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GENETIC DIVERSITY AMONG SELECTED KERSTING'S GROUNDNUT ACCESSIONS BASED ON SSR, SCoT AND RAPD MARKERS

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ABSTRACT

Kersting*'s groundnut is an underutilized but nutritious legume of West Africa. Adequate knowledge of genetic diversity among available accessions of* Kersting*'s groundnut will facilitate establishing a viable improvement program to enhance its potential as an alternative protein source. This study investigated the molecular diversity among 20* Kersting*'s groundnut accessions using Simple Sequence Repeat (SSR) markers, Start Codon Targeted (SCoT), and Random Amplified Polymorphic DNA (RAPD). The accessions were planted in the nursery farm of the Department of Botany, University of Ibadan, to raise seedlings of the accessions from where leaf samples were taken for molecular study. DNA extraction, PCR amplification using three molecular markers (five SSRs, seven SCoTs, and five RAPDs), and subsequent analysis using Power Marker version 3.25 software revealed significant genetic diversity and relationships among accessions. Higher polymorphism and higher gene diversities were found in SCoT 35 (86.4%, 0.88), SCoT 36 (80.8%, 0.83) and SCoT 3 (75.1%, 0.78), respectively. Higher allelic frequencies > 0.5 were observed in RAPD and SSR markers compared to the SCoT marker, indicating molecular variation. The SSR markers formed two main clusters at 10% genetic similarity. SCoTs dendrogram showed two clusters, with cluster 2 dividing into subclusters A and B. RAPDs grouped genotypes similarly. This study established genetic variability among the* Kersting*'s groundnut accessions wide enough to initiate a breeding program. The study underscored the potential usefulness of molecular technology in exploiting the genetic potential of the crop for improvement.*

Keywords: ***Genetic markers,*** **Kersting*****'s groundnut, Molecular diversity, Protein-rich crop***

INTRODUCTION

Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Maréchal & Baudet], is one of the many tropical crops known for its high nutrients-dense benefits and adaptability to dry climates but mostly neglected by research scholars and policymakers (Ayenan *et al*., 2016). It is a member of the Fabaceae family, and the taxonomic classification places it within the broader grouping of flowering plants characterized by their distinctive legume fruit, which typically contains seeds arranged in pods (Coulibaly 2022). Kersting's groundnut typically exhibits a diploid chromosome count, with 2n = 22 chromosomes and is primarily characterized as a self-pollinating species (Odo and Akaneme., 2021). Agronomically, Kersting's groundnut is classified as a leguminous crop, specifically falling within the category of grain legumes. *Macrotyloma geocarpum* is an important food crop in many regions where it is cultivated for its edible seeds, which are rich in protein and nutrients while its counterpart Kersting*iella geocarpa* is less commonly cultivated and has limited economic importance.

The lack of sufficient protein-rich food supplies in many developing countries has led to the exploration of new food sources to add to or replace the current protein sources available (Ahmed and Abdallah 2010). According to Singh (2013), approximately 795 million individuals suffer from malnutrition globally. Kersting's groundnut (*Macrotyloma geocarpum*), is a type of legume crop that is primarily cultivated by small-scale farmers in developing nations. This crop is native to West Africa and is considered one of the "neglected pulses." in the regions alongside bambara groundnut and pigeon pea (Coulibaly 2022). Kersting's groundnut seeds particularly stand out from other indigenous orphan crops with their high protein content (21.3%), crude fibre (6.2%), carbohydrates values, which are as high as 61.53–73.3% as well as its amino acids contents; lysine, arginine, histidine, and methionine richness, (Aremu *et al*., 2011, Abberton *et al.,* 2022). Furthermore, the seeds are known to possess other important minerals inclusive of, but not limited to Magnesium (Mg), Iron (Fe), Sodium (Na), Potassium (K), Phosphorus (P), and Calcium (Ca) (Aremu *et al*., 2011).

Overall, the combination of limited commercial value, low market demand, agronomic challenges, limited research and development, and policy priorities contribute to Kersting's groundnut being ranked among the lowest-priority crops in Nigeria. The Genetic Resources Center (GRC) of IITA, presently harbours only twenty-eight (28) Kersting's accessions out of 7920 of all underutilized presently housed by the center (Abberton *et al.,* 2022). Commonly, three main landraces based on seed coat colour (black, white, and brown) exist within the different climate and agricultural zones in the regions of Southern-Sudanian, Northern-Sudanian, and Northern-Guinean (Coulibaly *et al*., 2022). These landraces are well known for their low genetic variations thus limiting their extent of diversity. The tiny grain size, low yield, and a lack of improved cultivars are among the frequently cited causes of its decreased cultivation and possible extinction (Kafoutchoni *et al*., 2021). Other limitations are in their locational environmental condition for its cultivation to harness its full genetic resource conservation and breeding potential. Despite the low variations of Kerstin's groundnut as observed by various phenotypic studies, (Ayenan *et al*., 2016), the imminent climate change currently being experienced is forcing researchers to look into this group of underutilized crops as the currently most widely cultivated crops (rice, wheat, and maize) cannot withstand the anticipated harsh conditions.

The cultivation of crops such as Kerstin's groundnut, whose standout qualities include its ability to adapt to any marginal area and intense enhancement of soil fertility by fixing atmospheric nitrogen in the soil may be a solution to the impending extreme weather conditions to be experienced in the next decade, (Coulibaly *et al*., 2022). Exploiting variations in crops usually start with the phenotypic identification followed by molecular study which in its totality is anticipated to capture all inherent variation that might be within and among a germplasm. Earlier studies employing biochemical markers (allozyme) conducted by Pasquet *et al*. (2002) and agro-morphological investigations conducted by Akohoue *et al*., (2019), Assogba *et al*., (2015), and Bayorbor *et al*., (2010) have indicated a notably restricted genetic diversity within Kersting's groundnut. Overall, a deeper understanding and strategic utilization of the restricted genetic diversity in Kersting's groundnut have the potential to drive innovation and

advancement in food security, livestock nutrition, and agricultural sustainability.

The rapid advancement of molecular technologies has significantly enhanced our understanding of the genetic diversity present in various crops. When compared to morphological and biochemical markers, the majority of molecular markers are abundant and exhibit inheritance patterns that are both dominant (RAPD and AFLP) and co-dominant (RFLP and SSRs) (Ajayi *et al*., 2019; Olawuyi and Azeez., 2019; Singh *et al*., 2020), markers segregation based on single primer (Satya *et al*., 2015, Aboulila and Mansour, 2017). SSR (Simple Sequence Repeat) markers, recognized as vital tools in genetic research, exhibit high polymorphism and find broad applications in plant genomics. Varshney *et al*. (2018) in a recent study highlighted the effectiveness of SSR markers in uncovering genetic diversity and facilitating molecular breeding across various plant species.

Another noteworthy molecular marker, SCoT (Start Codon Targeted polymorphism) emerged as a novel and gene-targeted technique derived from the flanking ATG translation codon in plant genes. Thakur *et al*. (2021) emphasizes its authenticity in assessing genetic homogeneity, providing a valuable asset in molecular analyses. In addition, Random Amplified Polymorphic DNA (RAPD) markers, a subset of arbitrarily amplified dominant markers, offer a key advantage by enabling polymorphism analysis without prior knowledge of the organism's DNA sequence. Amiteye (2021) underscored the simplicity, resolvability, and cost-effectiveness of RAPD markers in assessing genetic variability. These markers individually play a crucial role in plant population genetics, DNA fingerprinting, and trait mapping.

Combining multiple genetic markers offers several advantages in genetic research. Firstly, it enhances resolution by capturing genetic variation at different scales, providing a detailed understanding of relationships among individuals or populations (Singh *et al*., 2020). Secondly, it reduces redundancy and bias inherent in any single marker type, leading to more reliable results (Sunar *et al*., 2020). Thirdly, it increases discriminatory power, allowing for accurate differentiation between closely related individuals or populations, especially beneficial in complex studies (Tran *et al*., 2022). In addition, comparing results from different marker types helps validate and confirm findings, strengthening research outcomes (Al-Khayri *et al*., 2022). Finally, utilizing a combination of markers provides comprehensive insights into genetic diversity, population structure, and evolutionary dynamics, offering a holistic understanding of biological processes. Overall, the synergistic use of multiple markers enhances the precision, reliability, and depth of genetic analyses, facilitating a more thorough understanding of genetic variation and its implications.

Thus, combining two or more of these markers for DNA fingerprinting is expected to reveal the extent of these variations which may help in defining the genetic relationship among these selected Nigerian Kersting's accessions. The objectives of this study were to (i) examine the molecular diversity among selected accessions of Nigerian Kersting groundnuts using SSR, SCoT, and RAPD and assess associations among results obtained using three markers.

MATERIALS AND METHODS

Plant Materials

Twenty accessions of Kersting's groundnuts were collected from the Genetic Research Centre of the International Institute of

Tropical Agriculture (Table 1). The potted experiment was conducted in the screen house, nursery of the Department of Botany, University of Ibadan, Nigeria. Four weeks young leaves of Kersting's groundnut were collected from each accession and DNA was extracted using the modified Doyle and Doyle, (1987) method, as reported by Borges *et al*. (2009). The concentration and purity of the DNA were assessed by utilizing a Nanodrop ND-1000 spectrophotometer, with the DNA samples being subsequently stored at -20ºC in TE buffer. CA, USA) for later use.

Table 1: List of Kersting's accessions used in this study.

S/NO	Accession Names	S/NO	Accession Names
1	TKg-15	11	TKg-34
2	TKg-18	12	TKg-35
3	TKg-24	13	TKg-36
4	TKg-25	14	TKg-37
5	TKg-26	15	TKg-38
6	TKg-27	16	TKg-39
7	TKg-28	17	TKg-40
8	TKg-30	18	TKg-41
9	TKg-32	19	TKg-43
10	TKg-33	20	TKg-45

TKg: Tropical **K**ersting's **g**roundnut

A set of primers consisting of five SSRs (SSR6733, SSR6717, SSR6188, SSR7079, SSR7000) seven SCoTs primers (SCoT3, SCoT16, SCoT18, SCoT28, SCoT33, SCoT35, SCoT36), and five RAPDs (OPB1, OPT19, OPB5, OPT4, OPH7) making a total of seventeen markers were used for the molecular studies (Table 2). The selection of SSR primers was guided by markers identified in cowpea, following the findings of Andargie, *et al*. (2011). ISSR primers from the UBC (University of British Columbia) series were chosen based on reports by Matasyoh (2012) and Patel *et al*. (2014). RAPD primers were randomly selected from the available markers for PCR amplification. For the three-marker set, the primers that exhibited the highest polymorphism were utilized in this study. (Table 2).

The final volume of the amplification was 25 µl, and each reaction contained 2.5 µl of PCR buffer, 2.5 mM dNTPs, 50 mM MgCl2, 0.1 unit of Taq polymerase, 1 µl of DMSO, 13.4 µl of PCR-grade water, 1 µl of each primer, and 3 µl of DNA. The reaction was performed in an Applied Biosystem Thermal Cycler using a standardized PCR program (Table 3). The samples that were amplified underwent the electrophoretic steps on a gel made of 1.5% agarose mixed in 1X TBE buffer. They were then treated with 1.5µl of ethidium bromide (1mg/ml) for staining. Each of the PCR products, measuring 3 µl, was mixed with 1.5µl of loading dye before being loaded onto the gel. The PCR product was subjected to electrophoresis at 100 volts for a duration of 1 hour. A 1Kb DNA ladder was used as a reference to compare the sizes of the bands produced by the amplified products. Visualization of the amplified fragment was done under a UV trans-illuminator after the electrophoresis. Amplified fragments were scored as 1 for present and 0 for absent for all primers. Dendrograms were generated by

the Unweighted Pair-Group Method and Arithmetic averages (UPGMA) method.

Molecular Data Analysis

The SSR, SCoT, and RAPD profiles for identical molecular weight fragments from each accession were rated as presence (1) or absence (0) in the data matrix. Using Jaccard's similarity coefficient, the data received from scoring the bands was put through a genetic similarity matrix (Jaccard, 1908; Solanki, *et al*., 2022). The NTSYS-pc software version 2.02 was used to perform cluster analysis utilizing the unweighted pair-group method with arithmetic averages (UGPMA) to find phylogenetic relationships with the NTSYS-pc software version 2.02 (Rohlf, 2000). Polymorphic information content (PIC) was calculated using the formula:

$$PIC = 1 - \sum pi^2$$

Where pi is the frequency of the ith allele.

Table 2: The primer name and sequence for the amplification of the 20 Kersting's accession

S/NO	Primer ID	5’ Forward Primer Sequence	3’ Reverse Primer Sequence
Simple Sequence Repeat Primers			
1	SSR-6733	CATGTCCAAGATGTATGTAGG	CCTGGGATTGCGGGATTGTT
2	SSR-6717	CCTCACTCTGAATTGCATAC	CTGAATCACCCAATTTGCTTCC
3	SSR-6188	CCACACCCTGTTCCGTACTC	ACCAGGTGCAATGCTTCTCT
4	SSR-7079	GCACGGGCATGTACTGAAAA	GTTTTTGGTGATCTGGACAT
5	SSR-7000	GAAGCTTAATCCACAGAATCTACGC	GGAAACTGTTTGCACTTTTATCCCA
SCoT Primers			
1	SCoT 3	CAACAATGGCTACCACCG	
2	SCoT 16	ACCATGGCTACCACCGAC	
3	SCoT 18	ACCATGGCTACCACCGCC	
4	SCoT 28	CCATGGCTACCACCGCCA	
5	SCoT 33	CCATGGCTACCACCGCAG	
6	SCoT 35	CATGGCTACCACCGGCCC	
7	SCoT 36	GCAACAATGGCTACCACC	
Rapid Amplified Polymorphic Primers (RAPDs)			
1	OPB 01	GTT TCG CTC C	
2	OPT 19	GTC CGT ATG G	
3	OPB 05	TGC GCC CTT C	
4	OPT 04	CAC AGA GGG A	
5	OPH 07	CTG CAT CGT G	

Table 3: Polymerase chain reaction program for the three sets of primers

	Program	Temperature	Time	Cycles
SSR primers				
	Denaturing	94	5 mins	
	Annealing	94	30 sec	
		55	30 sec	40 cycles
		72	1 mins	
	Final Extension	72	10 mins	
SCOTs Primers				
	Denaturing	94	5 mins	
	Annealing	94	30 secs	
		50	1 minute	35 cycles
		72	1 minute	
	Final Extension	72	10 mins	

RAPDs primers			
Denaturing	94	5 mins	
Annealing	94	30 sec	
	38	1 mins	48 cycles
	72	1 mins	
Final Extension	72	10 mins	

RESULTS

Qualitative and quantitative analysis of genomic DNA

The amplification profiles generated from Kersting's groundnut with the five SSRs, seven SCoTs, and five RAPDs showed a representative amplification profile generated for most of the genotypes (Plates 1-3). The quality of the DNA of the extracted samples in the ratio range at 260/280 and 260/230 gave a result of 1.60 to 2.23 purity to evidence good quality DNAs as determined by the Nano-drop spectrophotometer (Table 4).

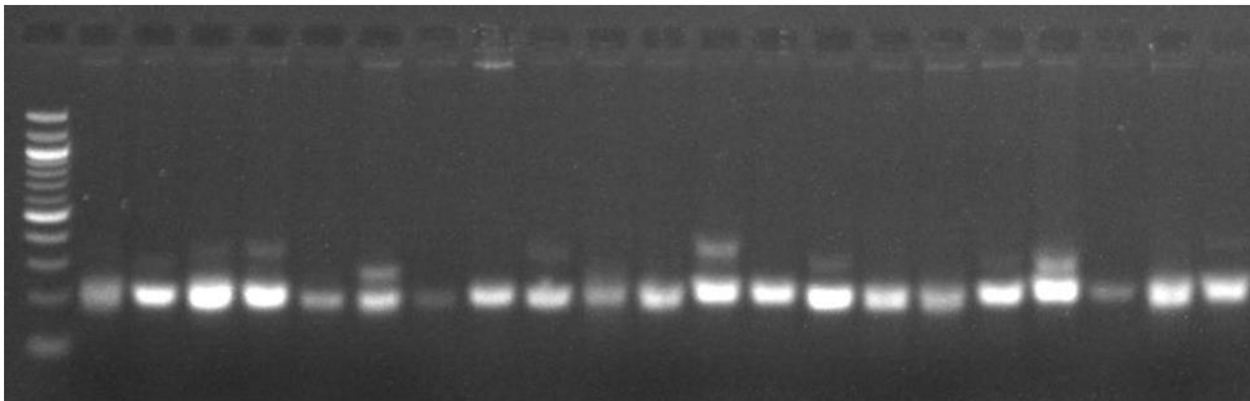

Plate 1: SSR- 6733 allele patterns among the 20 genotypes of Kersting's groundnut

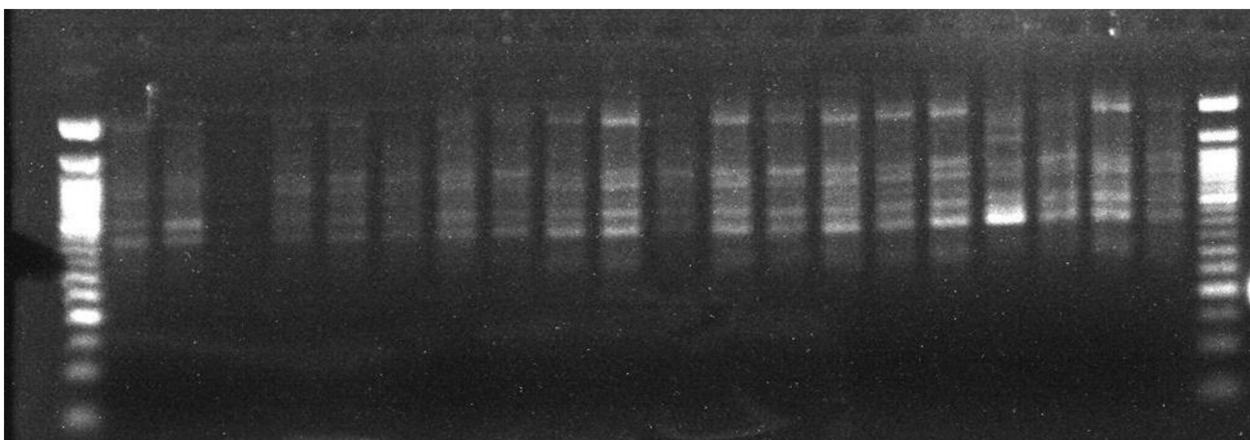

Plate 2: SCoT 18 allele patterns among the 20 genotypes of Kersting's groundnut samples

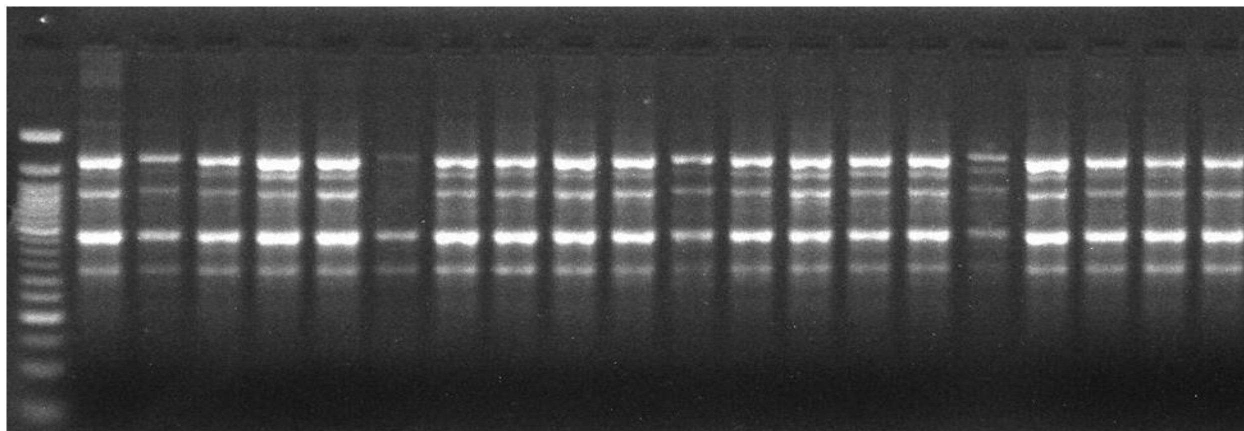

Plate 3: OPB19 allele patterns among the 20 genotypes of Kersting's groundnut samples

Table 4: Nanodrop Spectrophotometric concentration of extracted DNA from the 20 genotypes of Kersting's groundnut

S/N	Sample ID	Nucleic Acid (ng/µl)	DNA Conc (ng/uL)	
			260/280ng	260/230ng
1	TKg-24	531.92	1.98	1.84
2	TKg-45	1491.73	2.09	2.06
3	TKg-39	650.51	1.82	1.65
4	TKg-40	626.91	1.93	1.76
5	TKg-15	675.32	1.92	1.72
6	TKg-35	2474.81	2.05	2.07
7	TKg-34	1788.82	2.09	2.23
8	TKg-18	1361.83	2.00	1.87
9	TKg-33	962.15	2.04	2.18
10	TKg-36	1289.52	1.94	1.60
11	TKg-26	955.11	2.03	2.08
12	TKg-25	799.12	2.01	2.01
13	TKg-43	1653.12	2.07	2.07
14	TKg-27	839.91	1.96	1.86
15	TKg-37	908.12	2.03	1.93
16	TKg-41	1100.13	1.95	1.75
17	TKg-30	57.12	1.70	0.71
18	TKg-28	10831	2.01	1.73
19	TKg-38	713.31	1.87	1.79
20	TKg-32	2164.51	2.06	2.11

Cluster analysis of the 20* Kersting*'s groundnuts as revealed by the three markers

Analysis of all the seventeen marker loci showed that the markers were polymorphic and highly effective in discriminating the 20 genotypes of the Kersting's groundnut used in this study. The three combined primers (5 SSR, 7SCoT, and 5 RAPD) produced a total of 66 reproducible and scorable bands.

Individually, the three primers produced 13, 11 and 44 bands with averages of bands per primer being 6.0, 2.0, and 2.3 respectively. Each primer produced an amplification product in the range of 250–1500 bp. Number of scorable band varied from 2 (SCoT33, opb1, opt19, opt4, oph7, SSR 6717, SSR 7079), 3 (SCoT16, Scot28, opb5, SSR 6733, SSR 6188, and SSR7000), 7 (SCoT18), 8 (SCoT3) to 11 for SCoT35. The polymorphic information content as revealed by the seventeen markers ranged from 0.09 to 0.86 with a mean of 0.38. Markers SCoT35, SCoT36, SCoT3, SCoT18 and SSR 6733 gave high informative polymorphic information contents of 0.86, 0.81, 0.75, 0.58 and 0.53 respectively (Table 5).

Individual Genetic differentiation as revealed by the 5 SSRs, 7 SCoTs, and 5 RAPDs markers

At a genetic similarity of 10 %, two main clusters were formed in Figure 1 based on the SSR primers. Cluster 1 contained only TKg-41. Cluster 2 was divided into two sub-clusters, A and B at a genetic similarity of 20%. Sub-cluster B was further subdivided into B1 and B2 at 30% similarity. Cluster A consisted of TKg-18 and TKg-24. TKg-15, TKg-40, TKg-33, TKg-45, TKg-32, TKg-34, TKg-35, and TKg-39 formed cluster B1. Finally, cluster B2 consisted of TKg-30, TKg-25, TKg-36, TKg-26, TKg-28, TKg-37, TKg-43, TKg-27 and TKg-38. TKg-41 and TKg-38 had the furthest genetic distance between them (Figure 2).

The dendrogram generated by the SCoTs was divided into two main clusters 1 and 2. Cluster 2 is subdivided into two subclusters A and B. The main cluster 1 consisted of only TKg-35. Subcluster A consisted of TKg-25, TKg-36, TKg-34, TKg-15 and TKg-24. TKg-37, TKg-18, TKg-26, TKg-45, TKg-39, TKg-30, TKg-32, TKg-33, TKg-338, TKg-40, TKg-41, TKg-43, TKg-27 and TKg-28 were included subcluster B. In subcluster A, TKg-25 and TKg-36 were identical while TKg-34, TKg-15 and TKg-24 were also identical. From subcluster B, TKg-37, TKg-18 and TKg-26 were identical while TKg-30, TKg-32, TKg-33, TKg-338, TKg-40, TKg-41, TKg-43, TKg-27 and TKg-28 were alike. The furthest genetic distance is between TKg-35 and TKg-28 (Figure 3).

The constructed dendrogram based on the RAPDs also clustered Kersting's groundnut genotypes into 2 main clusters at a 5% level of genetic similarity. Cluster 2 was divided into 2 sub clusters A and B at 10 % genetic similarity. Subcluster B at 15% genetic similarity further divided into 3 three clusters B1, B2 and B3. Cluster 1 consisted of only TKg-45 while sub-cluster B1 consisted of TKg-24 and TKg-25. Sub cluster B2 contained TKg-39, TKg-38, TKg-43, TKg-26, TKg-28, TKg-32 and TKg-36. Genotypes TKg-34, TKg-40, TKg-27, TKg-15, TKg-18, TKg-15, TKg-18, TKg-41, TKg-30 and TKg-35 formed B3 cluster. In cluster B1, TKg-33 and TKg-37 were identical while TKg-38, TKg-43, TKg-26, TKg-28, TKg-32 and TKg-36 were similar and different genetically from the genotype TKg-39. In subcluster B3, TKg-27, TKg-15 and TKg-18 were identical in the same way TKg-41, TKg-30 and TKg-35 similar (Figure 4).

Table 5: The differentiation as revealed by SSR, SCoT, and RAPD markers.

	Markers	MAF	AN	GD	PIC
1	SSR6733	0.55	3	0.60	0.53
2	SSR6717	0.95	2	0.10	0.09
3	SSR6188	0.50	3	0.55	0.44
4	SSR7079	0.90	2	0.18	0.16
5	SSR7000	0.80	3	0.34	0.30
6	SCoT3	0.35	8	0.78	0.75
7	SCoT16	0.50	3	0.55	0.44
8	SCoT18	0.60	7	0.61	0.58
9	SCoT28	0.65	3	0.49	0.41
10	SCoT33	0.90	2	0.18	0.16
11	SCoT35	0.25	11	0.88	0.86
12	SCoT36	0.25	8	0.83	0.81
13	OPB1	0.75	2	0.38	0.30
14	OPT19	0.95	2	0.10	0.09
15	OPB5	0.90	3	0.19	0.18
16	OPT4	0.75	2	0.38	0.30
17	OPH7	0.95	2	0.10	0.09
	Mean	**0.68**	**3.88**	**0.40**	**0.38**

MAF: Major Allele Frequency, **AN:** Allele Number, **GD:** Genetic Diversity, **PIC:** Polymorphic Information Contents.

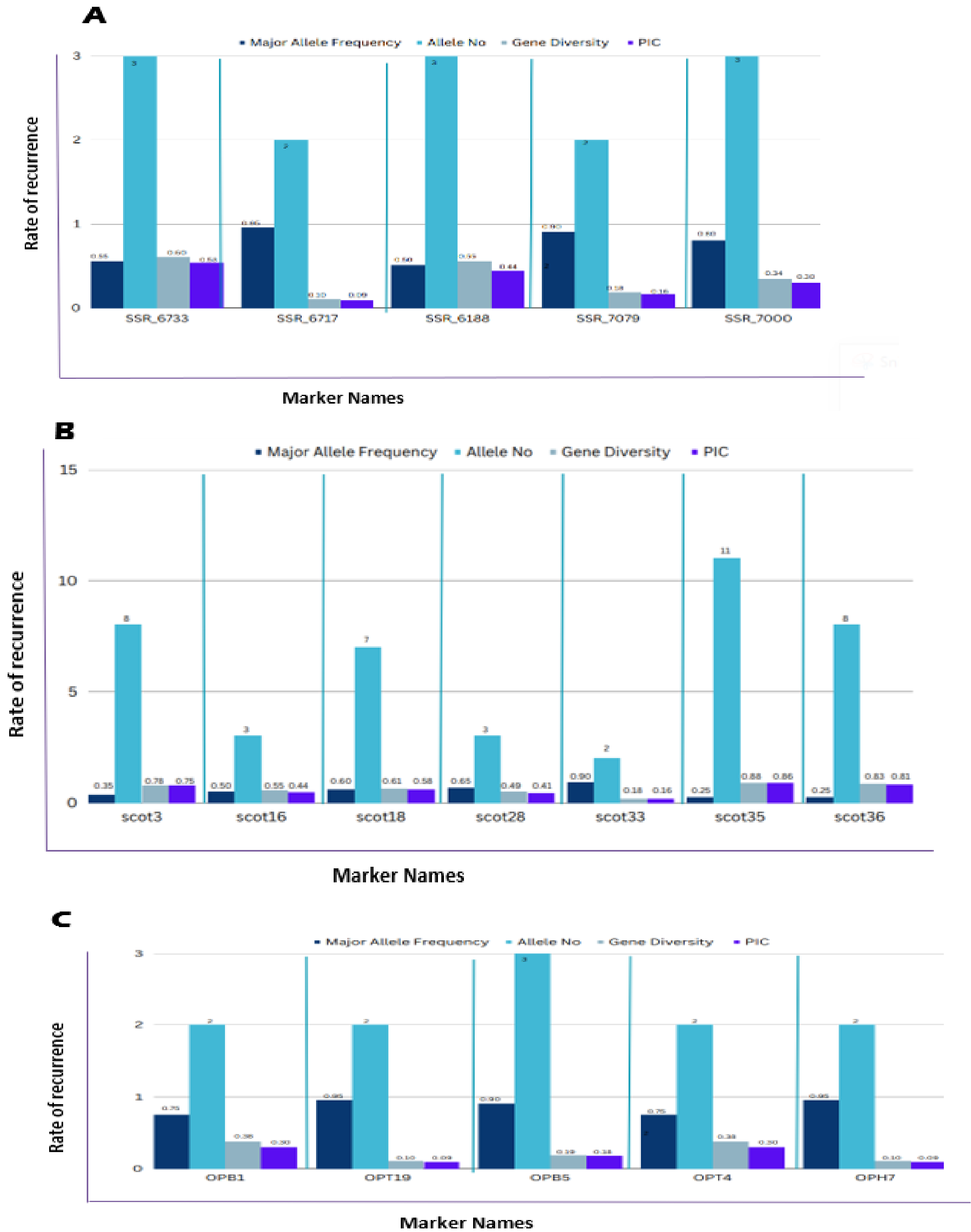

Figure 1: Bar chart depicting the Major Allele Frequency, Allele Number, Genetic Diversity, and Polymorphic Information Contents of the (A) SSR markers (B) SCoT markers (C) RAPD markers

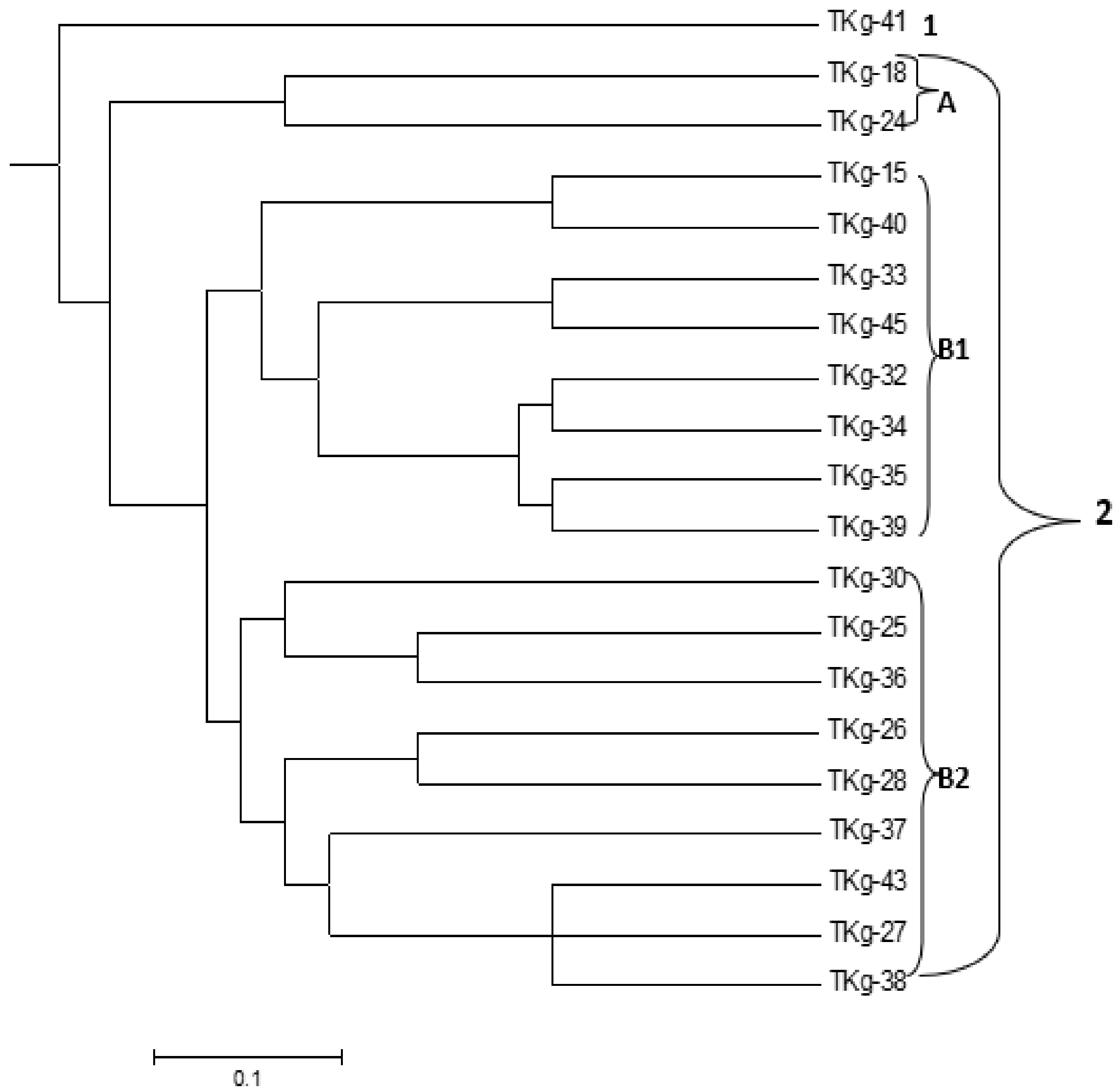

Figure 2: Dendrogram illustrating genetic distance based on the data generated by 5 SSRs among the 20 genotypes of Kersting's groundnut.

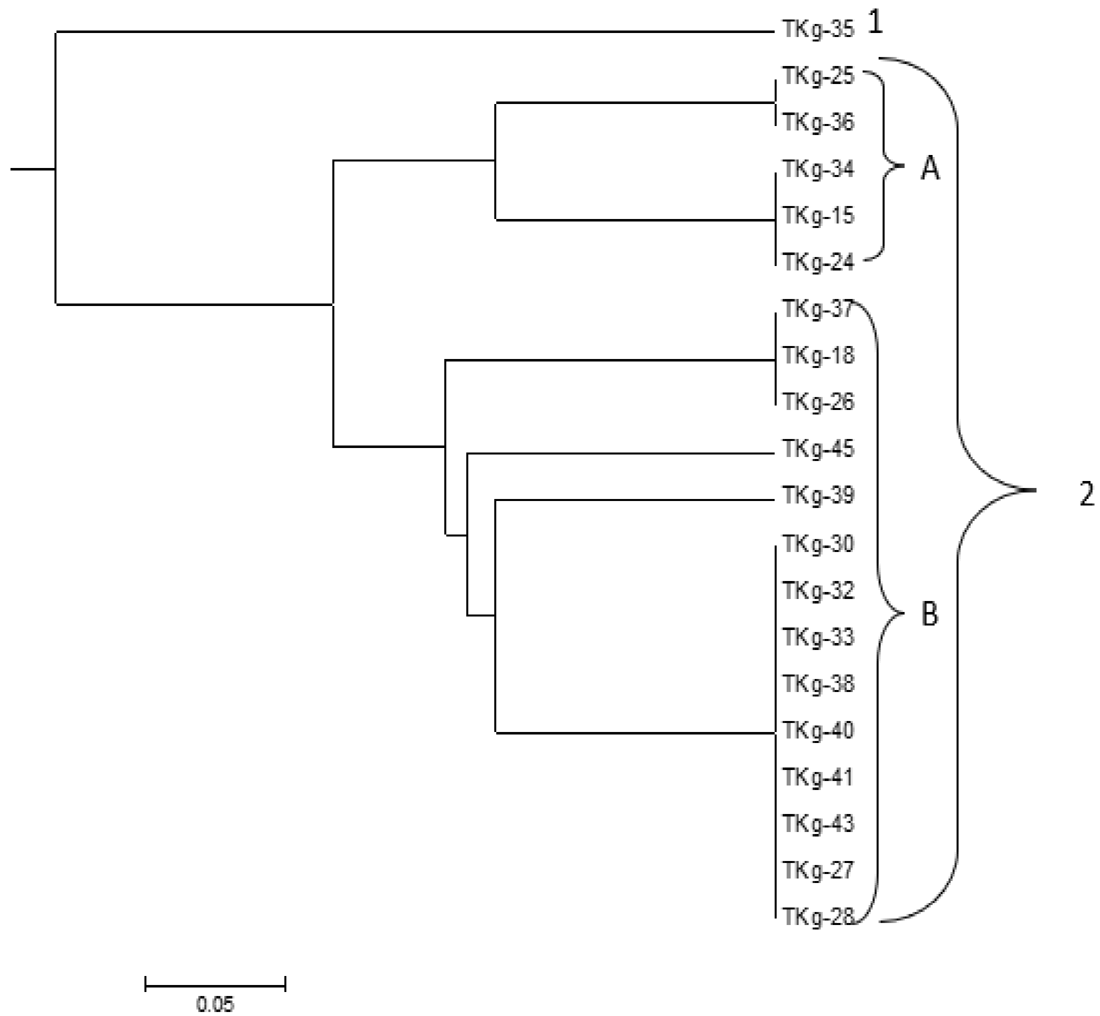

Figure 3: Dendrogram illustrating genetic distance based on the data generated by 7 SCoTs among the 20 genotypes of Kersting's groundnut.

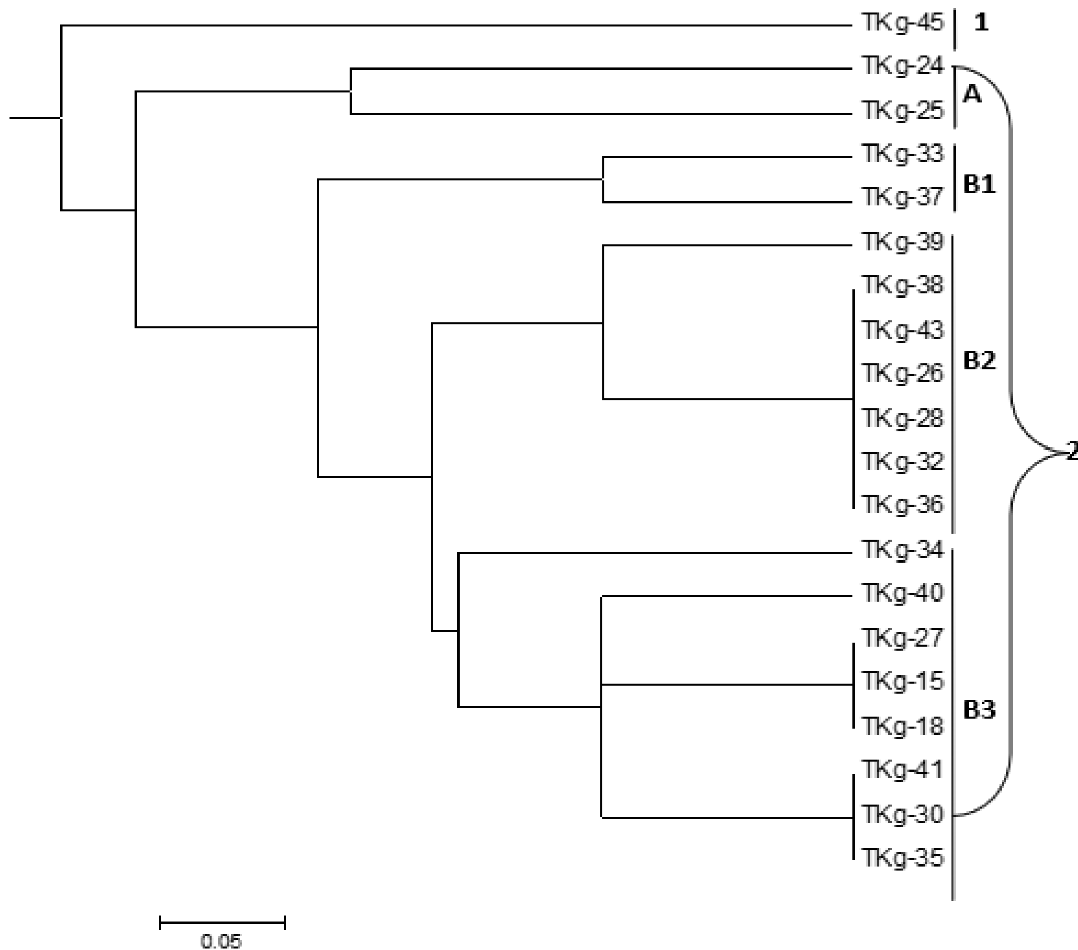

Figure 4: Dendrogram illustrating genetic distance based on the data generated by 5 RAPDs among the 20 genotypes of Kersting's groundnut.

DISCUSSION

DNA profiling or molecular marking is a valuable technique used to examine and understand genetic diversity among plants for specific plant variety identification. This process involves analyzing distinct and unique patterns of DNA sequences, known as markers, that are inherited from parent plants to offspring. A combination of the data of SSR, SCoT and RAPD on the Kersting's ground revealed their polymorphic nature and high efficacy in distinguishing among the 20 genotypes utilized in this investigation. Earlier studies on marker combinations include research on ISSR and RAPD profiles to study phenotypic and genetic diversity in many plant species (Sunar *et al*., 2016). Likewise, the combination of ISSR and RAPD for genetic diversity evaluations of 48 *Prunus mira* L. samples observed high levels of polymorphism (Tian *et al*., 2015). Amel *et al*. (2005) did a comparative analysis of genetic diversity in two Tunisian collections of fig cultivars based on Random Amplified Polymorphic DNA and Inter Simple Sequence Repeats fingerprints and established a considerable genetic variation

among the fig cultivars sampled from two regions.

In a similar context, the efficiency of RAPD and SCoT Markers was observed for the genetic diversity assessment of the common bean which further establishes a higher efficiency of SCoT markers compared to RAPD markers in the detection of the genetic diversity of the beans (Hromadová *et al.,* 2023).

Fewer studies focused on three marker combinations (Thakur *et al*., 2016), and for those studies, the marker combination showed a more distinct resolvable power of the marker's combination compared to single or double marker use. The SSRs, ScoTs and RAPDs analysis in this study also showed potential to distinguish the 20 Kersting's accessions. It is widely recognized that using multiple genetic markers, as opposed to relying on a single marker, can significantly enhance the accuracy and depth of genetic diversity analysis in plants. This is because multiple markers can provide a more comprehensive view of the genetic makeup of a plant, capturing a wider range of genetic information and variations. As a result, the use of multiple markers can lead to more reliable and nuanced insights into the genetic relationships and differences among plant cultivars.

CONCLUSION

Molecular marking has proven to be a valuable technique for examining and understanding genetic diversity among plants, especially for identifying specific plant varieties. The SSR, SCoT, and RAPD markers used in this study revealed high levels of polymorphism in the analyzed accessions of Kersting's groundnut. Among these, SCoT markers were the most informative, showing the highest level of polymorphism compared to the SSR and RAPD markers. Cluster analysis using SSR, SCoT, and RAPD markers effectively differentiated the accessions. SSR markers identified two main clusters further subdivided into subclusters. Similarly, SCoT and RAPD markers each revealed two main clusters, with further subdivisions at 10% and 15% genetic similarity, respectively. The markers effectively established a mode of wide genetic variability among the accessions, providing valuable information for planning successful breeding programs for the germplasm.

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