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Marker assisted identification of suitable candidates for biosystematics and crop improvement among groundnut (*Arachis hypogaea* L.) breeding lines

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ABSTRACT: This study aimed at identifying suitable groundnut candidates for biosystematics and crop improvement purposes amongst some breeding lines using a combination of Simple Sequence Repeats (SSR) and Randomly Amplified Polymorphic DNA (RAPD) molecular markers. A total of 96 ICRISAT breeding lines were selected for the present study. These lines were first subjected to preliminary field morphometric evaluation and identification. Molecular methods employed include FTA DNA extraction/purification, Conventional PCRs, Multiplex PCRs and Agarose Gel electrophoresis. Polymerase Chain Reactions (PCRs) were carried out using 15 groundnut specific SSR primers and 14 non-specific RAPD primers. Molecular analysis distinguished some breeding lines with unique DNA bands. The final partition of the SSR based dendrogram was divergent. Within cluster R^2 was 93.22% with an average distance of 0.946 from the centroid. Genetic distance among breeding lines ranged from 0.00 similarity level to 2.157 dissimilarity level. This resulted in 25 genetic circumscriptions. Eleven (11) outstanding breeding lines identified from SSR report were all ICRISAT breeds with known and unknown phenotypic links. Four botanical varieties identified morphologically were represented in molecular results. Molecular markers also expanded the number of varietal classification beyond the limit of morphological resolution. RAPD analysis of the 11 notable SSR breeding lines showed enormous polymorphism. Genetic similarity level ranged from 74.54 to 16.5% with a large distance of 0.834. G15 (ICGV-IS-13881) and G76 (ICGV-IS-141198) were the most divergent breeding lines. Breeding lines that have been distinguished by molecular markers could be placed under *Arachis hypogaea* L. taxon. Further dwindling in the similarity level may signal drastic evolutionary changes with possible speciation. Based on the available evidence, a classification system of five (5) sub species and ten (10) varieties in *A. hypogaea* is proposed. Thus, the germplasm is a genetically diverse collection of groundnut breeding lines undergoing evolutionary changes and divergence of characters, therefore, could serve as suitable candidate for biosystematics and crop improvement programmes.

Keywords: Biosystematics, breeding lines, crop improvement, groundnut, identification, molecular markers.

INTRODUCTION

Arachis hypogaea Linneaus, commonly known as peanut or groundnut, is classified under family Fabaceae or Leguminosae; tribe Aeschynomeneae; subtribe Stylosanthinae and section Arachis (Janila *et al*., 2013). It exhibits a monophyletic relationship with other wild species of *Arachis* but possesses divergent morphological and genetic characters. *Arachis hypogaea* is a valuable leguminous crop bearing edible seeds in the underground pods, hence the name groundnut (FAO, 2017). It ranks thirteenth among the world food crops, fourth as the most important source of edible oil, and third as the most

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important source of vegetable protein (FAO, 2017). Breeding effort has focused on producing quality varieties that are highly yielding, tolerant to harsh environmental conditions and resistant to pests and diseases. Creating inbred lines creates a gap that must be filled by plant taxonomists in the assignment of proper botanical nomenclature following the universally acceptable nomenclatural breeders must have assigned accession or varietal codes (Janila *et al*., 2013).

Molecular evidence provides the real genetic fingerprint that truly identifies taxa (Aguoru *et al*., 2015b, Wang *et al*., 2016). It is not a subjective approach as researcher's perception is not applied to give false result. Moreover, it is not influenced by environmental variables that hinder morphological evidence (Kumar and Gurusubramanian, 2011). Although molecular evidence requires high level of technical expertise and expensive resources (Kumar and Gurusubramanian, 2011), it is reliable, fast, specific, highly sensitive and accurate molecular characterization relies on the use of markers which are landmarks on Deoxyribonucleic acid (DNA) that are linked to various genes controlling all traits (Aguoru and Olasan, 2014). Randomly Amplified Polymorphic DNA (RAPD) marker permits detection of polymorphism within and between populations (Janila *et al*., 2013). It is the quickest way of detecting genetic polymorphism. RAPD marker requires no DNA probes and sequence information for the design of primers. It is simple, efficient, fast, and less expensive with easy protocols. As a non-specific marker, it can be used on any plant species. However, it is a dominant marker that cannot distinguish between homozygous and heterozygous loci. Hence, it is not recommended for breeding line identification (Cerasela *et al*., 2010). This is a major setback that makes RAPD primers less applicable in precise systematic research (Aljibouri *et al*., 2013). Simple Sequence Repeats (SSR) markers are highly distinguishing microsatellites. There are many microsatellite loci in every genome and every locus is highly polymorphic. A combination of several loci identifies every plant species or variety or sub variety. Unlike RAPD marker, SSR markers are highly specific and are commonly used for a particular plant species. This marker is highly variable, fast evolving and co-dominant (it can distinguish between homozygous and heterozygous loci). These properties make SSR markers much better than RAPD marker in the genetic identification and circumscription of taxa. However, SSR markers are expensive, time consuming and highly technical in usage (Egbadzor *et al*., 2014; Somta *et al*., 2011). Therefore, a combination of both RAPD and SSR markers would bring complementarity effects.

In groundnut research, SSR markers were previously applied in genetic diversity studies, construction of population genetic structure, characterization of germplasm, genome mapping, and marker assisted selection (Janila *et al*., 2013; Kumar and Gurusubramanian, 2011; Wang *et al*., 2016). Thus, the present study aimed at identifying suitable groundnut candidates for biosystematics and crop improvement purposes amongst some breeding lines using a combination of SSR and RAPD molecular markers.

MATERIALS AND METHODS

Preliminary field study and identification

Preliminary field evaluation was carried out on 100 genotype coded breeding lines using morphometric method from planting to harvesting. Data were taken on diagnostic vegetative and reproductive characters. Phenetic identification of botanical varietal taxon was undertaken using standard indented taxonomic keys (Krapovickas and Gregory, 2007).

A. Growth erect, branching sequential with light green leaflet, early flowering with early maturity (90-120 days). Yield low…….. **Subsp. *fastigiata***

A_1. Low bush or bunch, smaller foliate, reddish/green stem, pods dirtier/rough with higher pod yield and larger seeds per pods (Spanish type)….....var. *vulgaris*

A_2. Taller bush, larger foliate, reddish stem, pods cleaner /smoother with many seeds per pods (Valencia type)……...var. *fastigiata*

B. Growth decumbent/procumbent/prostrate. Branching alternate with dark green leaf, late flowering with late maturity (130-150 days).Yield high……**Subsp. *hypogaea***

B_1. Runner/prostrate, low bush, seeds medium sized……..…….var. *hirsute*

B_2. Bunch/decumbent/procumbent, tall bush, seeds very large (Virginia type)… ……var. *hypogaea*

Molecular studies

Exactly 96 breeding lines were randomly selected and labeled P1-P96 to represent DNA ID for molecular work (Table 1). Synthesized oligonucleotide primers and PCR master mix were procured from Inqaba Biotech company, South Africa. The master mix contained specified quantities of standard buffer, *Taq* DNA Polymerase, dNTPs (A, G, C, and T), $MgCl_2$, Tris-HCl of 9.0 pH, KCl and Green tracking dye. Storage was done at -20°C. PCRs were carried out using 15 groundnut specific SSR primers for breeding line identification and 14 non-specific RAPD primers for further detection of genetic polymorphism.

DNA extraction (FTA Card Method)

DNA extraction protocol of Aguoru *et al*. (2015a) was

Table 1. Groundnut breeding lines used for molecular analysis.

Genotype name	Origin	Genotype code	DNA ID
ICGV-IS 13846	ICRISAT	G116	P1
G-2-52	ICRISAT	G94	P2
ICGV 94379	ICRISAT	G81	P3
ICGV-IS 13011	ICRISAT	G101	P4
ICGV-IS 09932	ICRISAT	G111	P5
ICGX-IS11003-F2-B1-B1	ICRISAT	G104	P6
ICGV IS 141198	ICRISAT	G76	P7
ICGV-IS 07803	ICRISAT	G103	P8
ICGV-IS 13854	ICRISAT	G3	P9
SAMNUT 23	SAMARU	G96	P10
ICGV-IS 13811	ICRISAT	G114	P11
ICGV-IS 13851	ICRISAT	G118	P12
ICGV-IS 13810	ICRISAT	G113	P13
SAMNUT 25	SAMARU ZARIA	G97	P14
ICG 2106	ICRISAT	G44	P15
ICGV-IS 141156	ICRISAT	G60	P16
ICGV 97182	ICRISAT	G56	P17
ICGX 11057	ICRISAT	G48	P18
ICGV-IS 141198	ICRISAT	G76	P19
Dh-86	ICRISAT	G93	P20
HAUSA KANO	KANO	G98	P21
ICGV-IS 13850	ICRISAT	G117	P22
ICGV-IS 09996	ICRISAT	G102	P23
EX-DAKAR	DAKAR SENEGAL	G107	P24
ICGV-IS 141178	ICRISAT	G71	P25
ICGV-IS 13887	ICRISAT	G41	P26
ICGS 44	ICRISAT	G45	P27
ICGV 86024	ICRISAT	G80	P28
ICGV-IS 13863	ICRISAT	G100	P29
JL-24	ICRISAT	G91	P30
ICGV-IS 09926	ICRISAT	G110	P31
ICGV-IS 09992	ICRISAT	G112	P32
SAMNUT 24	SAMARU ZARIA	G87	P33
ICGV 91317	ICRISAT	G83	P34
TG-39	ICRISAT	G92	P35
ICGV-IS 13828	ICRISAT	G115	P36
ICGV-SM 01721	ICRISAT	G90	P37
ICGV-IS-89767	ICRISAT	G84	P38
ICGV-IS 13858	ICRISAT	G6	P39
ICGV-IS 07965	ICRISAT	G106	P40
J-11	ICRISAT	G78	P41
ICGV-IS 14906	ICRISAT	G74	P42
ICGV-IS 141145	ICRISAT	G66	P43
ICGV-IS 13943	ICRISAT	G28	P44
ICG 4729	ICRISAT	G43	P45

Table 1. Contd.

Genotype name	Origin	Genotype code	DNA ID
ICGV 87378	ICRISAT	G53	P46
ICGV-IS 07831	ICRISAT	G109	P47
TAG-24	ICRISAT	G95	P48
ICGV-IS 14867	ICRISAT	G61	P49
ICGX 13011	ICRISAT	G49	P50
ICGV-IS 141151	ICRISAT	G59	P51
ICGV 02189	ICRISAT	G54	P52
ICGV-IS 13967	ICRISAT	G79	P53
ICGV 02022	ICRISAT	G58	P54
ICGX 11010	ICRISAT	G46	P55
ICGV-IS 141193	ICRISAT	G69	P56
ICGV-IS 14898	ICRISAT	G72	P57
ICGV-IS 141176	ICRISAT	G67	P58
ICGV-IS 141144	ICRISAT	G73	P59
ICGV-IS 141063	ICRISAT	G75	P60
ICGV 99241	ICRISAT	G51	P61
ICGV-IS 13861	ICRISAT	G7	P62
MOSSTIGA	ICRISAT	G77	P63
ICGV-IS 141214	ICRISAT	G64	P64
ICGV-IS 141091	ICRISAT	G63	P65
ICGV-IS 141071	ICRISAT	G62	P66
ICGV 00308	ICRISAT	G57	P67
ICGV-IS 141199	ICRISAT	G68	P68
ICGV-IS 13927	ICRISAT	G25	P69
ICGV-IS 13953	ICRISAT	G30	P70
ICGV-IS 13896	ICRISAT	G19	P71
ICGV-IS 13856	ICRISAT	G4	P72
ICGV-IS 13875	ICRISAT	G12	P73
ICGS 11060	ICRISAT	G47	P74
ICGV-IS 13877	ICRISAT	G13	P75
ICGV-IS 13046	ICRISAT	G40	P76
ICGV-IS 13911	ICRISAT	G22	P77
ICGV-IS 13897	ICRISAT	G20	P78
ICGV-IS 13862	ICRISAT	G8	P79
ICGV-IS 13839	ICRISAT	G39	P80
ICGV-IS 13867	ICRISAT	G10	P81
ICGV-IS 13940	ICRISAT	G27	P82
ICGV-IS 13907	ICRISAT	G21	P83
ICGV-IS 13955	ICRISAT	G32	P84
ICGV-IS 13914	ICRISAT	G23	P85
ICGV-IS 13926	ICRISAT	G24	P86
ICGV-IS 13874	ICRISAT	G11	P87
ICGV-IS 13857	ICRISAT	G5	P88
ICGV-IS 13891	ICRISAT	G16	P89
ICG 4750	ICRISAT	G42	P90

Table 1. Contd.

Genotype name	Origin	Genotype code	DNA ID
ICGV-IS 13853	ICRISAT	G2	P91
ICGV-IS 13938	ICRISAT	G26	P92
ICGV-IS 13865	ICRISAT	G9	P93
ICGV-IS 13881	ICRISAT	G15	P94
ICGV-IS 09828	ICRISAT	G99	P95
ICGV-IS 13952	ICRISAT	G29	P96
ICGV-IS 13878	ICRISAT	G14	P97
ICGV 02271	ICRISAT	G50	P98
ICGV-IS 13893	ICRISAT	G18	P99
ICGV-IS 13971	ICRISAT	G36	P100

adopted. DNA was collected from 15-day old seedlings. A leaflet of each genotype was placed on the FTA™ Plant Card (Whatman®) followed by extraction in 200 µl of 70% ethanol and 200 µl of FTA purification reagent.

SSR and RAPD based PCR

For each genotype, 2 discs of DNA template were transferred to a labeled PCR tube. The following reagents were added: 12.5 µl Master Mix, 0.5µl of each SSR forward/reverse primer (or multiplexed SSR primers) and nuclease free water to make up a total reaction of 25 µl. Amplification was done on a thermal cycler (Applied Biosystem in Life Technology 2720 Model). SSR and RAPD protocols reported by Sai *et al*. (2016) were adopted after series of temperature/time optimization. Initial denaturation was programmed at 94°C followed by 35 cycles of 94°C for 30 seconds, annealing temperature at 59°C for 60 seconds, and extension temperature at 72°C for 2 minutes, and final extension at 72°C for 20 minutes. Temperature was held at 4°C.

Agarose Gel Electrophoresis (AGE)

Kirkhouse Trust (2010) AGE protocol was used. About 3% agarose gel was prepared in a conical flask (3 g of agarose powder in 300 ml TAE buffer) while 1 µl of ethidium bromide solution was added as stain (for DNA visualization on the UV light after electrophoresis). The electrophoresis tank (Galileo Bioscience tank connected to Consort EV243 electrophoresis power supply) was used and covered with TAE buffer. DNA bands were captured using digital camera (Canon SX120).

Data analysis

All DNA bands were scored using 0 and 1, for absence and presence of bands respectively. Minitab software (17.0) was used for cluster identification and construction of SSR based phylogenetic tree using the Average Linkage method based on Euclidean distance. The amalgamation profile of cluster was presented. Loci variability of SSR markers was revealed using graphical approach called the Individual Plot (IP). Identified breeding lines from SSR markers were profiled for genetic diversity using multiplex RAPD primers. Dendrogram was constructed using the McQuitty Linkage method measured on Average Correlation Coefficient Distance (ACCD) method. SSR based varietal classification was carried out on the SPSS software using the Ward's method measured on Euclidean distance.

RESULTS AND DISCUSSION

The Amalgamation Profile of cluster analysis is presented in Table 2. The final partition of the dendrogram was divergent. Within cluster sum of square was 93.22 with an average distance of 0.946 from the centroid. Maximum distance from the centroid is 1.91. Genetic distance among breeding lines ranged from 0.00 similarity level to 2.157 dissimilarity level. Some of the breeding lines were similar in genetic constitution based on the markers applied. 25 levels representing 21% of the total population were identified as dissimilar in genetic make-up (Figure 1). The 25 genetic circumscriptions have distinguished 11 distinct breeding lines identified by the SSR marker. Their pot codes are: P97, P7, P84, P62, P91, P82, P94, P51, P77, P71 and P39. The breeding line IDs are: G14, G76, G32, G7, G2, G27, G15, G59, G22, G19 and G6 respectively. The genotypes are listed as follows: ICGV-IS-13893, ICGV-IS-141198, ICGV-IS-13955, ICGV-IS-13861, ICGV-IS-13853, ICGV-IS-13940, ICGV-IS-13881, ICGV-IS-141151, ICGV-IS-13911, ICGV-IS-13896 and ICGV-IS-13858 respectively. The 11 SSR distinct breeding lines

Table 2. Amalgamation profile of cluster analysis using Euclidean Distance Average Linkage.

Final partition	Number of Cluster Observation	Within Cluster Sum of Squares	Average Distance from Centroid	Maximum Distance from Centroid	Similarity Level	Distance Level
Divergent	96	93.2188	0.9456	1.9143	11.946 to 100	0.000 to 2.157

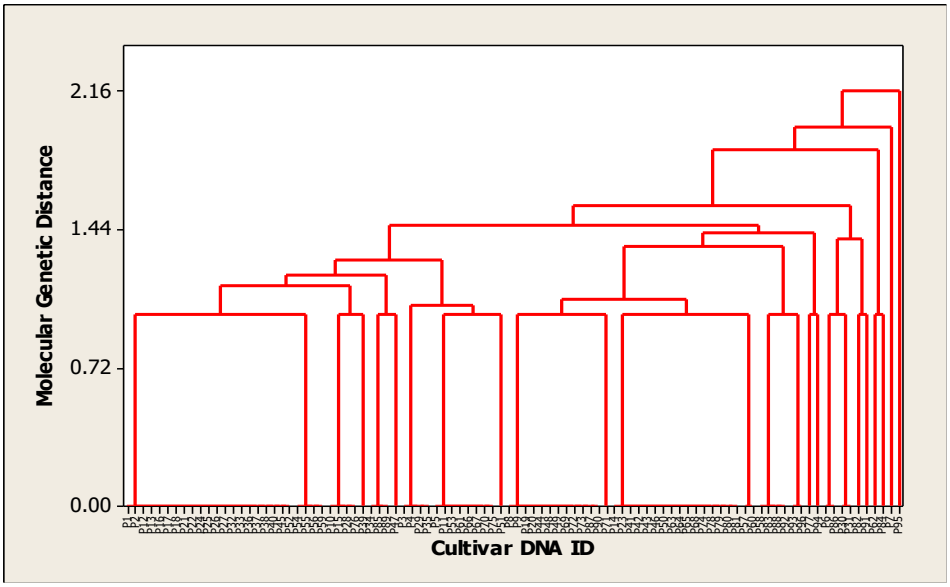

Figure 1. Identification and phylogenic construction of genetically circumscribed breeding lines using SSR markers. **Legend:** P1-P96 is breeding line DNA code. See table 2 for corresponding breeding line accession numbers and names.

were all ICRISAT breeds and none of the Nigerian landraces were included.

Table 3 lists all the 11 SSR identified breeding lines and their close links based on phenotypic outcomes. All varietal taxa identified morphologically were represented but dominated by the *A. hypogaea* subsp.*hypogaea* var.*hirsuta* and *A. hypogaea* subsp.*fastigiata* var.*vulgaris* varieties. Two unique breeding lines identified and re-named using morphological markers were resolved by SSR marker. They are: G76 and G7 representing ICGV-IS-141198 and ICGV-IS-13861 respectively. The four breeding lines previously suspected to be hybrids (G57, G63, G73 and G86) in morphological outcomes were not resolved in the SRR marker, therefore missing in the list. ICGV-IS-13893 and ICGV-IS-13896 were distinguished phenotypically for high disease resistance. ICGV-IS-13955, ICGV-IS-13940 and ICGV-IS-13811 were among breeding lines noted for high seedling vigour in phenotypic outcome. SSR resolved breeding lines with unknown phenotypic link are those with breeding line IDs G2, G59, G22 and G6.

The genetic variability of the SSR SEQ2B09 primer is shown in Figure 2. Out of 96 breeding lines, 79 (82.2%) produced bands at this locus while 17 breeding lines did not. The genetic variability of the SSR GMR165 primer is shown in Figure 3. Out of 96 breeding lines, 55 (57.3%) produced bands at this locus while 41 breeding lines did not. The genetic variability of the multiplexed SSR TC3E05 and GA5 primers is shown in Figure 4. Out of 96 breeding lines, 40 (41.7%) produced bands at this locus while 56 breeding lines did not. Figure 5 gives an insight into the levels of classification of botanical varieties. Ten (10) systematically circumscribed varieties were recognized (V1-V10) under 5 subspecies taxa (2 varieties per subspecies). There are micro classifications and associations among the genotypes of each botanical variety. V1 and V2 varieties have 6 and 8 genotypes respectively. Members are associated to some degrees. V3 (16 genotypes), V4 (12 genotypes) and V5 (21 genotypes) are varieties whose members are very closely

Table 3. The 11 SSR resolved breeding lines and their phenotypic link.

Pot ID	Cultivar ID	Cultivar	Phenotypic link	Taxonomic identity
P97	G14	ICGV-IS-13893	Disease resistance	Subsp.*hypogaea* var. *hypogaea*
P7	G76	ICGV-IS-141198	Unique morphotype	Nomenclature assigned
P84	G32	ICGV-IS-13955	High vigour	Subsp. *fastigiata* var. *vulgaris*
P62	G7	ICGV-IS-13861	Unique morphotype	Nomenclature assigned
P91	G2	ICGV-IS-13853	Unknown	Subsp.*hypogaea* var. *hypogaea*
P82	G27	ICGV-IS-13940	High vigour	Subsp. *fastigiata* var. *vulgaris*
P94	G15	ICGV-IS-13881	High vigour	Subsp.*hypogaea* var. *hirsuta*
P51	G59	ICGV-IS-141151	Unknown	Subsp.*hypogaea* var. *hirsuta*
P77	G22	ICGV-IS-13911	Unknown	Subsp.*hypogaea* var. *hirsuta*
P71	G19	ICGV-IS-13896	Disease resistance	Subsp. *fastigiata* var. *fastigiata*
P39	G6	ICGV-IS-13858	Unknown	Subsp. *fastigiata* var. *vulgaris*

Figure 2. Variability of SEQ2B09 Locus using the Individual Plot Method.
Legend: P1- P96 is breeding line DNA code.

associated. V6 to V10 varieties have genotypes that varied widely in genetic constitution. Exactly 7 new circumscribed varieties emerged from the Ward's classification as designated with letter "N". Their DNA Identification codes are: P95, P71, P47, P39, P7, P51 and P62. The corresponding breeding line accession numbers are: G99, G19, G109, G6, G76, G59 and G7 respectively.

Figure 6 shows the genetic diversity and relationship among the 11 unique breeding lines (selected based on SSR result) using multiplex RAPD markers. Only 8 breeding lines gave sharp bands in the RAPD primers. They are represented by DNA identification codes: P97, P7, P84, P62, P91, P82, P94 and P51. The breeding line IDs are: G14, G76, G32, G7, G2, G27, G15 and G59. RAPD analysis of the isolated breeding lines showed huge polymorphism. Genetic similarity level ranges from 74.54% (G27 and G22) to 16.5% (G14 and G59) with a wide distance ranging from 0.255 to 0.834 respectively. G15 (ICGV-IS-13881) and G76 (ICGV-IS-141198) were the most divergent breeding lines.

Molecular markers have proven to be effective in the taxonomic audit of *Arachis hypogaea* L. This is because the germplasm was accurately x-rayed using RAPD and SSR markers. Taxa identification, classification, circumscription and determination of phylogenetic relationships have been provided. These are the targeted taxonomic entities commonly considered in any systematic research (Ncube Kanyika *et al*., 2016). SSR markers, as

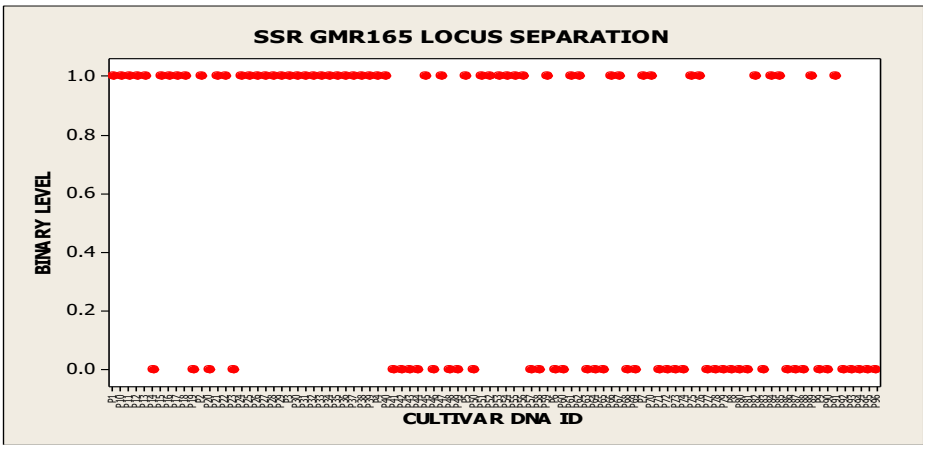

Figure 3. Variability of GMR165 Locus using the Individual Plot Method.
Legend: P1- P96 is breeding line DNA code.

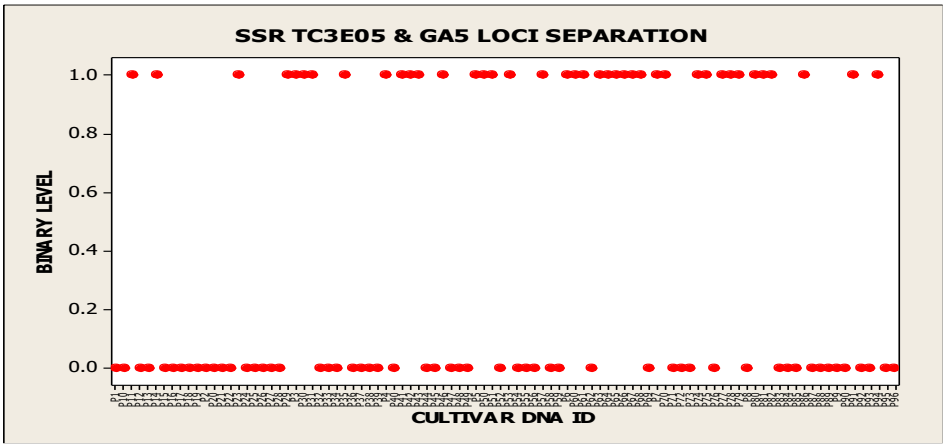

Figure 4. SSR TC3E05 & GA5 Loci using the Individual Plot Method
Legend: P1- P96 is breeding line DNA code.

distinguishing specific microsatellites, have been confirmed to be useful in breeding line identification. This view has been widely reported among molecular geneticists (Ncube Kanyika *et al*., 2016; Somta *et al*., 2016; Wang *et al*., 2016). In most molecular systematic reports, SSR marker is applied because of its high resolving power in revealing true genetic identity (Ncube Kanyika *et al*., 2016). Multiplex RAPD approach has further revealed the diversity and relationships among the few breeding lines identified through SSR markers. This confirms the relevance of RAPD markers in groundnut systematic research if appropriately applied as employed in other crops (Aljibouri *et al*., 2013; Singh *et al*., 2016). The present outcome has challenged groundnut researchers who find RAPD markers less useful in systematic application apart from detecting genetic polymorphism (Al-Saghir and Abdel-Salam, 2015; Sai *et al*., 2016; Siva *et al*., 2014).

The results of molecular characterization obtained in the present study agree with morphological studies. Despite minor differences in the pattern of information, the content of the information provided are complimentary. Molecular analysis has distinguished some breeding lines with unique DNA bands (Plate 1). They are: G-2-52, ICGX-11010, ICGV-2106, ICGV-IS-13858, ICGV-IS-13914, ICGV-IS-1301, ICGV-IS-13811, ICGV-IS-141151, ICGV-IS-13854, ICGV-IS-13896, ICGV-IS-09996, ICGV-IS-141063, ICGV-IS-13907, ICGV-IS-13952, ICGV-IS-13952, ICGV-IS-13881, ICGX-IS11003-F2-B1-B1, ICGV-IS013926, JL-24, ICGV-IS-13867, ICGV-IS-13867, ICGV-IS-13853, ICGV-IS-13861, ICGV-IS-13955, ICGV-IS-141198, ICGV-IS-09828 and ICGV-IS-07831. This has resulted in 25 genetic circumscriptions representing 21% of the population analysed, signifying huge genetic

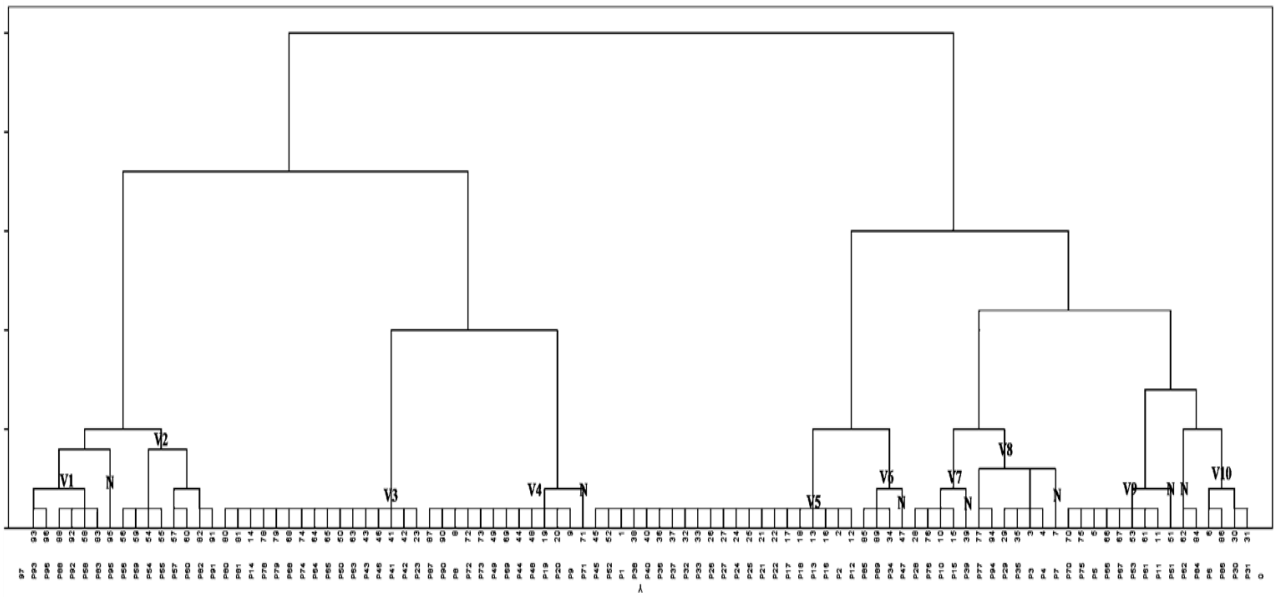

Figure 5. Varietal classification of groundnut breeding lines using SSR markers. **Legend:** P1- P96 is breeding line DNA code.

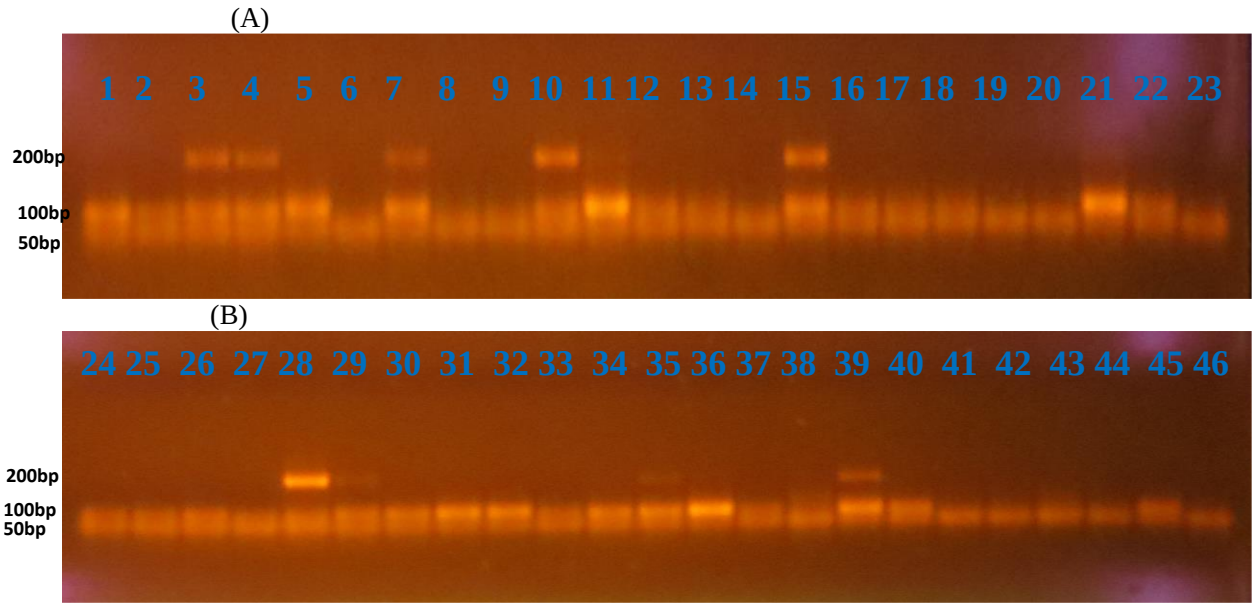

Plate 1 (A-B). DNA Band Amplified by SSR Primer GM2165 (1- 46 Breeding lines).

polymorphism among members of the *A. hypogaea* germplasm (Olasan *et al*., 2017, 2018). The wide genetic variability also emanating from morphological analysis is fully supported.

A total of 11 outstanding breeding lines have been identified using SSR marker. They are: ICGV-IS-141198, ICGV-IS-13861, ICGV-IS-13893, ICGV-IS-13896, ICGV-IS-13955, ICGV-IS-13940, ICGV-IS-13811, ICGV-IS-13853, ICGV-IS-141153, ICGV-IS-13911 and ICGV-IS-13858. They are among those with high disease resistance and those with best seedling performances reported in this study. This list also includes the two outstanding breeding lines named using morphological evidence. For example, G32 (ICGV-IS-13955), G27

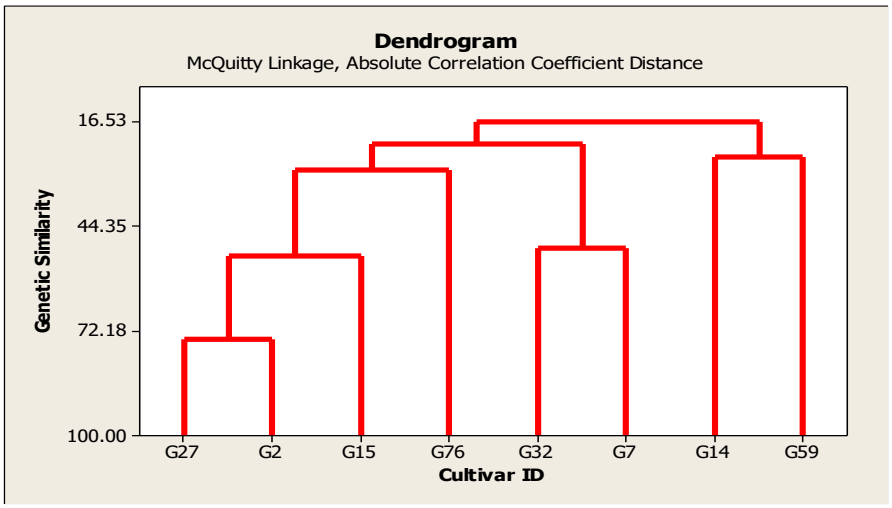

Figure 6. Genetic diversity of SSR-identified breeding lines using Multiplex RAPD marker.

(ICGV-IS-13940) and G15 (ICGV-IS-13881) are unique molecularly and physiologically. G7 (ICGV-IS-13861) and G76 (ICGV-IS-141198), re-named using morphological evidence, are validated by molecular evidence. This outcome agrees with popular views that physiological activities are coded for by the gene (Aguoru *et al*., 2017). It could be inferred that morphological traits are phenotypic expression of genetic information (Bayat *et al*., 2017). This view corroborates popular position that phenotypic expression of an organism is an interplay or interaction of environment and genetic constitution called genotype by environment interaction (G X E) effect (Bayat *et al*., 2017; Desawi *et al*., 2014; Janila *et al*., 2013; Singh *et al*., 2016). Though there are gene-environment interactions, the genetic constitution of an organism is unchanged if mutation is excluded (Aguoru *et al*., 2015a; Janila *et al*., 2013).

Among the groundnut breeding lines, none of the Nigerian landraces had unique genetic constitution. Divergent breeding lines were all ICRISAT breeds. It may signal the dominance of distinct alleles in the gene pool of new entrants and subsequent genetic erosion of the landraces. Calls for urgent conservation of Nigeria's landraces have been made (Mshelmbula *et al*., 2017) as a strategy to overcome loss of biodiversity.

Molecular markers have expanded the number of varietal classification beyond the resolution of morphological marker in *A. hypogaea*. This study proposes a classification system of five (5) sub species and ten (10) varieties in *Arachis hypogaea* L. It deviates from the existing 2 by 4 system of classification (Krapovickas and Gregory, 2007; Kotzamanidis *et al*., 2006). However, molecular systematics partially agrees with morphological outcome of 2 varieties per sub species. This report shows that *Arachis hypogaea* is a genetically diverse crop undergoing fast evolutionary process and divergence of characters. This position contradicts the view of authors who reported slow evolution in *Arachis hypogaeai* (Janila *et al*., 2013; Krapovickas and Gregory, 2007; Kotzamanidis *et al*., 2006). The contribution of the investigated germplasm to the genetic divergence cannot be ruled out because it is an assemblage of genetic resources that have been improved upon by breeders to meet specific aims (ICRISAT, 2015). These may include improvement for quality, yield, taste, disease resistance and tolerance to extreme environmental conditions (Janila *et al*., 2013). Crop improvement may have generated diverse genetic resources of groundnut that are fast evolving.

Ward's method of varietal classification has further revealed the nature of relationship among members of different levels (groups or clades) using molecular approach. The first two varietal levels (V1 and V2) are genetically related to an extent. V3 (16 members), V4 (12 members) and V5 (21 members) varietal levels have members that are completely related in genetic composition. They are not candidates of plant breeding (Janila *et al*., 2013) and evolution process may be partially frozen, a view widely reported among authors (Wang *et al*., 2016). The V6, V7, V8, V9 and V10 varietal levels have members that are widely diverse in genetic constitution. These are candidates that may be targeted for crop improvement and genetic exchange (Janila *et al*., 2013). Geneticists interested in discovering novel groundnut genes may also target these varieties. Evolution among the members may oscillate and therefore drastic evolution cannot be overruled in future. Members may undergo speciation into different species under the genus *Arachis*;

section Arachis (Belamkar et al., 201; Leal-Bertioli *et al*., 2015; Moretzsohn *et al*., 2013). *Arachis hypoaea* is a unique species of *Arachis* that is likely to diverge and evolve rapidly. This view was upheld in the report given by Koppolu *et al*. (2010) using SSR markers. RAPD analysis of the identified breeding lines showed huge polymorphism. Genetic similarity level ranges from 74.54 to 16.5%. This wide gap further supports the circumscription of the varietal taxon. However, due to the huge level of interrelatedness and convergence observed among the breeding lines in the phylogenetic tree, they may still be placed under *Arachis hypogaea* L. taxon. Further dwindling in the similarity level may signal evolutionary changes, implying speciation. In this case, they may exist as different species under genus *Arachis,* section Arachis, family Fabaceae.

Conclusion

The studied germplasm could be described as a genetically diverse collection of groundnut genetic resources undergoing evolutionary changes and divergence of characters, therefore a good candidate for crop improvement programme. Eleven divergent breeding lines were reported as good candidates for crop improvement, biosystematics and evolutionary studies. They were: ICGV-IS-141198, ICGV-IS-13861, ICGV-IS-13893, ICGV-IS-13896, ICGV-IS-13955, ICGV-IS-13940, ICGV-IS-13811, ICGV-IS-13853, ICGV-IS-141153, ICGV-IS-13911 and ICGV-IS-13858. SSR and RAPD markers have also proven useful as molecular evidence that could be used to solve challenges arising from the botanical identification and biosystematics of groundnut breeding lines as provided in the report.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Aguoru, C. U., & Olasan, J. O. (2014). Introduction to techniques in molecular genetics. A Text Book for Undergraduate and Postgraduate Students. Kingdom Press, Makurdi, 105pp.

Aguoru, C. U., Omoigui, L. O., & Olasan, J. O. (2015a). Molecular characterization of *Solanum* species (*Solanum aethiopicum* complex; *Solanum macrocarpon* and *Solanum anguivi*) using multiplex RAPD primers. *Journal of Plant Studies*, *4*(1), 27-32.

Aguoru, C. U., Azua, E. T., & Olasan, J. O. (2015b) Approaches to minimizing and overcoming current biodiversity loss. *British Journal of Environmental Sciences*, *3*(3), 12-26.

Aguoru, C. U., Ogbodo, C., & Olasan, J. O. (2017). Chemosystematics studies on six varieties of *Mangifera indica* L. *Agricultural Sciences*, 8(3), 253-259.

Aljibouri, A. A. M., Zokian, S. A. Y., Almusawi, A. H. (2013). RAPD-PCR analysis of some species of *Euphorbia* grown in University of Baghdad Campus in Jadiriyah. *African Journal of Biotechnology*, 12(49), 6809-6816

Bayat, M., Amirnia, R., & Rahimi, M. (2017). Phenotypic and genotypic relationships between traits in saffron (Crocus sativus L.) as revealed by path analysis. *Journal of Applied Research on Medicinal and Aromatic Plants*, *5*, 33-40.

Belamkar, V., Selvaraj, M. G., Ayers, J. L., Payton, P. R., Puppala, N., & Burow, M. D. (2011). A first insight into population structure and linkage disequilibrium in the US peanut minicore collection. *Genetica*, *139*(4), 411-429.

Cerasela, P., Ciulca, S., Lazar, A., Irina, P., Giancarla, V. (2010). Assessment variability of alfalfa regenerants using RAPD molecular markers. *Journal of Horticulture, Forestry Biotechnology*, *14*(3), 172-176.

Desawi, H. T., Sentayehu, A. K., Daniel, E. G. (2014). Assessment of genetic variability, genetic advance, correlation and path analysis for morphological traits in *Sesame* genotypes. *Asian Journal of Agricultural Research*, 8(4), 181-194.

Egbadzor, K. F., Ofori, K., Yeboah, M., Aboagye, L. M., Opoku-Agyeman, M. O., Danquah, E. Y., & Offei, S. K. (2014). Diversity in 113 cowpea [*Vigna unguiculata* (L) Walp] accessions assessed with 458 SNP markers. *SpringerPlus*, *3*(1), 1-15.

Food and Agricultural Organization (FAO) (2017). Peanut. Food and Agricultural Organization.

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (2015). Annual Report of 2015. Retrieved from https://www.icrisat.org/

Janila, P., Nigam, S. N., Pandey, M. K., Nagesh, P., & Varshney, R. K. (2013). Groundnut improvement: use of genetic and genomic tools. *Frontiers in plant science*, *4*, Article Number 23.

Ncube Kanyika, B. T., Lungu, D., Mweetwa, A. M., Kaimoyo, E., Njung'e, V. M., Monyo, E. S., Siambi, M., He, G., Prakash, C.S., Zhao, Y., & de Villiers, S. M. (2015). Identification of groundnut (*Arachis hypogaea*) SSR markers suitable for multiple resistance traits QTL mapping in African germplasm. *Electronic Journal of Biotechnology*, *18*(2), 61-67.

Kotzamanidis, S. T., Stavropoulos, N., & Ipsilandis, C. G. (2006). Classification and evaluation of Greek groundnut (*Arachis hypogaea* L.) using 17 main agronomic and quality traits. *Pakistan Journal of Biological Science*, *9*(6), 1021-1027.

Koppolu, R., Upadhyaya, H. D., Dwivedi, S. L., Hoisington, D. A., & Varshney, R. K. (2010). Genetic relationships among seven sections of genus *Arachis* studied by using

SSR markers. *BMC Plant Biology*, *10,* Article number 15.

Krapovickas, A., Gregory, W. C., Williams, D. E., & Simpson, C. E. (2007). Taxonomy of the genus Arachis (Leguminosae). *Bonplandia*, *16*, 7-205.

Kumar, N. S., & Gurusubramanian, G. (2011). Random amplified polymorphic DNA (RAPD) markers and its applications. *Colloquium, 11*(3), 116-124.

Leal-Bertioli, S. C., Santos, S. P., Dantas, K. M., Inghs, P. W., Nielen, S., Araujo, A. C., Silva, J. P., Cavalcante, U., Guimaraes, P. M., Brasleiro, A. C., Carrasquilla-Garca, N., Penmetsa, R. V., Cook, D., Moretzsohn, M. C., & Bertioli, D. J. (2015). *Arachis batizocoi*: A study of its relationship to cultivated peanut (*A. hypogaea*) and its potential for introgression of wild genes into the peanut. *Annal of Botany*, 115(2), 237-249.

Moretzsohn, M. C., Gouvea, E. G., Inghs, P. W., Leal-Bertioli, S. C. M., Valls, J. F. M., & Bertioli, D. J. (2013). A study of the relationship of cultivated peanut (*Arachis hypogea* L.) and its most closely related wild species using intron sequence and microsatellite markers. *Annals of Botany*, 111(1), 113-126.

Mshelmbula, B. P., Jummai, B. F., Mallum, S. M., & Zacharia, R. (2017). Studies on four breeding lines of groundnut grown in Mubi, Adamawa State Nigeria. *Journal of Technological Research*, *12*(1), 1-6.

Olasan, J. O., Aguoru, C. U., Ugbaa, M. S., & Ekefan, E. J. (2017). Physiological characterization of groundnut (*Arachis hypogaea* L.) seedlings using vigour and performance indices. *Proceedings of the Genetic Society of Nigeria 41*st *Annual Conference, University of Agriculture Makurdi*, Pp. 84-91.

Olasan, J. O., Aguoru, C. U., Omoigui, L. O., & Ekefan, E. J. (2018). Character association studies in groundnut (*Arachis hypogaea* L.). *American Journal of Plant Sciences*, 9(7), 1531-1543.

Al-Saghir, M. G., & Abdel-Salam, A-S. G. (2015). Genetic diversity of peanut (Arachis hypogea L.) cultivars as revealed by RAPD markers. *American Journal of Plant Sciences*, *6*(14), 2303-2308.

Sai, D. K., Rani, K. R., Usha, R. (2016). Assessment of genetic diversity in groundnut (*Arachis hypogaea* L.) genotypes using PCR based RAPD markers. *Biotechnological Research*, *24*(4), 142-147.

Singh, S., Prakash, A., Chakraborty, N. R., Wheeler, C., Agarwal, P. K., & Ghosh, A. (2016). Genetic variability, character association and divergence studies in *Jatropha curcas* for improvement in oil yield. *Trees*, 30(4), 1163-1180.

Siva, G., Kamalakkannan, K., Sivakumar, S., Premkumar, G., Gurusaravanan, P., Vinoth, S., & Jayabalan, N. (2014). Analysis of genetic variability in *Arachis hypogaea* L. Using RAPD markers. *Journal of the Swamy Botanical Club*, *31*, 29-36.

Somta, P., Chank, S., Rungnoi, O., & Srinive, P. (2016). Genetic diversity of the Bambara groundnut (*Vigna subterranea* (L.) Verdc.) as assessed by SSR markers. *Genome*, 54(11), 898-910.

Wang, H., Khera, P., Huang, B., Yuan, M., Katem, R., Zhuang, W., Harris-Schultz, K., & Moore, K. M. (2016). Analysis of genetic diversity and population structure of peanut and breeding lines from China, India and the U.S. using SSR markers. *Journal of Integrative Plant Biology*, 58(5), 452-465.

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