventional breeding methods, the need to increase efficiency and precision, and save time, resources and efforts, has motivated the application of new breeding strategies. Association mapping (AM) is a relatively recent quantitative trait loci (QTL) mapping approach (Filippi et al., 2015), that has the potential for resolution to the level of individual genes (alleles) (Oraguzie et al., 2007). In contrast to classical QTL mapping techniques used in the analysis of complex traits, AM is a method that detects relationships between phenotypic variation and gene polymorphisms in existing germplasm collections, without development of mapping populations (Fusari et al., 2012; Jorde 2000). There is dearth of knowledge of the genetic constitution and variability among commonly grown rice germplasm in Nigeria.

The population structure estimate of several AM studies was commonly carried out using SSR derived information because of the proven usefulness of this type of markers for population genetics inferences and their higher information content when compared to biallelic markers (Reif et al., 2013; Fusari et al., 2012; Dreisigacker et al., 2005; Roussel et al., 2005). Moreover, SSR markers are considered to be appropriate for assessment of population structure and variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently (Smith et al., 1996; Varshney et al., 2005; Ijaz, 2011). Here, some commonly grown elite rice genotypes were selected because of their susceptibility to biotic and abiotic stresses brought about by loss of genetic diversity (Adegbaju et al., 2016). Also, some traditional rice varieties or landraces reputed for its wide genetic variability were also included in this study. The objective of the study was to assess the population structure and genetic diversity of selected rice genotypes using gSSR markers.

MATERIALS AND METHODS

Rice germplasm for this study consisted of 21 genotypes including; 5 landraces from Benue and Ebonyi States in Nigeria, 7 NERICAs (upland varieties) and 3 lowland and 6 upland elite varieties collected from the gene bank of Africa Rice Center at Ibadan Oyo state (Table 1).

A total of 19 trait-linked SSR markers sourced from Inqaba office at Ibadan Nigeria, were used for analysis, out of which 5 were linked to grain width, 3 markers were each linked to tiller number and panicle length, 2 markers were each linked to seedling height and panicle number while 1 marker was linked to rice bran and tiller length (Table 2).

Seeding

Seeds of respective genotypes were pregerminated by soaking inside distilled water for 2 days, after which the water was removed, and the seeds kept damp for the next 4 days. Sprouted seeds were carefully planted inside sterile soil in well labelled pots at the net house and wetting was done daily after sowing.

Genomic DNA isolation and PCR amplification

Fresh leaf sample from 17 day-old seedlings were harvested and used to extract DNA through cetyltrimethyl ammonium bromide (CTAB) protocol described by George et al. (2002, 2004), Thermo Fisher Scientific, USA. The quality and quantity of DNA were estimated using Spectrophotometry (Nanodrop system). PCR amplifications were performed in DNA thermal cycler (Model: ALS 1296, BioRad, USA and G-STORM, GSI, England, Serial no: GT-11620) with a total volume of 10 µL for each PCR mixture containing 3 µl of diluted genomic DNA, 0.5 µl of each forward and reverse primer, 0.25 µl of 10 mM dNTPs, 1.5 µl of 10x buffer (100 mmol/L Tris, PH 9.0, 500 mmol/L KCL and 0.1% gelatin), 0.2 µl of *Taq* polymerase, 1.8 µl of MgCl2 and 2.25 µl of ddH_2O. The PCR profile was carried out as follows; initial denaturation step for 5 min at 94°C (hot start and strand separation), annealing (55°C) and primer elongation (72°C) for 30 seconds each and then a final extension at 72°C for 5 min. The PCR profile took place for 34 cycles and amplified products were stored at -20°C. The amplified products for each of the 19 SSR markers were resolved by electrophoresis on 10% polyacrylamide gels in TBE buffer and the gel, after electrophoresis, was stained with ethidium bromide for 35 min, kept in the dark, and then

Table 1. List of rice genotypes and where they were collected.

Genotype	Source of collection	Genotype	Source of collection
Mars	Benue state	CK73	Africa Rice Center
CP/Millina	Benue state	BG 90-2	Africa Rice Center
Igbemo	Benue state	FARO-44	Taiwan
Ofada	Ogun state	Wab56-104	Africa Rice Center
Kirikiri/Cheria	Benue state	UPIA-3	Africa Rice Center
NERICA-1	Africa Rice Center	FARO-57	Africa Rice Center
NERICA-2	Africa Rice Center		
NERICA-3	Africa Rice Center		
NERICA-4	Africa Rice Center		
NERICA-5	Africa Rice Center		
NERICA-7	Africa Rice Center		
NERICA-8	Africa Rice Center		
Abakaliki	Farmers gate in Akure Ondo State		
Moberekan	Africa Rice Center		
Wita-4	Africa Rice Center		
CK73	Africa Rice Center		

scanned using an UVPRO (Uvipro Platinum, EU) gel documentation unit linked to a computer. The reproducibility of amplification products was confirmed twice for each primer.

Assessment of polymorphism information content (PIC) and resolving power (RP)

PIC values were calculated as described by Chesnokov and Artemyeva (2015), with the following formula for Co-Dominant markers:

$$PIC=1-(^{k}\Sigma\ ^{i=1}\ pi^{2}) - ^{k-1}\Sigma\ ^{i=1k}\Sigma\ ^{j=1}\ 2\ pi^{2}\ pj^{2}$$

Where K=number of alleles; pi and pj = frequencies of i and j alleles in the population.

Resolving power (RP) values were calculated as described by Gilbert et al. (1999) with the following formula:

$$Rp=\Sigma Ib,$$

Where band informativeness, $Ib=1\ (2x\left|\ 0.5p\right|)$ and p is the proportion of genotypes containing the band I.

Genetic diversity analysis

The genetic relationships among the 21 genotypes studied was determined by scoring all the polymorphic markers. Scoring was done in binary matrix, ‘1’ was used to represent band presence while ‘0’ represent absence of band. Computed binary matrix was used to calculate Jaccard coefficient of similarity (Table 5) (Sneath and Sokal, 1973) by means of NTSYS version 2.02 software (Numerical Taxonomy and Multivariate Analysis system, Exeter Software, New York). The formula for similarity coefficient calculation was:

Jaccard = N *AB*/ (N*AB*+N*A*+N*B*)

Where N*AB* is the number of marker bands shared by two samples (*A* and *B*) and N*A* and N*B* are the marker band present only in sample *A* or *B*.

Cluster analysis was conducted to construct phylogenetic tree based on the results of the calculated Jaccard similarity coefficient, via unweighted pair group method with arithmetic averages (UPGMA) to group the rice genotypes into different clusters (Figure 1).

Population structure analysis

The population structure in the Nigerian rice genotypes was inferred using the software structure 2.3.2 (Pritchard et al., 2000) based on the amplification pattern of the 19 SSR markers. STRUCTURE 2.3.2 analyses differences in the distribution of genetic variants amongst populations with a Bayesian iterative algorithm by placing samples into groups whose members share similar patterns of variation. It both identifies populations from the data and assigns individuals to that population representing the best fit for the variation patterns found (Porras-Hurtado, 2013). Most of the parameters were set to their default values as advised in the user’s manual of structure 2.0 (Pritchard and Wen, 2003). Particularly, the admixture model and the option of correlated allele frequencies between populations was chosen, because these configurations

Table 2. A total of 19 Traits linked microsatellite markers were used to assess the extent of genetic diversity across the 21 cultivars.

PRIMER	Forward and Reverse sequence	Traits
RM 349	(F)TTGCCATTCGCGTGGAGGCG (R) GTCCATCATCCCTATGGTCG	Rice bran
RM236	(F) GCGCTGGTGGAAAATGAG (R) GGCATCCCTCTTTGATTCCTC	Panicle number
RM 264	(F) GTTGCGTCCTACTGCTACTTC (R) GATCCGTGTCGATGATTAGC	Grain width
RM 175	(F) CTTCGGCGCCGTCATCAAGGTG (R) CGTTGAGCAGCGCGACGTTGAC	Seedling height
RM 225	(F) TGCCCATATGGTCTGGATG (R) TGCCCATATGGTCTGGATG	Culm (Tiller) number
RM 5	(F) TGCAACTTCTAGCTGCTCGA (R) GCATCCGATCTTGATGGG	Culm (Tiller) Length
RM 17	(F) TGCCCTGTTATTTTCTTCTCTC (R) GGTGATCCTTTCCCATTTCA	Seedling height
RM 215	(F) CAAAATGGAGCAGCAAGAGC (R) TGAGCACCTCCTTCTCTGTAG	Panicle length
RM 279	(F) GCGGGAGAGGGATCTCCT (R) GGCTAGGAGTTAACCTCGCG	Grain width
RM 256	(F) GACAGGGAGTGATTGAAGGC (R) GTTGATTTCGCCAAGGGC	Panicle length
RM 289	(F) TTCCATGGCACACAAGCC (R) CTGTGCACGAACTTCCAAAG	Grain width
RM 555	(F) TTGGATCAGCCAAAGGAGAC (R) CAGCATTGTGGCATGGATAC	Grain width
RM 84	(F) TAAGGGTCCATCCACAAGATG (R) TTGCAAATGCAGCTAGAGTAC	
RM 341	(F) CAAGAAACCTCAATCCGAGC (R) CTCCTCCCGATCCCAATC	Grain length
RM 229	(F) CACTCACACGAACGACTGAC (R) CGCAGGTTCTTGTGAAATGT	Tiller length
RM332	(F) GCGAAGGCGAAGGTGAAG (R) CATGAGTGATCTCACTCACCC	Panicle length
RM 280	(F) ACACGATCCACTTTGCGC (R) TGTGTCTTGAGCAGCCAGG	Panicle number
RM 340	(F) GGTAAATGGACAATCCTATGGC (R) GACAAATATAAGGGCAGTGTGC	
RM 210	(F) TCACATTCGGTGGCATTG (R) CGAGGATGGTTGTTCACTTG	Culm (Tiller) number

were considered best in cases of subtle population structure by Falush et al. (2003). Similarly, the degree of admixture alpha was allowed to be inferred from the data. To estimate the number of subpopulations (the K parameter), the dataset was analyzed allowing the value of K ranging from 1 to 10 and length of the burn-in and MCMC (Markov chain Monte Carlo) was set at 10,000 according to the reports of Evanno et al. (2005). Inference of true K which is the optimal value for number of sub-populations was calculated based on the second order rate of change of the likelihood of ad hoc quantity (ΔK). This can be expressed with the formula as given below:

$\Delta K = m([L''K])/s[L(K)]$

Where: L(K) = an average of 20 values of Ln P(D); L'(K) = L(K)n – L(K)n-1; and L''(K) = L'(K)n – L'(K)n-1 (Evanno et al., 2005).

A bar plot of line membership in each population was generated using STRUCTURE 2.3.2.

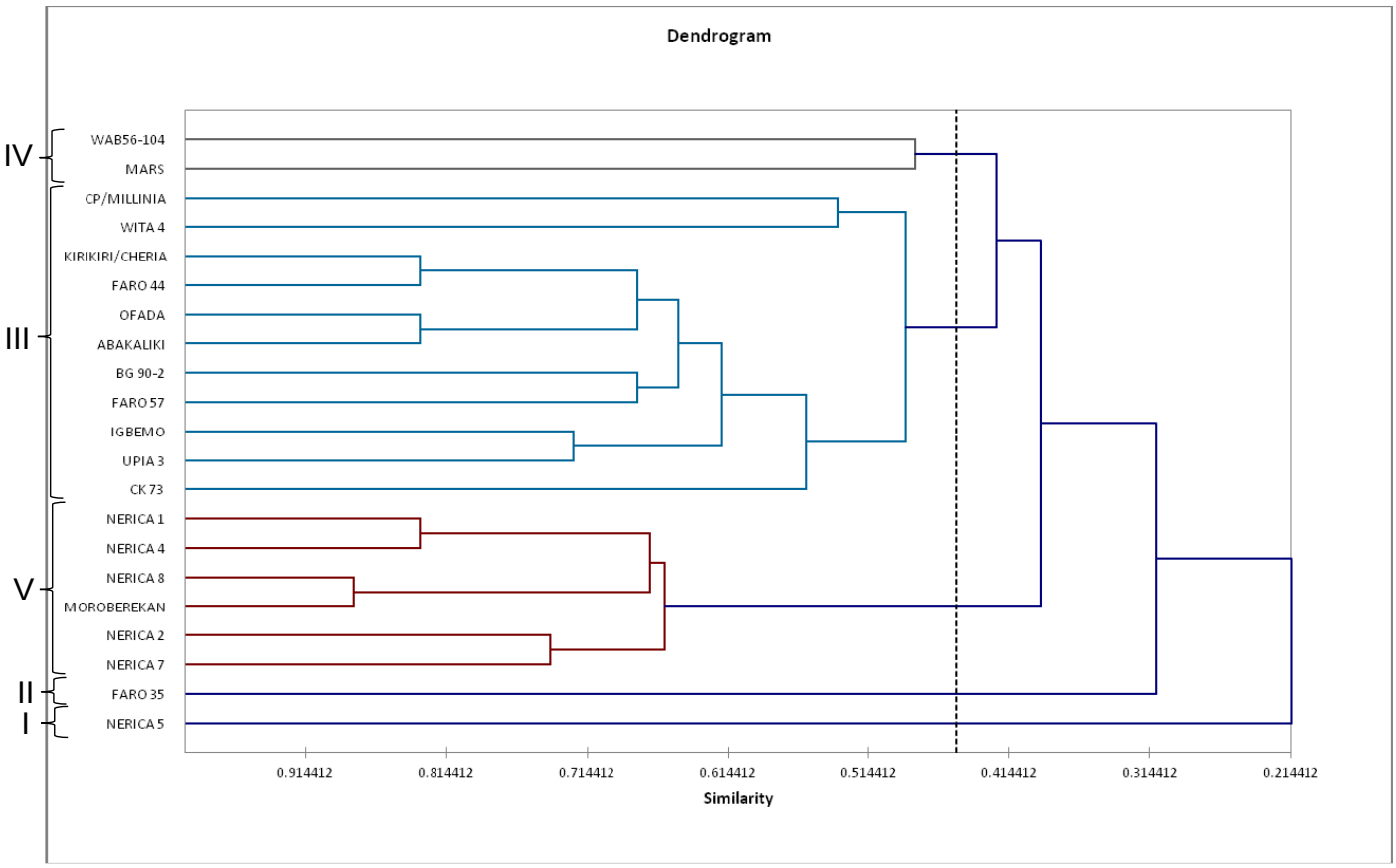

Figure 1. Unweighted pair group method with arithmetic mean (UPGMA) based dendrogram of the 21 rice genotypes.

RESULTS

SSR Markers Analysis

A total of nineteen (19) trait linked SSR primers (Table 2) were used to investigate the population structure and genetic diversity amongst the selected twenty-one cultivars. Out of the 12 chromosomes of rice, only chromosome 7 and 10 had no representation among the 19 SSR marker sets used. All the 19 SSR markers used in this study generated polymorphic bands for the 21 rice genotypes, and no band was found to be monomorphic. Markers RM175 and RM210 showed heterozygosity at the loci they amplified for all the 21 cultivars. These 19 Polymorphic markers amplified a total of 53 alleles and the size for the detected alleles produced ranges from 75 to 260bp. The number of alleles per primer varied from 1 in RM 215 and RM555 to 7 in RM341 and the PIC values ranges from 0.01 (RM555) to 0.96 (RM341). The three markers with highest resolving power were RM 175, RM17 and RM210 with values 5.52, 3.61 and 3.14 respectively (Table 2), while RM84 and RM215 had the lowest values 1.7 and 1.8 respectively. The primers with higher values were able to differentiate between the genotypes and the resolving power summation of all the 19 primers were 37.43.

Population structure

The population analysis of the 21 rice genotypes with the polymorphic markers separated the genotypes into three sub-populations (Table 4). These genotypes have been listed by their primary membership in each of these sub-populations, however, where admixture values Q, for the population of primary membership were <0.50, genotype was considered as mixed within their population of primary membership. Each genotype's estimated membership in each of the three populations is depicted in a STRUCTURE bar plot (Figure 2) and exact membership proportions are provided in Table 3.

The population structure was delineated base on Q values and the maximum ΔK was detected at K = 3, cluster I, II and III comprises of 7, 8, and 5 genotypes, respectively, where all the NERICA varieties was grouped into a single cluster and majority of the landraces/local selections were also separated into a cluster. The variety WITA-4 had the least membership proportion (Q) value of 0.556; the (Q) value was used in assigning genotypes to corresponding sub-populations. This variety shares secondary membership with sub-populations III (0.369) and I (0.075), also BG 90-2, CK73 and landrace MARS in sub-population III shared secondary membership with sub-

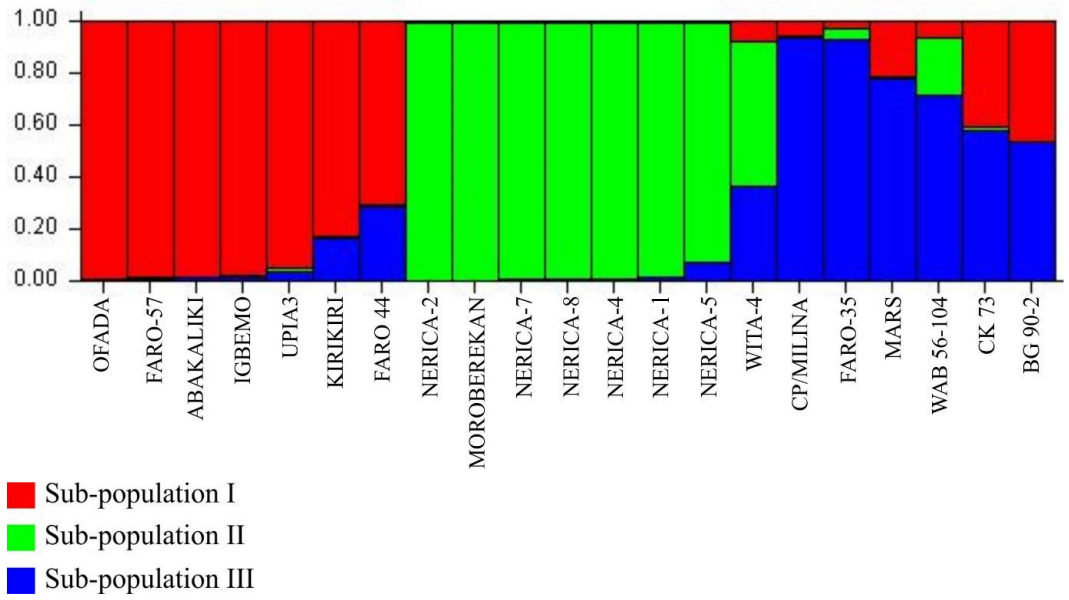

Figure 2. Diagrammatic representation of the population structure estimate, with each variety in a population represented by bar plot proportional to Q values of the 21 genotypes.

Table 3. Estimated membership inference cluster values among rice varieties.

Genotypes	Cluster I	Cluster II	Cluster III
OFADA	0.992	0.002	0.0006
FARO57	0.982	0.004	0.014
ABAKALIKI	0.979	0.003	0.019
IGBEMO	0.978	0.004	0.018
UPIA 3	0.978	0.016	0.036
KIRIKIRI	0.827	0.005	0.170
FARO 44	0.706	0.006	0.289
NERICA-8	0.004	0.992	0.004
NERICA-2	0.005	0.991	0.005
NERICA-7	0.005	0.986	0.009
MOROBEREKAN	0.007	0.984	0.010
NERICA-4	0.005	0.981	0.014
NERICA-1	0.005	0.976	0.020
NERICA-5	0.007	0.919	0.074
WITA-4	0.075	0.556	0.369
CP/MILINA	0.053	0.009	0.935
FARO 35	0.026	0.039	0.935
MARS	0.214	0.006	0.781
WAB 56-104	0.064	0.221	0.715
CK 73	0.407	0.008	0.584
BG 90-2	0.461	0.002	0.536

population I. Whereas, WAB56-104 which was the recurrent parent of the NERICA varieties used in this study was grouped into sub-population III but it shared secondary membership with sub-population II comprising all the NERICA varieties.

Genetic diversity based on molecular markers

Similarity indices for the cultivars used was calculated using the binary data from the 19 polymorphic SSR primers. The similarity coefficient base on the 19 SSR

Table 4. Distribution of rice genotypes into different sub-populations based on population structure analysis.

Cluster	No. of genotypes	Genotypes	Remarks
I	7	OFADA, FARO 57, ABAKALIKI, IGBEMO, UPIA 3, KIRIKIRI, FARO 44	Comprise mainly of the Landraces and three elite varieties
II	8	NERICA-8, NERICA-2, NERICA-7, MOROBEREKAN, NERICA-4, NERICA-1, NERICA-5, WITA-4	The NERICA varieties are elite rice developed from the cross of CG14 (*O. glaberrima* Steud) & WAB 56-104 (*O. Sativa* a Subspecies Japonica).
III	6	CP/MILINA, FARO-35, MARS, WAB 56-104, CK 73, BG 90-2	Comprise mainly of lowland elite varieties and two landraces

Table 5. Jaccard coefficient of similarity.

	NERICA 5	FARO 35	WITA 4	CP/MILLINIA	MARS	WAB 56-104	NERICA 7	ABAKALIKI	FARO 57	OFADA
NERICA 5	1	0.130	0.200	0.179	0.179	0.174	0.296	0.152	0.231	0.194
FARO 35	0.130	1	0.440	0.444	0.300	0.375	0.242	0.375	0.267	0.303
WITA 4	0.200	0.440	1	0.536	0.387	0.423	0.452	0.412	0.400	0.469
CP/MILLINIA	0.179	0.444	0.536	1	0.533	0.429	0.412	0.500	0.552	0.471
MARS	0.179	0.300	0.387	0.533	1	0.481	0.371	0.545	0.552	0.471
WAB 56-104	0.174	0.375	0.423	0.429	0.481	1	0.355	0.406	0.345	0.333
NERICA 7	0.296	0.242	0.452	0.412	0.371	0.355	1	0.262	0.424	0.268
ABAK ALIKI	0.152	0.375	0.412	0.500	0.545	0.406	0.262	1	0.613	0.833
FARO 57	0.231	0.267	0.400	0.552	0.552	0.345	0.424	0.613	1	0.633
OFADA	0.194	0.303	0.469	0.471	0.471	0.333	0.268	0.833	0.633	1
NERICA 4	0.231	0.267	0.500	0.500	0.500	0.444	0.679	0.389	0.467	0.361
NERICA 1	0.231	0.267	0.500	0.500	0.452	0.500	0.679	0.351	0.419	0.324
UPIA 3	0.250	0.281	0.500	0.412	0.412	0.355	0.351	0.559	0.567	0.677
NERICA 2	0.391	0.188	0.400	0.364	0.324	0.258	0.741	0.282	0.419	0.289
IGBEMO	0.207	0.323	0.552	0.455	0.455	0.313	0.351	0.656	0.621	0.677
FARO 44	0.179	0.345	0.536	0.643	0.586	0.481	0.371	0.700	0.667	0.667
KIRIKIRI/CHERIA	0.24	0.370	0.64	0.517	0.467	0.407	0.353	0.633	0.654	0.714
MORO BEREKAN	0.222	0.300	0.483	0.484	0.353	0.429	0.714	0.342	0.364	0.316
NERICA 8	0.259	0.250	0.517	0.424	0.343	0.367	0.750	0.333	0.394	0.342
CK 73	0.138	0.300	0.483	0.394	0.438	0.333	0.263	0.645	0.452	0.613
BG 90-2	0.207	0.242	0.406	0.455	0.371	0.273	0.282	0.656	0.679	0.677

marker ranges between 0.13 to 0.88, maximum diversity was observed between Faro 35 and NERICA 5 (similarity coefficient of 0.13), followed by NERICA 5 and Abakaliki, WAB56-104, CP/MILLINIA and MARS (similarity coefficient of 0.152, 0.174, 0.179, 0.179 respectively). Whereas, maximum similarity was observed between NERICA 8 and MOROBEREKAN (0.88) followed by those between FARO 44 and KIRIKIRI/CHERIA (0.833), OFADA and ABAKALIKI (0.833) also NERICA 1 and NERICA 4 (0.833). The 21 rice genotypes were grouped into 5 major cluster by

Table 5. Cotd.

	NERICA 4	NERICA 1	UPIA 3	NERICA 2	IGBEMO	FARO 44	KIRIKIRI /CHERIA	MOROB EREKAN	NERICA 8	CK 73	BG 90-2
NERICA 5	0.231	0.231	0.250	0.391	0.207	0.179	0.240	0.222	0.259	0.138	0.207
NERICA 5	0.267	0.267	0.281	0.188	0.323	0.345	0.370	0.300	0.250	0.300	0.242
WITA 4	0.500	0.500	0.500	0.400	0.552	0.536	0.640	0.483	0.517	0.483	0.406
CP/MILLINIA	0.500	0.500	0.412	0.364	0.455	0.643	0.517	0.484	0.424	0.394	0.455
MARS	0.500	0.452	0.412	0.324	0.455	0.586	0.467	0.353	0.343	0.438	0.371
WAB 56-104	0.444	0.500	0.355	0.258	0.313	0.481	0.407	0.429	0.367	0.333	0.273
NERICA 7	0.679	0.679	0.351	0.741	0.351	0.371	0.353	0.714	0.750	0.263	0.282
ABAK ALIKI	0.389	0.351	0.559	0.282	0.656	0.700	0.633	0.342	0.333	0.645	0.656
FARO 57	0.467	0.419	0.567	0.419	0.621	0.667	0.654	0.364	0.394	0.452	0.679
OFADA	0.361	0.324	0.677	0.289	0.677	0.667	0.714	0.316	0.342	0.613	0.677
NERICA 4	1	0.833	0.382	0.63	0.424	0.552	0.433	0.667	0.704	0.406	0.343
NERICA 1	0.833	1	0.382	0.630	0.382	0.500	0.387	0.667	0.643	0.406	0.343
UPIA 3	0.382	0.382	1	0.382	0.724	0.548	0.586	0.412	0.441	0.5	0.563
NERICA 2	0.63	0.630	0.382	1	0.382	0.406	0.387	0.552	0.643	0.324	0.343
IGBEMO	0.424	0.382	0.724	0.382	1	0.655	0.704	0.412	0.441	0.600	0.613
FARO 44	0.552	0.500	0.548	0.406	0.655	1	0.833	0.484	0.469	0.586	0.655
KIRIKIRI/CHERIA	0.433	0.387	0.586	0.387	0.704	0.833	1	0.419	0.452	0.571	0.643
MORO BEREKAN	0.667	0.667	0.412	0.552	0.412	0.484	0.419	1	0.880	0.314	0.333
NERICA 8	0.704	0.643	0.441	0.643	0.441	0.469	0.452	0.880	1	0.343	0.361
CK 73	0.406	0.406	0.500	0.324	0.600	0.586	0.571	0.314	0.343	1	0.500
BG 90-2	0.343	0.343	0.563	0.343	0.613	0.655	0.643	0.333	0.361	0.500	1

using UPGMA-based analysis, cluster III presented the highest number of genotypes followed by cluster V, cluster IV, while Cluster II and Cluster I has same number of genotypes.

DISCUSSION

In Nigeria, rice farmers cultivate elite rice varieties and landraces indigenous to different agroecological zones and contrary to elite varieties, landraces still maintain broad diversity and great genetic potential unlike the elite rice varieties which have lost their diversity because of genetic erosion (Xu, 2010). Genetic variability present among the selected rice genotypes can therefore serve as the basis for rice improvement. Simple sequence repeats (SSRs) have been used to characterize germplasm and in genetic diversity (Graner et al., 2003). Also, genomic SSR (gSSR) were reported to be an attractive marker for population structure studies because of their abundance, reproducibility and high levels of polymorphism (Filippi et al., 2015). The SSR primers used in this study shows variation in their ability to identify differences among the 21 genotypes as revealed by the resolving power RP values. The primers with the highest RP are the most versatile in differentiating among the genotypes used in this study. However, the RP values of most of the primers were mostly low, this trend may be as a result of many closely related genotypes used in this study for example, six NERICA varieties was among the genotypes used. Despite low RP values recorded for most of the primers, the combination of primers RM175 and RM 17 or RM 210 was not only adequate in identifying differences among the genotypes but it will be adequate in identifying genotype differences among genotypes far higher than 21 in number. This is simpler to the suggestion made by Amiryousefi et al. (2018), where combination of two primers with RP values of 3.97 and 4.77 were recommended for accessions >100 in number. The average PIC Value is 0.66, this was lower than those obtained by Kumbhar et al. (2015) in landraces and improved rice varieties in India and Das et al. (2013) in rice landraces. The reason for the slight difference between the result in this study and those mentioned above may be due to the number of genotypes used and the closeness of some of the genotypes used in this study. The average PIC value was however higher than that obtained in the study by Aljumaili et al. (2018), even though 51 rice accessions were used, the genetic

diversity of the accession used was low. The population structure analysis separated the genotypes into three sub-populations, and the landraces were categorized into a unique sub-population while the elite varieties were distributed into two sub-populations. These results were consistent with the result gotten from the study of Kunusoth et al. (2015) where 24 genotypes from India were distributed into three sub-populations. However, when the population structure of 50 rice genotypes also from India were investigated by Kumbhar et al. (2015), the genotypes were distributed into five sub-populations. Whereas, the landraces used in the study by Kunusoth et al. (2015) were separated into distinctive sub-populations as in the case of this study, the observed difference in the number of sub-populations obtained in study of Kumbhar et al. (2015) may be because of the numbers of genotypes used or the maximum ΔK value detected. Most of the genotypes in cluster I and cluster II shared less similarity with other sub-population, this is because, cluster I comprise mostly of the landraces used in this study, which is an indication that they are still very pure in their genetic composition. Also, cluster II which comprise mostly of the NERICAs may have been subjected to intense selection during its breeding, which must have made their genome to comprise mostly that of the recurrent parent. This result is contrary to the result of Aljumaili et al. (2018), where more genetic differences was observed within the sub-population than without. The reason for such discrepancies may be because the numbers of genotypes used was more (51) and fewer landraces was used in their study. All the genotypes in cluster II except the duo of MOROBEREKAN and WITA-4 comprise of the NERICA varieties. However, WITA-4 shows significant and moderate relationships with genotypes in sub-populations III (0.369) and I (0.075). Shared ancestry and complex evolutionary trends in the development of WITA-4 may be responsible for the shared relationship with other sub-populations (NACGRAB, 2012). WITA-4 though a lowland variety, is reputable for its tolerance to iron toxicity and drought (NACGRAB, 2012), these attributes are also common to NERICA varieties, whose donor parent C14 possesses the aforementioned attributes. WAB 56-104 shared secondary relationship with sub-population II; this may not be unconnected to its involvement in the parentage of all the NERICA varieties in sub-population II, because WAB56-104 was the recurrent parents of NERICA's 1 to 11(Somado et al., 2008). However, the 21 genotypes used in this study were grouped into 5 clusters based on their similarity coefficients. This was different from the result obtain from the population structure analysis. This may not be unconnected with the different methods used in genotype grouping. When the result of the population analysis was compared with that of cluster analysis, most of the NERICAs except NERICA5 were all group in same sub-population and clusters. This shows that even though both methods were different, both were able to identify common parentage shared by the NERICAs.

Conclusion

According to this study, the population structure analysis of selected rice genotypes based on (gSSR) markers clearly identifies genetically related genotypes and separated them into common sub-populations. The knowledge generated on the population structure of these genotypes is an important contribution to crop breeding and conservation. It is however pertinent to note that Nigeria have several landraces indigenous to different agro-ecological zones, hence, a population structure analysis and genetic diversity involving more landraces and local collections is very essential in developing improved rice varieties which will be better adapted to vast agro-ecological zones in Nigeria.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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