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Molecular analysis of sesame (*Sesamum indicum* L.) seeds sampled from northern Nigeria

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Abstract

Sesame seed, commonly called benniseed, is among the most valued seeds and seed oil with significant nutritional and medicinal uses to an everyday man. Nigeria is ranked the 3rd largest in (*Sesamum indicum* L.) cultivation. Rated the worlds healthiest food as well as its cash crop values, cultivations by farmers in the region are still mostly based on heterogenous landraces which are less productive but harbours useful genes needed for further improvement. The diversity of 45 landraces sampled from three states out of the six geo-political zones of North Central (Nasarawa), North Eastern (Bauchi), and North Western (Kano states) in Nigeria where sesame is commonly cultivated was assessed using Simple Sequence Repeat (SSR) markers. Phylogenetic relations were determined using UPGMA cluster analysis, multivariate grouping and polymorphic information content (PIC) were calculated using standard procedures. Primers SEM8, SEM9 and SEM10 gave 100% polymorphism. The landraces were grouped into six clusters depicted in the dendrogram and this was at a similarity coefficient of 67%. Var01 (KBi1) collected from Bichi LGA of Kano state was genetically distinct from all others at a dissimilarity coefficient of 0.33. The values in the polymorphic information contents was in the range 0.137 for (SEM9) to 0.712 (SM8). Primer SM8 was most informative with the highest PIC (0.712). This study revealed that the 45 sesame landraces sampled from three northern states in Nigeria constitute six major genetic clusters which grouped all into 25 landraces basically distributed around the northern regions of Nigeria.

Keywords: Genetic diversity, Factorial coordinate analysis, Landraces, microsatellite markers, Polymorphism

Introduction

Sesame is an essential oil producing crop otherwise called the 'Queen of oil' owing to the quality of oil it produces (Adu-Gyamfi *et al.* 2019). Sesame is the most identified member among the family Pedaliaceae (Singh *et al*., 2015) with the cultivated specie (*Sesamum indicum* L) been reported as having 2n =26 chromosome number (Anilakumar *et al*., 2010). Cultivation of sesame are mostly in Africa, particularly the tropical region, Asia, and the southern temperate regions of the world. (Anilakumar *et al*., 2010). Sesame seeds which is commonly used in soups has a high nutritive value with about 18-25% protein and 13.5% carbohydrates, in addition to its higher oil content of 44-58%, in comparison to other oil seed crops (peanut, soybean and rapeseed. Other beneficial minerals in considerable amount include calcium, copper, magnesium, manganese,

phosphorous, thiamin, iron, vitamin B6 and zinc. Sesame is reported to be high in antioxidants (sesamolin, sesamol and sesamin) alongside the fatty acids content with both guaranteeing exceptional attributes of the long shelf life characterised by the oil (Chung *et al*., 2004; Suja *et al*., 2004). The high antioxidants, proffers on it the antibacterial and insect repellent properties while its use for the treatment of tuberculosis had also been reported (Lim *et al*., 2007; Wei *et al*., 2011; Singh *et al*., 2015). The perilla leaves are edible when fresh, the stems could be burnt as fuel when dry and the collected ash can further be used in making local soaps.

Amidst all its industrial attributes, it is still a poorly cultivated crop and often referred to as an orphan crop (Bisht *et al*., 1998). The low yield potential of Nigerian sesame is in its difficulties in establishment and harvest which have discouraged growers in the major sesame cultivating regions. Sesame production in Nigeria lost its ranks as the 3rd largest producer after India and China due to the neglect and inadequate cultivation of the crop (FAOSTAT, 2012). The inadequacy in cultivation is usually brought about by the low yield coupled with problems encountered during its establishment and harvest, which have further discouraged growers in the major sesame cultivating regions (Falusi and Salako, 1998), thus leading to a decline in the total area given to its cultivation. Likewise, landraces of these crop are still commonly cultivated by the farmer with no breeding concerted improvement for higher production. Studies of morphological characterization showed extensive variation exists in sesame (Bedigian *et al.,* 2003). Crop improvement strategy are more informative when done at the molecular level (Singh *et al*., 2015) using the DNA fingerprinting platform which usually provides a gene-gene information on the crop of interest thus providing a better and unique identification of organisms beyond the phenotypic characters.

Molecular approach is extensively used for testing the distinctiveness and unique attribute peculiar to different crops (Singh *et al*., 2015). Amplified Fragment Length Polymorphism (AFLP) (Laurentin and Karlovsky, 2007: Ali et al., 2007), Inter Simple Sequence Repeats (ISSR) (Parsaeian *et al*., 2011 Kumar *et al*., 2012), Sequence-Related Amplified Polymorphisms (SRAP) (Zhang *et al*., 2012), Random Amplified Polymorphic DNA (RAPD) (Abdellatef *et al*., 2008; Pham *et al*., 2011) are some of the molecular markers that had been used for diversity screening of diverse sesame genotypes and most recently, Simple Sequence Repeats (SSR) was also used in screening sesame sampled from Northern Ghana (Adu-Gyamfi *et al.,* 2019). Previous SSR markers screening by Nweke *et al.,* (2015) on 30 Nigeria sesame seeds was in relation to phytochemicals while recent studies by Olorunsola (2019) identified variations in 23 Nigeria sesame landraces using RAPD markers. These previous researches have helped identify molecular approach as a better platform for cultivar identification and giving the unique attributes of SSR markers as the most preferred due to its high reproducibility tendencies, hypervariability, abundance, co-dominance and extensive genome coverage (Uncu *et al*., 2015), it is believed that, diversity study on these 45 sesame landraces using SSR markers will provide additional information on the wider variations that exist among these larger pool of sesame landraces, thereby helping to identify potential landraces that can further be used for breeding purposes.

Materials and Methods

Plant Materials

Seeds from Forty-five (45) Sesame landraces were collected from three (3) major sesame-producing states in Nigeria using the Simple random sampling technique (SRS) (FAO, 1989). The collected samples were from the North Central (Nasarawa), North East (Bauchi), and North West (Kano states) three out of the six (6) geo-political zones of Nigeria. Seeds of each landrace were planted in pots containing sterile soil. Table 1 shows the list of landraces collected in each State and Local Government Area.

DNA Extraction

Two weeks old leaf tissues of the planted seeds were sampled for DNA extraction using the Zymo Research Plant/Seed DNA extraction kit (Inqaba Biotechnology South Africa) following supplier's instructions. Extracted DNAs was quantified and stored at -20ºC in TE buffer. CA, USA).

SSR profile Analysis

Five arbitrary primers (Table 2) synthesized by Inqaba Biotec, South Africa was used for the molecular analysis of the 45 Nigeria sesame seeds collection. The polymerase chain reaction was performed using a thermocylcer (Gene Amp PCR system 9700, Applied Biosystems, USA) in 20µl total volume containing 2.0µl 10X Buffer, 1.2µl of 25mM MgCl2, 1.0 µl dNTP, 2 µl of 50ng/µl. and 0.5 µl each of 25uM forward and reverse primers, 0.25 µl of 5 units taq polymerase (Bioline Massachusetts, USA) and 13.55 µl dH2O. PCR protocol for marker analysis was 3 min initial denaturation at 94ºC, then 35 cycles of 30 sec of denaturing at 94ºC, 45sec at 55-60ºC for annealing, and extension at 72ºC for 10 min and stored at 4ºC. PCR products was run on 2 % agarose gel in 1X TBE buffer and visualized under UV light.

Molecular Data Analysis

Data matrix of SSR profiles obtained for fragments of similar molecular weight from each individual was scored as present (1) or absent (0). The data was subjected to genetic similarity matrix using Jaccards' similarity coefficient (Jaccard, 1908, Ojuederie *et al.*, 2014). DARwin software package Version 5.0.158 (Perrier and Jacquemoud-Collet, 2006), was used for the Multivariate groupings. Cluster analysis was performed to generate a phylogenetic using NTSYS-pc software. Polymorphic information content calculation of Ojuederie *et al*. (2014) was adopted using the formula:

$PIC = 1- \sum p_i^2$

Where;

p_i is the frequency of the i^{th} allele.

Results and Discussion

Molecular characterization of 45 landraces of *Sesamum indicum* with 5 arbitrary SSR markers identified a total of 33 polymorphic bands (Table 3) of polymorphism with only three out of the screened five primers amplified fragments across the 45 landraces studied. Fragments amplified per marker were from 25 (primer SEM10) to 27 (primer SM8). Polymorphic fragments were generated by SSR makers SM8, SEM10, and SEM9 (Figs 1 and 2) while primers SEM18 and SEM44 failed to amplify. Polymorphic information content (PIC) values ranged from 0.137 – 0.712 with SEM 9 giving the least level of polymorphism (0.137) while SM8 gave the highest polymorphism (0.712). The SSR banding profiles of the 45 sesame using primers SM8 and SEM9 are shown in plates 1 and 2. For primer SM8, the band sizes ranged from 200bp to 600bp while for SEM9 it was from 400bp to 500bp.

At a similarity coefficient of 67%, the landraces were grouped into six clusters (Fig 3) with the similarity coefficient been from 0.33 to 1.00. Only var01 (KBi1) was placed in cluster one (1), cluster two (2) had eight landraces that were closely related. These include; var02 (KBi2), var33 (NL3), var03 (KBi3), var05 (KBi5), var04 (KBi4), var15 (KD5), var29 (BGi4) which were from the three different northern Nigeria states. Cluster three (3) had the highest number (27) of landraces grouped together. The landraces were further grouped into 3 sub-clusters inclusive; sub-cluster I, II and III.

Sub-cluster I consisted of 12 landraces; var6 (KBa1), var8 (KBa3), var9 (KBa4), var10 (KBa5), var12 (KD2), var30 (BGi5), var39 (ND4), var7 (KBa2), var11 (KD1), var20 (BGa5), var22 (BN2) and var17 (BGa2). In sub-cluster group I, var8 (KBa3), var9 (KBa4), var10 (KBa5), var12 (KD2), var30 (BGi5), and var39 (ND4), were all closely related, as well as var11 (KD1), var20 (BGa5) and var22 (BN2). Also, in this sub-cluster, var6 (KBa1), var7 (KBa2) and var17 (BGa2) were genetically isolated from every other landrace. Sub-cluster II had the following landraces in the same cluster; var13 (KD3), var18 (BGa3), var19 (BGa4), var21 (BN1), var27 (BGi2), var24 (BN4), and var25 (BN5), as well as var13 (KD3), var 18 (BGa3), var 19 (BGa4), var21 (BN1), var27 (BGi2) var24 (BN 4), and var25 (BN5).are closely related.

The Factorial coordinate analysis (FCO) grouped the 45 landraces of Sesame into 6 groups just as in the dendrogram but with a total of number of 25 landraces with the remaining 20 landraces overlapping within the established clusters (Fig 4). Var01 (KBi1) in group 1 was genetically isolated from every other genotype as confirmed in the phylogenetic tree (Fig 4). Var14, var31, var2 and var33 were all clustered together in group 2 with var2 and var33 overlapping on each other as sister genotypes that might have originated from same source. Group 3 had var32, var26, var17 and var34 all clustered together but at a different gradient. Group 4 had a total of 7 landraces with 2 of the landraces overlapping each other as single or sister genotypes. Group 5 had var44, var28 and var42, genetically isolated from the overlapping landraces as depicted in fig2. Group 6 had the highest number of landraces clustered together with about 3 having a distinct overlap.

In nascent cultivars, as well as their wild relatives, the level of the genetic variation that could be identified among them determines to a large extent possible success that would be achieved in the course of its improvement (Govindaraj *et al*., 2015). The diversity of the 45 *Sesamum indicum* were explained by the total of 33 amplicons which were all polymorphic giving an average percent polymorphism of 100%. This was higher than the 93% polymorphism reported by Laurentin and Karlovsky (2006) using AFLP in thirty-two sesame accessions. Polymorphic information content (PIC) values ranged from 0.137 (SEM9) to 0.712 (SM8) and considering that markers with polymorphic information content greater than 0.5 are considered highly informative (Molosiwa, 2012), then the markers used in this study can be regarded to be significant to discriminate sesame. The average number of alleles (33) as observed in this study compared to 365.14 obtained by Wu *et al*. (2014) was lower but higher than the average of 4 alleles and 6.3 alleles reported by Park *et al*., (2014) and Uncu *et al*. (2015) respectively. Primer SM8 had a higher PIC value than the PIC obtained by Wu *et al*. (2014) which ranged from 0.215 to 0.456 using SSR markers, as well as values reported by Laurentin and Karlovsky (2006) using AFLP markers. The range in values of 0.33 to 0.87 for the Jaccard similarity coefficient between pairs of landraces studied is very similar to 0.38 to 0.85 obtained by Laurentin and Karlovsky (2006) evidencing a higher genetic diversity among these sesame group. Although our molecular analysis method involved only few numbers of SSR markers, yet it was found to be highly effective in discriminating the 45 sesame landraces.

With the high genetical similarity indices identified with Var02 (KBi2): Var33 (NL3), Var03 (KBi3) and Var05 (KBi5), Var15 (KD5) and Var 29 (BGi4), in cluster 2 and the combination of Var11 (KD1), Var 20 (BGa5) and Var 22 (BN2) as well as Var 08 (KBa3), Var 09 (KBa4), Var 10 (KBa5), Var 12 (KD2), Var 30 (BGi5) and Var 39 (ND4) in cluster 3, it therefore shows that farmers constantly exchange seeds among themselves from one LGA to another within or across States while the sub clusters formed is also suggestive to have been based on its close pedigree. Factorial coordinate analysis (FCoA) failed to differentiate the samples according to their area of origin as most of the landraces from other LGAs were compressed from the 45 landraces to 25 to demonstrates the close genetic relationship of the landraces.

Similar to the dendrogram groupings, the FCoA of studied landraces depicted with the principal axes 1 and 2 identified var01 (KBi1) from Bichi LGA of Kano state, to be very distinct from all other studied landraces at a dissimilarity coefficient of 0.33 and this accession was also identified to be specifically distinct in the morphological studies involving plant height and pods production as it was the tallest of all the accessed landraces and also the one with the highest number of pods. Var01 can thus be singled out as a potential accession for subsequent breeding research. Just like the previous molecular report on 68 accessions of Sesame using isozyme Isshiki *et al.* (1997), the genetic study of exotic sesame and Indian germplasm using RAPD, and the high level of differentiation using fourteen ISSR markers Kim *et al.* (2002) this SSRs also helped understand the diversity of 45 accessions sampled in the northern part of Nigeria as factorial analysis also assisted in picking out Var01 (KBi1) as a potentials for further research work, perhaps to domestication stage with promise of higher yields for the future.

Conclusion

The study has shown that the population of Sesame landraces from northern Nigeria, are genetically distinct and SSR markers can be effectively employed to assess genetic diversity. In depth understanding of this distinctiveness among these pool of sesame landraces will determines to a large extent possible success that would be achieved on its improvement.

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Conflict of Interests

The authors declare no conflict of interests

Tables, Figures and Charts

Table 1: List of the sampled Sesame landraces used in this study

S/NO	Landrace given names	State of collection	LGA
1	var01 (KBi1)	Kano State	Bichi
2	var02 (KBi2)	Kano State	Bichi
3	var03 (KBi3)	Kano State	Bichi
4	var04 (KBi4)	Kano State	Bichi
5	var05 (KBi5)	Kano State	Bichi
6	var06 (KBa1)	Kano State	Bagwai
7	var07 (KBa2)	Kano State	Bagwai
8	var08 (KBa3)	Kano State	Bagwai
9	var09 (KBa4)	Kano State	Bagwai
10	var10 (KBa5)	Kano State	Bagwai
11	var11 (KD1)	Kano State	Dawanki
12	var12 (KD2)	Kano State	Dawanki
13	var13 (KD3)	Kano State	Dawanki
14	var14 (KD4)	Kano State	Dawanki
15	var15 (KD5)	Kano State	Dawanki
16	var16(BGa1)	Bauchi State	Ganjuwa
17	var17(BGa2)	Bauchi State	Ganjuwa
18	var18(BGa3)	Bauchi State	Ganjuwa
19	var19 (BGa4)	Bauchi State	Ganjuwa
20	var20 (BGa5)	Bauchi State	Ganjuwa
21	var21 (BN1)	Bauchi State	Ningi
22	var22 (BN2)	Bauchi State	Ningi
23	var23 (BN3)	Bauchi State	Ningi
24	var24 (BN4)	Bauchi State	Ningi
25	var25 (BN5)	Bauchi State	Ningi
26	var26 (BGi1)	Bauchi State	Giyade
27	var27 (BGi2)	Bauchi State	Giyade
28	var28 (BGi3)	Bauchi State	Giyade
29	var29 (Gi4)	Bauchi State	Giyade
30	var30 (BGi5)	Bauchi State	Giyade
31	var31 (NL1)	Nasarawa State	Lafia
32	var32 (NL2)	Nasarawa State	Lafia
33	var33 (NL3)	Nasarawa State	Lafia
34	var34 (NL4)	Nasarawa State	Lafia
35	var35 (NL5)	Nasarawa State	Lafia
36	var36 (ND1)	Nasarawa State	Doma
37	var37 (ND2)	Nasarawa State	Doma
38	var38 (ND3)	Nasarawa State	Doma
39	var39 (ND4)	Nasarawa State	Doma
40	var40 (ND5)	Nasarawa State	Doma
41	var41 (NE1)	Nasarawa State	Eggon
42	var42 (NE2)	Nasarawa State	Eggon
43	var43 (NE3)	Nasarawa State	Eggon
44	var44 (NE4)	Nasarawa State	Eggon
45	var45 (NE5)	Nasarawa State	Eggon

*Landraces names were formed from the location where they were sampled
LGA-Local Government Areas of sampling.

Table 2: SSR markers and sequences for diversity studies on Nigerian Sesame landraces

S/NO	Primer ID	Primer Sequences
1	SM8	CACGCTCGAACTCTCTCCTT (F)
		GACTTGTCCGACCATCCATC (R)
2	SM18	GCTAGCAGAATCACGATTTAATCTC (F)
		TTGGTGTTGGTGTTGCTGTT (R)
3	SEM9	TTCCCGGAACATTCTGATTC (F)
		GCTTACCTCCCCCAAAAGTC (R)
4	SEM10	GGACCATGTAATCCCAGCAC (F)
		GGGGCACAGAGTGGATGTAG (R)
5	SEM440	TTTTCACGCTATCATCAAACC (F)
		CCTCCTCACCCTTGAACTGA (R)

Source: Badri *et al.* (2014) synthesized by Inqaba Biotec South Africa (2016) **www.inquababiotec.co.za**

Table 3: Polymorphism obtained from four SSR primers on 45 Sesame landraces

SSR Primers	Number of amplified bands	Polymorphic bands	% Polymorph	No of landraces identified	Number of alleles	Allele frequency	PIC
SM8	47	47	100	24	47	0.2625	0.712
SEM9	27	27	100	27	27	0.1698	0.137
SEM10	25	25	100	26	25	0.1592	0.360
SM18	-	-	-	-	-	-	-
SEM440	-	-	-	-	-	-	-
Average	33	33	100	25.6	33	0.1972	0.403

PIC-Polymorphic information content

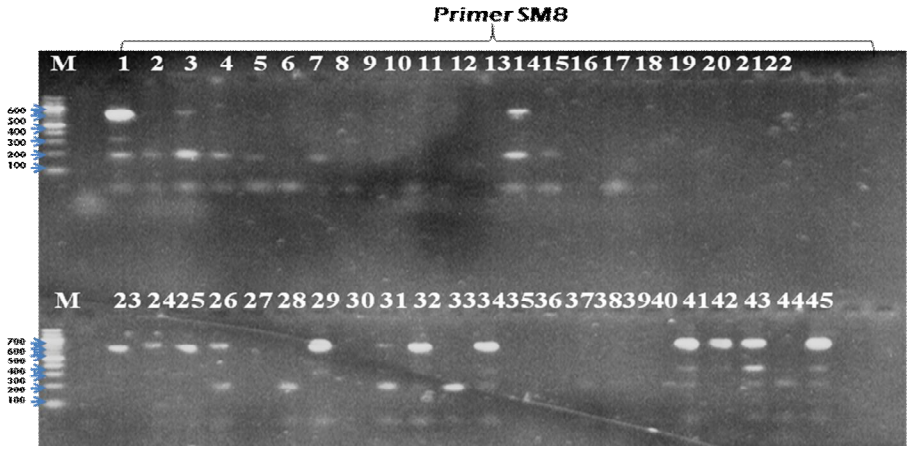

Figure 1: SSR banding profile of 45 Sesame landraces using primer SM8 Lane M-100bp, Lanes1-45 represents SM8 DNA profile from 45 sesame landraces.

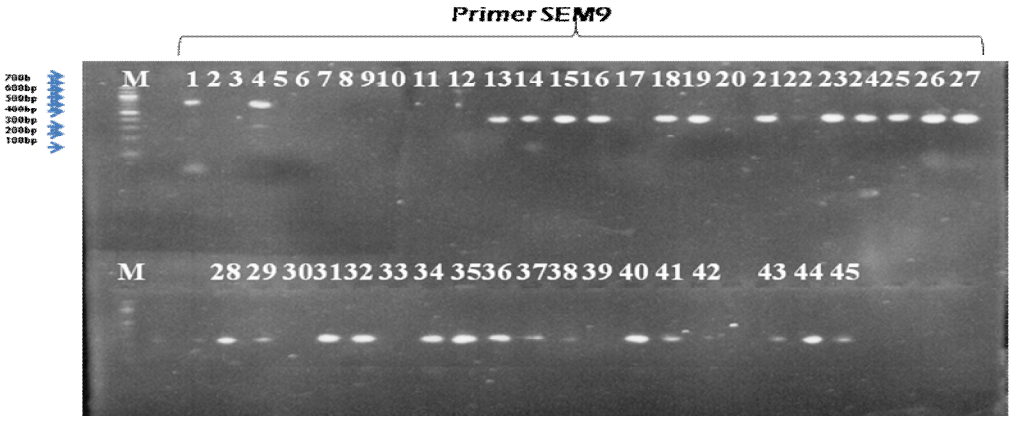

Figure 2: SSR banding profile of 45 Sesame landraces using primer SEM9 Lane M-100bp, Lanes1-45 represents SEM9 DNA profile from 45 sesame landraces.

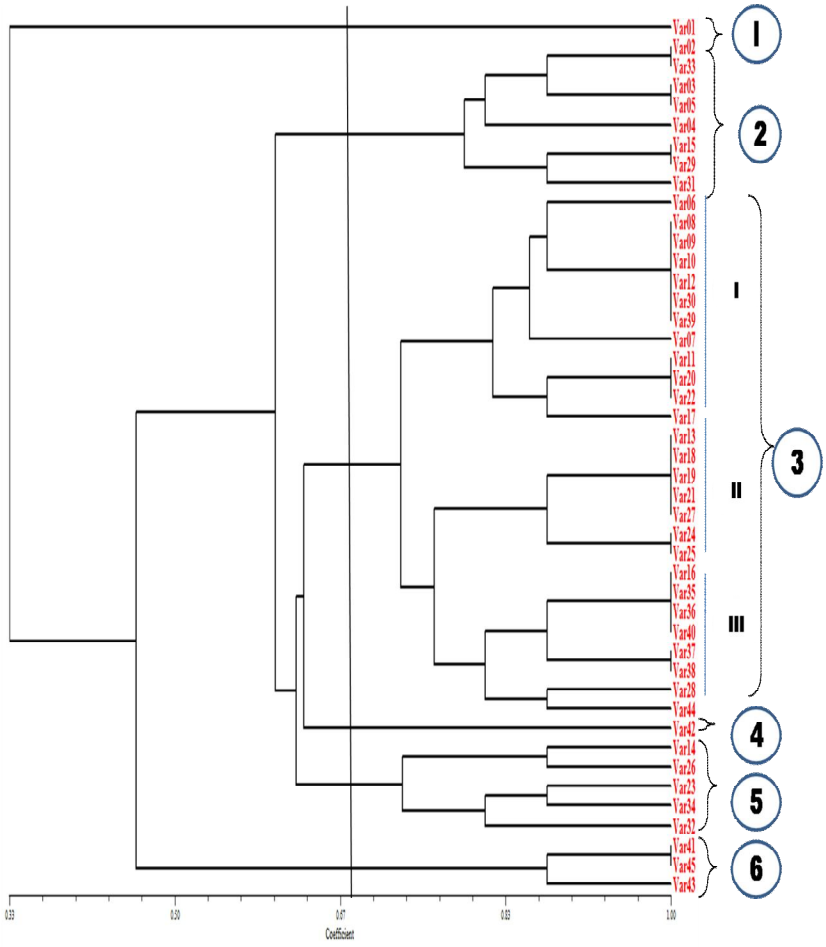

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Figure 3: Dendrogram of 45 Sesamum indicum landraces revealed by UGPMA cluster analysis using jaccards similarity coefficient.

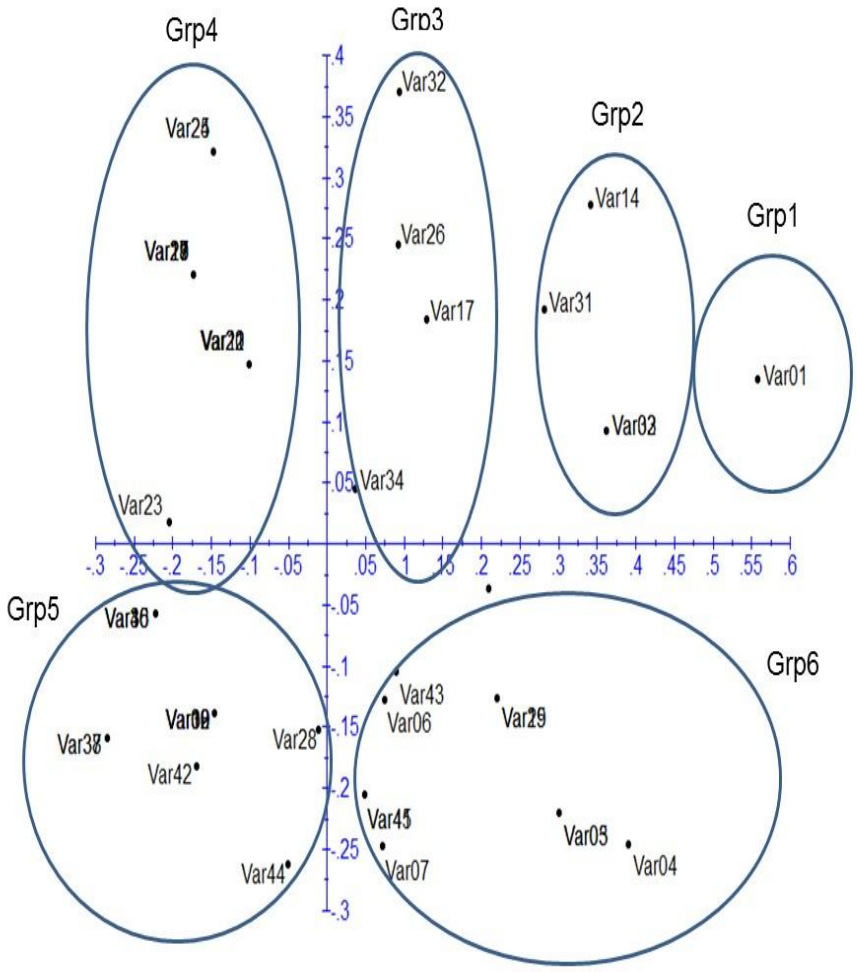

Fig 2: Factorial coordinate analysis of 45 *Sesame indicum* landraces

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