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Genetic Polymorphism Among Groundnut Genotypes Using Simple Sequence Repeat (SSR) Markers

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Abstract

Nowadays, breeders can specifically target traits for improvement and also enhance the overall efficiency of breeding program using molecular breeding approaches. Assessment of genetic diversity in groundnut through identification of polymorphic molecular markers is prerequisite to the identification of target markers to traits of economic importance for integration of molecular breeding in groundnut improvement. Assessment of genetic diversity in groundnut through identification of polymorphism at the molecular level will also help breeders in judicious selection of genotypes that show DNA polymorphism to generate genetically diverse breeding populations and facilitate the identification of valuable germplasm for use in linkage mapping and genetic enhancement of specific traits in groundnut. The objective of the study therefore was to identify genetic polymorphism among selected groundnut genotypes using SSR markers. Five (5) Simple Sequence Repeat markers were used to identify genetic polymorphism among 40 groundnut genotypes. DNA was extracted from leaf tissue of one month old plants using FTA PlantSaver cards. Extracted DNA was amplified via polymerase chain reaction (PCR) in a 25 µL mixture (final volume). A 10µL of the final PCR product was electrophoresed on a 2% agarose gel with ethidium bromide staining. The ethidium bromide-stained gel was visualized on an UV transilluminator and photographed using a digital camera for binary scoring of '1' and '0' to indicate the presence and absence of bands respectively. Out of six amplified bands produced by five SSR primers (AH3, AH4-101, GM694, TC3G03, and S118), four were found to be polymorphic (66.7%). Average number of bands and polymorphic bands per primer were 1.6 and 0.8 respectively. Polymorphism Information Content (PIC) ranged from 0.00 (GM694 and TC3G03) to 0.78 (AH4-101). Resolving power ranged from 0.00 to 2.19. Euclidean distance revealed two main clusters (A and B) and four sub-clusters. Genotypes TMV-2, ICGV 91328 and ICGV-IS-89767 were singly clustered. ICGV-SM-12991 and JL-24 clustered in pairs as well as ICGV 94309 and ICGV 00068 making them the most divergent genotypes. The divergent genotypes can be explored by breeders to capture wide variability for groundnut improvement programs. The information from the study will be useful to taxonomists.

Keywords

Polymorphism Information Content, Resolving Power, Clusters, Euclidean Distance, Variability

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1. Introduction

Groundnut (*Arachis hypogaea* L.), (2n = 4x = 40) also known as peanut or earthnut, a self-pollinated legume [1], is one of the world's principal oilseed crops. It is the 13th most important food crop, 4thoil seed crop of the world and important source of vegetable protein. Analysis of the groundnut seed showed that is a rich source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium [2].

Vigorous debate has trailed the origin of cultivated groundnut. However, according to Seijo et al. [3], it has become common believe that a single hybridization event involving two wild diploid species, *Arachis duranensis* (AA genome) and *Arachisipaensis* (BB genome), followed by chromosome duplication, gave rise to a wild allotetraploid (plausibly the still extant *Arachis monticola*) that was fertile and capable of reproducing itself. Its earliest known traces were found in jars of food at burial sites near Lima, Peru. The domestication of the wild allotetraploid and improvement for use as human food then resulted in the present day cultivated peanut [3].

Statistics by the FAO [4] shows that it is grown on about 28 million ha worldwide with a production volume of about 45.9 million metric tons, and a global average yield of 1.6 t ha^{-1}. In Africa, Nigeria and Sudan rank top in production with over 2.9 million tonnes [4]. Despite this fact, the productivity of the crop in Nigeria is still low with an average yield of around 0.9 t ha^{-1} compared with global average of 1.6t ha^{-1} [4].

The improvement of productivity through conventional approaches like selection and introduction has been ongoing however; evaluation of genetic diversity based on morphological features may not be efficient as they are highly influenced by environments. It is possible to specifically target traits for improvement, and also enhance the efficiency of overall breeding program through use of molecular breeding approaches [5]. Assessment of genetic diversity in groundnut through identification of polymorphic molecular markers is prerequisite to the identification of target markers to traits of economic importance for integration of molecular breeding in groundnut improvement [6]. Assessment of genetic diversity in groundnut through identification of polymorphism at the molecular level will also help breeders in judicious selection of genotypes that show DNA polymorphism to generate genetically diverse breeding populations and facilitate the identification of valuable germplasm for use in linkage mapping and genetic enhancement of specific traits in groundnut. Low to moderate levels of molecular genetic polymorphism have been detected among cultivated groundnut accessions using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) [7]. This necessitate the need to use Simple Sequence Repeat (SSR) molecular markers which are multi-allelic, highly abundant, analytically simple and highly transferable [8] to identify genetic polymorphism in Groundnut (*Arachis hypogaea*) genotypes. The objectives of the study therefore were to identify genetic polymorphism among selected groundnut genotypes using SSR markers.

2. Materials and Methods

2.1. Plant Materials and SSR Markers

Five (5) Simple Sequence Repeat markers (Table 1) were used to screen forty (40) groundnut genotypes obtained from International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Kano, Nigeria. The screening was done at the Molecular Biology Laboratory of the Federal University of Agriculture, Makurdi.

Table 1. Primer sequences for DNA analysis in this study.

Marker	Primer Sequence
GM694 (GA165)	F: ATTTGTGCCCTACCACCTTCT R: TCCCTCCTAGAGGTTGACTTGA
AH3	F: AATGCATGAGCTTCCATCAA R: AACCCCATCTTAAAATCTTACCAA
AH4-101	F: GCACCATCGCCACAAGATTAAC R: GCACAGGAAAGAGCGCATTAGA
S118	F: TATATGATGCTTGATTGAGACT R: CATGTAGAAGGCTTGGAGGGTAT
TC3G03 (GM35)	F: ATCTGCAGCCTCAAGCTGAT R: GCCGGTATGAGAGATTGGAG

2.2. DNA Extraction Using Fast Technology Application (FTA)

The forty (40) genotypes were planted in pots in the screen house of the Molecular Biology Laboratory. Genomic DNA was extracted from leaf tissue of one month old plants using the Fast Technology Application (FTA) Plantsaver cards.

The excised young leaf was placed on the FTA Plantsaver card covered with parafilm paper, and brief pressure was gently applied with a pestle until plant material was sufficiently transferred to the card. The cards were allowed to dry for one hour; plant material was brushed off with tissue paper. After air drying, FTA cards was placed in a paper pouch and stored at ambient temperature in a desiccator. DNA was made PCR ready as described by Omoigui et al. [9].

2.3. PCR Analysis

Extracted DNA was amplified via Polymerase chain reaction (PCR). The PCR mixture (25 µL final volume) contained, in addition to the purified 2 mm FTA DNA disc containing the DNA sample, a final concentration of 18 µL of molecular

grade water, 2.5 µL each of balanced dNTPs mix and 10 x PCR buffer, 0.05 µL of Taq polymerase, and 1 µL of each of the forward and reverse primers (synthesized by Integrated DNA Technologies, Coralville, IA). PCR reactions were performed on a heated lid thermal cycle (iCycle

thermocycler from Bio-Rad) operated as follows: 35 cycles of denaturation at 94°C for 30 sec, followed by annealing temperature at 57.5°C for 30 sec and extension at 72°C for 2 min. A final extension cycle of 10 min at 72°C was added to ensure completion of the final amplification products as described by Omoigui et al. [9].

2.4. Analysis of PCR Product

A 10µL of the final PCR product was electrophoresed on a 2% agarose gel stained with ethidium bromide (10mg/ml of H_20) using horizontal gel electrophoresis system (GALILEO bioscience). The gel was run for approximately 1 h 30 min at 120 voltages in 1 X Tris acetic acid (TAE) buffer (45 mmol L-1 glacial acetic acid, 0.5 mmol l-1 ethylenediaminetetra acetic acid (EDTA), (pH, 8.4). A 1 kb DNA molecular marker ladder loaded in the first well for band size determination of PCR products. The ethidium bromide-stained gel was visualized on a Benchtop UV Transilluminator (M-26V) and photographed using a digital camera for scoring.

2.5. DNA Band Scoring Data Analysis

Clear and unambiguous of SSR markers were scored to generate marker profile for genotypes. The scores '1' and '0'were used to indicate the presence and absence of bands, respectively. Percentage polymorphism was calculated using the following equation:

$$\% \text{ Polymorphism} = \frac{\text{Polymorphic bands}}{\text{Total number of bands}} x100$$

Polymorphism information content (PIC) for each polymorphic band of a primer was calculated as $PIC = 1 - p^2 - q^2$ where P = band frequency and q = no band (absence of band) frequency. The average PIC for a polymorphic primer was the sum of PIC of all polymorphic bands divided by the number of polymorphic bands [10]. Resolving power for each primer was calculated as $RP = \sum 1_b$ where $\sum 1_b = 1 - [2 \text{ x}(0.5\text{-p}]$ and P = proportion of genotypes containing the band [11].

Based on the marker profiles from screening of groundnut genotypes, a dendrogram was constructed using the Average Linkage (Between Group) method of the SPSS software (20.0 Version) using Complete linkage and Euclidean distance.

3. Results and Discussion

Out of the five (5) primers used for the study, 2 primers (GM694 and TC3G03) were non polymorphic while 3 (AH3, AH4-101 and S118) were polymorphic (Figure 1 and Figure 2). The PCR amplification using these markers on 40 groundnut genotypes yielded six (6) amplified bands (Table 1) out of which four bands, representing 66.7% were polymorphic. The percentage polymorphism observed in this study is higher than other studies by Gautami et al. [8] (57%), Roomi et al. [12] (50%), Pandey et al. [13] (37.30%), He et al. [14] (33%) and Ferguson et al. [15] (28%). This could be an indication that, the molecular markers used in this study are more informative than the markers used by these researchers.100% polymorphism was detected among the polymorphic primers. Average number of bands and polymorphic bands per primer of 1.6 and 0.8 (Table 1) is low compared with the findings of Gautami et al. [8] (2.3) among 8 polymorhpic markers and 2.25 alleles per primer by Jiang et al. [16]. Higher values of 15.4 alleles per locus were obtained by Noelle et al. [17] for wild accessions and Pandey et al. [13] (3.2) alleles. This agrees with the earlier finding by Varshney et al. [18] that, abundant DNA polymorhpism in wild *Arachis* species has been observed where as little variation has been reported in cultivated peanut. None the less, the highest PIC of 0.78 and mean PIC of 0.58 in this study indicates that sufficient variability exists in the investigated germplasm that can be exploited for improvement. Also, the difference could be the large number of markers; 4,485 used by Pandey et al. [13], 31 by Noelle et al. [17].

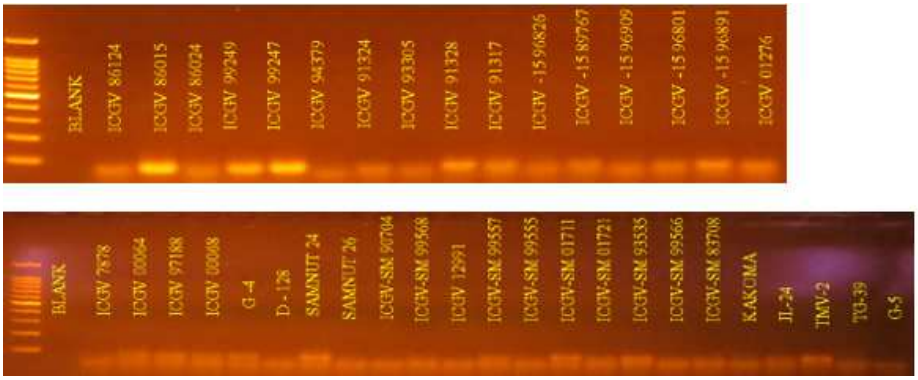

Figure 1. A sample of non-polymorphic marker using GM694 primer.

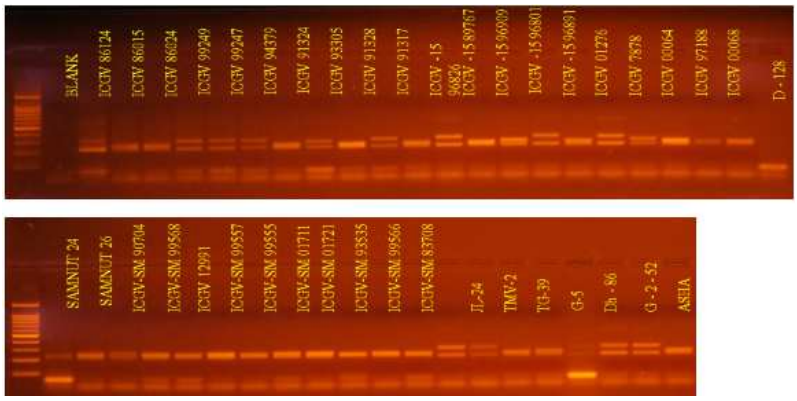

Figure 2. A sample of polymorphic marker using AH4-101 primer.

Table 2. Molecular polymorphism, PIC values and RP values of SSR Markers among 40 groundnut genotypes.

S/No	Marker	Total bands	Polymorphic bands	Percentage (%) Polymorphism	PIC	RP
1	AH3	1	1	100	0.50	1.55
2	AH4-101	2	2	100	0.78	2.19
3	GM694	1	0	0	0.00	0.00
4	S118	1	1	100	0.47	1.22
5	TC3G03	1	0	0	0.00	0.00
	Total	06	04			
	Mean	1.6	0.8	66.7		

Polymorphism information content (PIC) values for polymorphic markers ranged from 0.47 to 0.75 (Table 1). The high polymorphism and multiallelism observed in this study, and as reported from similar studies in groundnut by Gautami et al., Pandey et al., He et al., Ferguson et al., Jiang et al., Noelle., Varshney et al. [8, 13-18] using SSR markers is an indication of the abundance of microsatellites in the wild and cultivated groundnut genome, and their highly allelic nature. These findings resonate with Rakoczy-Trojanowska and Bolibok [19] that SSR markers appear to have wider application because of their presence in genomes of all living organisms and their high level of allelic variation. Thus, Pandey et al. [13] asserts that SSR markers have remained the common marker of choice by scientists as a routine tool in the breeding and genetic analysis of groundnut.

Clustering pattern using complete linkage and Euclidean distance revealed two main clusters (A and B) and four sub-clusters, two under main cluster A (AI and AII) and two under main cluster B (BI and BII) as shown in Figure 3. The result agrees with other workers who reported similar grouping of two major clusters in their diversity analysis in groundnut using molecular markers Pandey et al., Jiang et al., Noelle., Varshney et al., Oteng-Frimpong [13, 16-18, 20]. Genotypes TMV-2, ICGV 91328 and ICGV-IS-89767 were singly clustered. ICGV-SM-12991 and JL-24 clustered in pairs as well as ICGV 94309 and ICGV 00068 making them the most divergent genotypes.

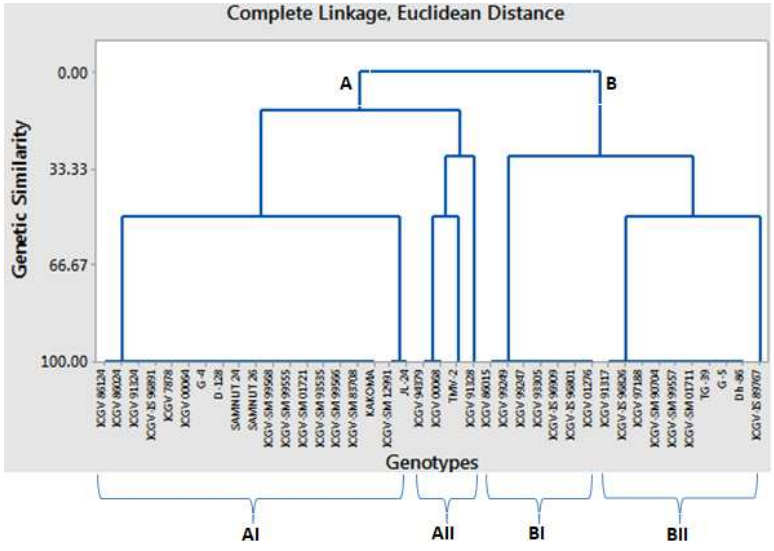

Figure 3. Dendrogram showing relationship among groundnut genotypes based on SSR profile.

The clusters of genotypes in this study were examined side by side with the cluster groups reported by Olasan et al. [21] who carried out a morphological diversity study of the same group of genotypes in this study using various traits. The examination showed that groundnut genotypes in this study clustered independent of their phenotypic attributes. According to Janila et al., Pandey et al. [1, 13] this further confirms the low level of genetic variation in cultivated groundnut. The relatively low genetic diversity observed could because these are advanced breeding genotypes generated from a breeding program. That SSR markers could discern variations and differentiate between the closely related genotypes, makes this technology a powerful tool for genomic characterization of groundnut [19].

The divergent genotypes are strongly recommended to be explored by breeders and genetic engineers to capture wide variability for groundnut improvement programs, and the information from the study will be useful to taxonomists.

4. Conclusion

Out of six amplified bands produced by five SSR primers (AH3, AH4-101, GM694, TC3G03, and S118), four were found to be polymorphic (66.7%). Average number of bands and polymorphic bands per primer were 1.6 and 0.8 respectively. Polymorphism Information Content (PIC) ranged from 0.00 (GM694 and TC3G03) to 0.78 (AH4-101). Resolving power ranged from 0.00 to 2.19. Euclidean distance revealed two main clusters (A and B) and four sub-clusters. Genotypes TMV-2, ICGV 91328 and ICGV-IS-89767 were singly clustered. ICGV-SM-12991 and JL-24 clustered in pairs as well as ICGV 94309 and ICGV 00068 making them the most divergent genotypes. The divergent genotypes can be explored by breeders to capture wide variability for groundnut improvement programs. The information from the study will be useful to taxonomists.

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