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GENETIC DIVERSITY FOR YIELD COMPONENTS IN SESAME (*Sesamum indicum* L.)

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DADOS CURRICULARES DO AUTOR

Abdullahi Ibrahim Gadanya was born on October 11, 1982 in Gadanya town, Bagwai Local Government Area of Kano State, Nigeria.

I had my Primary Education at Gadanya Central Primary School in 1994 and later attended Government Secondary School Bichi where I obtained Senior Secondary Certificate in 2000. I also proceeded to Federal College of Education (Technical) Bichi, where I obtained Nigeria Certificate in Education (NCE). I also moved to Bayero University Kano where I pursued my Bachelor of Science Degree in Agricultural Education in 2016.

I was admitted to the department of Agronomy (plant breeding and Genetics) for a Master's degree in 2021 and depended dissertation on 28/02/2024. Academic at Faculty of Agriculture and Veterinary Sciences – Unesp, Campus of Jaboticabal, in the Graduate Program on Agronomy (plant breeding and Genetics)

I am currently a lecturer III at Federal College of Education (Technical) Bichi, Kano State, Nigeria.

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Summary

ABSTRACT

The study on genetic diversity for yield components in sesame (*Sesamum indicum* L, Pedaliaceae family) was done at the Jaboticabal campus, in the Screen house and Molecular laboratory of Universidade Estadual Paulista, (Unesp) Brazil. This was aimed to elucidate the diversity among some sesame varieties (*Sesamum indicum* L) in Nigeria using agro-morphological parameters and Inter-simple sequence repeats (ISSR) molecular markers. Eight sesame varieties: NCRIBEN-01M, NCRIBEN-02M, NCRIBEN-04E, NCRIBEN-05E, Black seeded, E8, Gujarat, and Yandev-55 were planted in the screen house. Leaves were collected at 12 days after planting and processed for molecular evaluation. Furthermore, each variety were evaluated morphological agronomic parameters in screen house potted experiment the varieties varied in seed coat colour of four groups: black, brown, light brown, white and ovate leaf lobe in E8 variety. Plant height (cm) ranged from 96.4±1.1 to 144.3±2.0, days for 50% flowering and days to maturity ranged from 55±1.0 to 68±1.0 and 91±1.0 to 130±2.0 days respectively. Yield influencing parameters had ranges of 35±2.0 to 63±1.0 for capsule per plant, 52±2.0 to 76±2.0 for seed per capsule and 2.12±1.56 to 4.43±0.1 for 1000 seed weight (g). Principal components matrix revealed that the PC1, which accounted for the highest variability was mostly related to yield related traits like number of capsules plant', number of primary branches plant^{-1} and 1000 seed weight. PC2 was dominated by growth related traits. Dissimilarity dendrogram of the studied sesame genotypes, established using Agglomerative clustering at 0.821 coefficient, revealed three clusters. These results showed variability in yield traits of these sesame varieties studied. Eleven ISSR primers were used for analysis eight different sesame -populations from Nigeria. The primers showed legible and reproducible genomic profiles. The 11 primers amplified 472 bands of which 228 (48.3 %) were polymorphic. PIC (Polymorphic Information Content) PIC in this study with 11 sesame genotypes varied from 0.330 to 0.50 an indication of the informativeness of ISSR markers as well as their relevance in discriminating the populations studied. There is therefore diversity among the sesame genotypes studied. Further studies on genetic diversity of Nigerian sesame accessions using ISSR markers using greater numbers of markers and genotypes will further unravel their degree of diversity is recommended

Keywords: Genetic characterization, molecular markers, Sesame varieties, Polymorphism.

Introduction

Sesame (*Sesamum indicum* L. Pedaliaceae) is one of the prehistoric oilseed crops, dating back to 3050- 3500 BC and has been cultivated from ancient times (Bedigian, 2003). It is one of the first oilseed crops used by humans, occupying the ninth position among the most cultivated in the world (Bezerra *et al*., 2010; Perin *et al*., 2010; Mesquita *et al*., 2013; Pinto *et al*., 2014; Silva *et al*., 2014). Originating from East Africa and in India (Nayar and Mehra, 1970; Bedigian, 2003) sesame thrives well on moderate temperatures (25 and 30 ºC), lowland (below 500 m) and low rainfall (500 to 650 mm) (Arriel *et al*., 2009). It is extensively cultivated worldwide and naturalized in dry habitats of tropical and subtropical regions of the old world (Bedigian, 2003). The crop is highly drought tolerant, grows well in most kind of soils, regions and is well suited to different crop rotations. Subsistence farmers grow sesame using low inputs and in low and less water (Cagirgan, 2006). Major world leading producers of sesame are Sudan, Myanmar, India, Tanzania, Nigeria, China, Ethiopia and Burkina Faso (FAO, 2021). Asian and African countries cultivate about 90 percent of the world sesame production area.

Sesame is one of the most important oil seed crops in the world widely used in baked goods, confectionery products (because it is stable against oxidative changes than other vegetable oils), pharmaceutical industries (Falusi and Salako, 2001) and alternative medicine (Mushtaq *et al*., 2020). Oil extract is used the industry to produce soap, perfume, carbon paper and pharmaceutical products (Adu-Gyamfi *et al*., 2019). Its edible nutritious seeds and leaves are used for making vegetable soup and oil cake for animal feed (Kafiriti and Deckers, 2001; Alam *et al*., 2007).

Despite its much priced position in food and oil production with great export potential, sesame yield has been low. In Nigeria, for instance 788.5 kg average yield in 2020 is far below its potential of 1,800 kg/ha. The fact that average yield per hectare in Nigeria from 2011 to 2020 was above the mean for Africa and world (FAO, 2021) lays credence to poor sesame yield the world over.

The most important challenging traits of sesame the world over include lack of widely adapted varieties, poor stand establishment, susceptibility to diseases and pests, poor responses to fertilizer, lack of profuse branching, non-synchronous

maturity, shattering of capsules at maturity and low harvest index (Wijnands *et al*., 2007; Mahajan *et al*., 2007). One other reason for low seed yield and reduced acreage under cultivation is the persistent use of traditional practices and lack of improved varieties for use by the farmers. Even when modern varieties are present, their **c**ultivation is reported to be limited due to insufficient genetic information on them (Alege *et al*., 2021).

Limited genetic variability in sesame germplasm is a result of long history of domestication, continuous cultivation on rain fed marginal lands, hence survival via natural selection (Tripathy *et al*., 2019). Genetic drift and selection by nature have reduced diversity. Therefore, possible using broad-based genetic resources of wild types, land races and improved varieties offers better opportunity for sesame genetic improvement. This is because broad based genetic resources provide important genepool of untapped genetic resource for abiotic and biotic stress resistance and yield increase as well as maintaining and enhancing resilience of production systems (Tripathy *et al*., 2019).

Picture of flowering sesame plants

Crop genetic diversity can be determined with the aid of morphological, biochemical and molecular markers (Stuber, 1992) or in combination. Morphological and molecular markers help scientists and plant breeders in screening and selection (Pham *et al*., 2011) of germplasm and their use in breeding program. However, for the sesame plant and especially in Nigeria, not much progress has been made on, markers assisted selection (Bhat *et al*. 1999). Several studies have been reported on the Nigerian sesame agronomic and yield evaluations (Babajide, and Oyeleke, 2014; Ijoyah *et al*., 2014; Paul, 2020; Olonuriha, *et al*., 2021), Agro-morphological characteristics and variability studies (Ismail and Usman, 2014; Falusi, *et al*., 2015; Nweke *et al*., 2019). Nweke (2015) characterized the variability in phytochemical composition among the sesame plant genetic resources in Nigeria.

Selection of plant varieties based on these traits alone has proved to be ineffective due to problems of low heritability, strong influence of the environment, and genetic complexity. Fortunately, advances in molecular technologies have enabled proper identification, selection, and use of germplasm in crop improvement programmed (Spandona *et al*., 2012). DNA markers in particular are useful and reliable to assist phylogenetic relationships, parental line selection, and population structure and allele distribution among germplasm lines and to design breeding strategies while remaining stable under different environmental conditions (Ferdinandez *et al*. 2001).

Moreover, the advent of next-generation sequencing (NGS) and genotyping by sequencing (GBS) technologies has provided a means for examining genetic diversity and population structure of crop-plants, which can facilitate the genetic dissection of agronomic traits and integrate them in breeding programs. Genome-wide association studies (GWAS) are a promising approach that connects phenotypic variation and genetic markers to detect genomic regions underlying complex traits (Tibbs *et al*., 2021). GWAS were applied successfully for various crop plants, such as maize (Li *et al*., 2012), rice (Zhao *et al*., 2011), and soybean (Sonah *et al*., 2015) and Sesame (Wei *et al*., 2015; Asekova *et al*., 2021). The relatively small genome size (~ 357 Mbps), sesame qualifies as an ideal crop for genetic diversity studies. This is more so for a crop with wide genetic diversity (Dossa *et al*., 2017, Wang *et al*., 2016)

Despite high economic with and high nutritional value, sesame is still lagging behind in the achievement of breeding perspective, only few reports are available on the use of molecular markers on Nigerian sesame. According to Animasaun *et al*. (2022) despite the fact that Nigeria accounts for a significant quantity of sesame production in Africa, there is insufficient information on the diversity of sesame accessions in cultivation, hence only a little progress is made on sesame breeding and development of elite cultivars in the country. Even when modern varieties are present, their **c**ultivation is reported to be limited due to insufficient genetic information on them (Alege *et al*., 2021). Furthermore, there is no report of use of ISSR in the study of genetic diversity for Nigerian sesame.

This study aims to investigate genetic diversity of eight Nigerian sesame genotypes based on selected morpho-agronomic qualitative and quantitative traits as well as ISSR molecular markers. This study tends to bridge this gap while studying sesame gene pool from Nigeria and provide bases for selected genotypes to be used in sesame improvement in Nigeria and Africa at large,

Material and Method

Experimental site

The experiment was done at the Jaboticabal campus at Universidade Estadual Paulista (Unesp), Brazil, in the Department of Biology.

Experimental materials

The eight genotypes used for this study are" NCRIBEN-01M, NCRIBEN-02M, NCRIBEM-04E, 05E, BLACK, E-8, GUJARAT and YANDEV-55 The eight sesame varieties were collected from National Cereals Research Institute (NCRI) Badeggi, Nigeria. The NCRI has the national mandate for collection and improvement of sesame among several other crops in Nigeria. These genotypes were to further research on the diversity of these Nigerian sesame.

Figure 1: Pictures showing the eight varieties used in this study

Table 1: Description of eight sesame varieties used in the study culled from NAERLS Bulletin

S/No.	Variety	Days to maturity	Seed colour	Seed size (mm)	Oil content (%)	Potential yield (Kg/ha)
1	NCRIBEN-01M	102-115	White	3.0	45	1000
2	NCRIBEN-02M	102-115	Light brown	3.0	45	750
3	NCRIBEN-04E	122	White	2.99	45	1300
4	NCRIBEN-05E	102	Light brown	3.6	50	1200
5	BLACK	90-120	Black	3.3	45	250
6	E8	90	Light brown	3.6	50	1000
7	GUJARAT	86	White	3.4	49	770
8	YANDEV-55	125	Light brown	2.5	45	600

Experimental design

The experiment was laid in a randomized complete block design (RCBD) with three replicates using 22 cm height pots laid out 1m between pot bases. There were 12 pots each within a block which gave the total number of 96 pots for the study, with spacing of 15x75 cm was adopted for the research, weeding was done manually at 2 and 6 weeks after planting to ensure weed free from pots. All data were collected within the total of 5 plants were tagged for data collection within each plot. These parameters were based on The International Plant Genetic Resources Institute (IPGRI) descriptors for sesame (IBPGR and NBPGR, 2004). The parameters include: plant height taken with the aid of measuring tape from the base of the plant to the tip from 5 plants that was tagged day of flower, days of 50 % flowering, days of maturity, yield and yield related characters such as number of pod, number of seed per pod, number of capsule per leaves axis, pod weight and 1000 seed weight were recorded

Planting for sample collection

Seeds of each variety of sesame used in the study were planted in well labelled separate seed trays. These were placed in a screen house and watered when required. Leaves of the varieties were picked and placed in separately labelled containers. These leaves were used for DNA extraction.

DNA extraction

The method employed for DNA extraction, as adapted from Lohdi *et al*. (1994), prescribes that for a 200-mL stock solution in a sterilized beaker, the following were added: 150 mL of distilled water. 16.4 g NaCl (decant the genetic material) 2.44 g of TRIS (hydroxymethyl) aminomethane 1.48 g EDTA (disodium salt) 4.0 g CTAB (cetyltrimethyl ammonium bromide, a detergent that breaks the cell wall);

These were added one by one in a beaker using an electromagnetic stirrer. The pH of the solution maintained between 7.0 and 8.0. When necessary, pH was correct by slowly adding 40% HCl until it reaches the ideal ph. Plastic or glass stick (never metal) was used to stir to avoid lumps. Distilled water was used as a top-up to complete 200 mL.

Working solution

Prepared in autoclaved recipients, for each sample of plant tissue, you have: 1 mL stock buffer solution; 2 L of mercaptoethanol (an antioxidant that is crucial to avoid oxidation of the DNA); 0.009 g of PVP (polyvinylpyrrolidone) (another antioxidant); Tip**:** Always extrapolate 1 unit more, e.g., if you are extracting from 4 samples, you calculate the amounts of reagents 5 times (5x 1 mL stock buffer solution + 5x 2 L B-mercapto + 5x 0,009 g PVP).

DNA extraction process

Isopropanol 100% and ethanol 70% were stored in the freezer before starting this process (to be ice cold), and the incubator (heater) was turned on. For each sample, the following were prepared in a microtube tray: 2 microtube of 2 mL, 1 mortar, and 1 pistil; Plant leaf samples were crushed using liquid nitrogen, pouring cautiously, macerating slowly, and cantering until it become a powder. Then, 1 mL of the working solution was added and macerated until it homogenizes, but quickly and accurately to avoid DNA oxidation. The liquid was then transferred to a well-labelled microtube for identification of the sample. Microtube was kept in the incubator set at 60°C for 25 minutes, this was manually inverted every 5 minutes. The samples were allowed to cool to room temperature, using the chloroform isoamyl alcohol (24:1) out of the fridge to bring them to room temperature, 1mL of chloroform isoamyl alcohol was added or topped up to the 2.0mL mark (it’s important that they are all even in liquid quantity) and gently inverted for 5 minutes with the hands; The refrigeration switch of the centrifuge was turned on before using it, and then the microtube arranged opposite each other, generating a balance in the centrifuge rotor. The centrifuge was then programmed at 10,000 rpm for 15 min at 24°C. The aqueous phase (supernatant) is transferred to another microtube, half volume of 5M NaCl is added, and the mixture is completed with ice-cold 100% isopropanol up to the 2.0mL mark. - The aqueous phase (supernatant) is transferred to another micro tube, half a volume of 5M NaCl is added and completed with ice-cold 100% isopropanol up to the 2.0 mL mark.

DNA quantification using Nano Drop

The solution was incubated in the super freezer (-80°C) for 5 min while the centrifuge was turned on at 4°C for 5 minutes. Centrifuged at 6.000 rpm for 5 min at 4°C and then at 10.000 rpm for 10 min at 4°C; the supernatant is discarded. The small, thin gel at the bottom of the microtube is the pellet (DNA). The pellet was washed with 500 l of ice-cold 70% ethanol and centrifuged at 10,000 rpm for 5 min at 4°C. The supernatant is again discarded, leaving the pellet at the bottom to dry completely for 1-3h upside down on a paper towel. The pellet was the dissolved in 25mL of autoclaved distilled water (which is best for PCR) and mixed delicately by tapping the microtube with a finger; The pellet is then allowed to rest overnight before measuring the DNA concentration in the Nano drop.

A spectrophotometer is capable of measuring the amount of DNA accurately. The computer is turned on, and the Nano Drop program is opened in nucleic acid mode. Two 2μL of autoclaved distilled water is placed in the equipment, and ‘blank’ is clicked for it to capture what water is. This is cleaned with tissue paper, the 1mL of the sample (pellet) is placed in the place of the distilled water, and ‘measure’ is clicked on. Results of DNA concentration was then recorded (for PCR, the ideal is 10 ng/L), absorbance reason 260nm/280nm (evaluates the purity of the DNA, which is ideal 1. 7 to1.8 by the presence of proteins, contaminants, or pH change), and absorbance reason 260nm/230nm (second measure of purity for other contaminants, which is ideal 2.0 to 2.2. After measuring each sample, the equipment is cleaned with paper tissue before applying another sample. After measuring all samples, it is blanked again with water, cleaned, and turned off.

ISSR markers and their polymerase chain reaction (PCR)

Sixteen ISSR markers were optimized for the PCR (Table 2). Eleven ISSR markers produced distinct polymorphic bands. However, the primers 817, 818, 819 and 820 did not produce polymorphic bands.

PCR amplification was done in 20μL reaction mixture containing 0.2μmoL ISSR primers, 0.2μL of each dNTPs, 2 mmolL−1 MgCl2, 10 X PCR buffer and 0.5 unit Taq polymerase and 50 ng sample DNA. The program for ISSR and was carried out in a

DNA thermocycler (model-Pro MJ RESEARCH, INC.PTC - 100) United State of America. The program was set up as follows: denaturation at 94°C for 3min; 94°C for 1min; 47°C for 2min; 72°C for 2min; 30 cycles at 72°C for 7min; 4°C ∞ depending on apropos annealing temperature of the primer, and finally extension at 72°C for 7min. Amplified PCR products were separated on a 1.4% agarose gel stained with ethidium bromide. A 50 base pair ladder marker (GeneRuler 50 bp DNA Ladder, Thermo Scientific, USA) was used to estimate PCR fragment size. 16 ISSR primers (UBC-University of British Columbia (Table 2) were tested and selected based on the best amplification pattern and loci isolation quality, and the x insert in the column of the table shows that the primers are not amplifying.

Table 2: Sixteen (16) ISSR primers used in this study

Primer code	Sequence (5'-3')	AT (°c)
811	5'-GAG AGA GAG AGA GAG AC -3'	47
812	5' GAG AGA GAG AGA GAG AA -3'	47
813	5'-CTC TCT CTC TCT CTC TT -3'	x
814	5' CTC TCT CTC TCT CTC TA -3'	47
815	5' CTC TCT CTC TCT CTC TG -3'	47
816	5' CAC ACA CAC ACA CAC AT -3'	47
817	5'- CAC ACA CAC ACA CAC AA -3'	x
818	5'- CAC ACA CAC ACA CAC AG -3'	x
819	5'- GTG TGT GTG TGT GTG TA -3'	x
820	5'- GTG TGT GTG TGT GTG TC -3'	x
834	5' AGA GAG AGA GAG AGA AYT -3'	47
835	5' AGA GAG AGA GAG AGA GYC -3'	47
836	5' AGA GAG AGA GAG AGA GYA -3'	47
840	5' GAG AGA GAG AGA GAG AYT -3'	47
844	5' CTC TCT CTC TCT CTC TRC -3'	47
847	5' CAC ACA CAC ACA CAC ARC -3'	47

Note: AT= annealing temp.,

Data analysis

. All morpho-agronomic data collected were subjected to analysis of variance (ANOVA), while least significant difference (LSD) at 5% level of probability was used in separating the means. Cluster analysis, Multivariate principal component analysis (PCA) and Pearsons's correlation coefficient were done using the software Past 4.03 (Hammer *et al*., 2001)

The amplified bands in each of the 8 genotypes were scored manually for their presence (1) or absence (0) for each primer. The binary data matrix was prepared for each primer separately and merged as combined data for overall analysis. For each primer, the number of total bands (TB), polymorphic bands (PB) and polymorphism percentage (P %) and polymorphic information content (PIC) were calculated. Genetic associations among individual genotypes were determined using the dissimilarity matrix and a Neighbour-Joining (NJ) tree using Phylogenic Analysis Using Parismony (PAUP 4a169) (Swofford, 2003).

Allele-frequency based analyses of genetic diversity and structure were performed using AFLPsurv version 1.0. (Vekemans, 2002) which is based on the methods described by Lynch and Milligan (1994). Allelic frequencies at ISSR loci were estimated from the binary presence/ absence matrix, under the assumption of Hardy–Weinberg equilibrium, from the observed frequencies of fragments using the Bayesian approach proposed by Zhivotovsky (1999). A non-uniform prior distribution of allelic frequencies was assumed with its parameters derived from the observed distribution of fragment frequencies among loci. Nei's (1973) gene diversity (also known as expected heterozygosity) as well as global and pairwise genetic differentiation (FST) values were computed. Significance of the genetic differentiation between groups was tested by comparison of the observed FST with a distribution of FST under a hypothesis of no genetic structure, obtained by means of 1,000 random permutations of individuals among groups.

RESULTS AND DISCUSSIONS

The performance of eight varieties of sesame on vegetative part was described in Table 3 and morphologically, the sesame varieties were observed to have some similarities. These include, all had whitish pink flowers and sparse flower petal hairiness. They were all semi erect in branching habit, with medium stem hairiness. Their leaves arrangements were opposite and they all bear elongated capsules which shatter partially along the suture lines. However, seven of the eight varieties have laceolate leaf lobes, E8 have ovate leaf lobes. The other morphological dissimilarity of these varieties is the seed coat colour. Four seed coat colours shown where White for 01M, light brown for 02M, E8, and YANDEV-55, brown for 04E, 05E and Gujarat and black seed coat colour for the black sesame variety (Figure 2).

There were significant differences observed among the varieties for all the quantitative traits studies (Table 4, Figure 2, 3). Significant differences ($P < 0.01$) in plant habit, seed colour, capsule characteristics and other yield parameters support the findings of strong variability in these traits among sesame genotypes (Bedigian *et al*., 2010; Tabatabaei *et al*., 2011; Tanwar and Bisen, 2017). On plant height significance difference was observed among the variety that was used, with varieties Black having taller plants followed by E8 which could be attributed to soil condition, environmental condition and probably genetic makeup of such varieties. This finding is in conformity with the findings of Anon (2004) and Mulkey *et al.* (2017). The plant heights in these genotypes were similar to the range of 104-161 cm reported by Pham *et al*. (2010) in south-East Asia but higher than the reports of Animasaun *et al*. (2022) who worked

Table 3: Morphological features of eight varieties of sesame means were calculated form five representative plants of each

Qualitative traits	01m	02m	04E	05E	Black	E8	Gujarat	Yandev-55
Flower petal colour	whitish pink flower	whitish pink flower	whitish pink flower	whitish pink flower	whitish pink flower	whitish pink flower	whitish pink flower	whitish pink flower
Flower petal hairiness	Sparse	Sparse	Sparse	Sparse	Sparse	Sparse	Sparse	Sparse
Branching habit	Stem erect	Stem erect	Stem erect	Stem erect	Stem erect	Stem erect	Stem erect	Stem erect
Stem hairiness	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Leaf arrangement	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite
Leaf lobes	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Ovate	Lanceolate	Lanceolate
Capsule hairiness	Hairy plants	Hairy plants	Hairy plants	Hairy plants	Hairy plants	Hairy plants	Hairy plants	Hairy plants
Capsule shape	Elongated	Elongated	Elongated	Elongated	Elongated	Elongated	Elongated	Elongated
Capsule dehiscence	Partially shattering	Partially shattering	Partially shattering	Partially shattering	Partially shattering	Partially shattering	Partially shattering	Partially shattering
Seed coat colour	White	Light Brown	Brown	Brown	Black	Light Brown	Brown	Light Brown

With similar materials in Nigeria. The differences may be due to the use of pots of 30 x 25cm size by the authors which may not have been enough soil for the full expression of the growth potential of the crop. On number of branches significant difference existed between the varieties used with Black having higher number of branches followed by 04E which is not far from the fact that environmental condition, genetic make-up and agronomic practice might have caused the difference, this work is supported by the findings of Magashi and Yusuf (2013) reported that vegetative growth leading to establishments of branches are influence by environmental condition/factors and mostly by crop genetic make-up. Significant difference was observed on number of branches among the varieties where Black had higher number of leaves followed E8, this could be influenced by genetic make-up of the plant which plays a very vital role in intercepting solar radiation for photosynthetic activities as reported by Day *et al*. (2002). Georgiev (2000) also lend support to the above accretion in his work on problems and prospect of selection of sesame. Says that environmental factors, agronomic practice and genetic make-up plays an important role in plant floral part there by affecting the crop yield. The black seeded variety produced significantly more branches (10) bearing a greater number of capsules per plant (63) and seeds per capsule (76). These are result of greater Photosynthate partitioning into forming seeds and will contribute to the yield of this crop. The variety 02M flowered (58 days) and matured late (116 days) while the Black variety flowering at 51 days and maturing at 102 days was the earlier completing of life cycle. This phonological parameters of days to maturity (CV = 2.51) and days to flowering (CV = 2, 69) are the least quantitative variable traits (Table 4). The capsule per leaf axil, capsule internode and 1000 seed weight with CV values of 21.85%, 21.77% and 21.59% respectively, had the most variability's (Table 4).

Table 4: Agronomic quantitative traits of eight varieties of sesame means ± sd, least significant difference, standard error and Cumulative variance calculated from five samples representative plants of each genotype

Varieties	01M	02M	04E	05E	Black	E8	Gujarat	Yandev-5	Mean	LSD (P < 0.05)	SE±	CV
Stem girth (cm)	3.41±0.99	3.27±0.50	1.19±0.14	2.70±0.74	3.34±0.13	2.09±0.2	3.20±0.45	2.25±0.26	2.7	0.657	0.32	19.0
Primary branches	5.0±0.71	5.2±0.69	9.0±1.00	7.0±1.41	10.0±1.46	7.2±1.30	2.0±0.42	7.0±1.22	6.6	1.401	0.69	16.6
Leaf size (cm)	5.476±0.38	4.18±0.66	8.55±0.40	8.14±0.68	7.49±0.43	9.50±0.74	4.61±0.31	5.60±0.32	6.7	0.665	0.33	7.7
Plant height (cm)	100.0±10.78	98.7±1,06	97.6±2.52	134.0±6.52	144.3±6.73	96.4±1.87	101.0±3.61	110.0±4.12	110.3	7.127	3.5	5.0
Days to flower	52.6±0.51	58,0±0.45	53.2±0.58	56.8±0.86	44.0±0.55	51.0±0.71	57.0±0.71	48.6±0.60	52.7	1.822	0.89	2.7
Days to maturity	105.2±2.28	116.0±2.00	106.4±2.61	113.6±3.85	88.4±1.14	102.0±3.16	114.0±3.16	97.0±2.00	105.3	3.411	1.67	2.5
Capsule/plant	39.0±2.92	60.0±4.47	48.0±4.86	51.0±2.85	63.0±2.24	62.8±1.35	35.0±3,81	58.0±2.49	52.1	4.267	2.09	6.4
Capsule internodes	3.0±0.71	3.0±0.71	2.9±0.10	2.01±0.01	3.0±0.00	2.58±0.99	2.02±0.71	2.50±0.35	2.6	0.737	0.36	21.9
Seeds/capsule	62.0±1.58	67.0±2.00	58.0±1.58	60.0±2.00	76.0±3.81	58.2±2.56	54.0±1.00	52.0±1.86	60.9	2.827	1.39	3.6
1000 seed weight (g)	3.08±0.68	3.67±0.20	2.98±0.71	2.71±0.93	4.30±0.50	4.42±0.43	2.15±1.11	3.01±0.71	3.3	0.915	0.45	21.6
Capsule width (cm)	0.67±0.01	0.60±0.02	0.79±0.07	0.68±0.00	0.78±0.01	0.68±0.04	0.38±0.02	0.23±0.01	0.6	0.039	0.02	5.1
Locules/capsule	4±0.71	3.0±0.71	4.0±0.00	2.0±0.00	2.6±0.55	2.6±0.55	4.0±1.00	4.0±0.00	3.3	0.734	0.36	17.4
Capsule/leaf axil	2±0.00	2.0±0.00	2.2±0.45	2.4±0.89	4.0±0.71	2.0±0.14	2.0±0.21	2.0±0.71	2.3	0.654	0.32	21.8

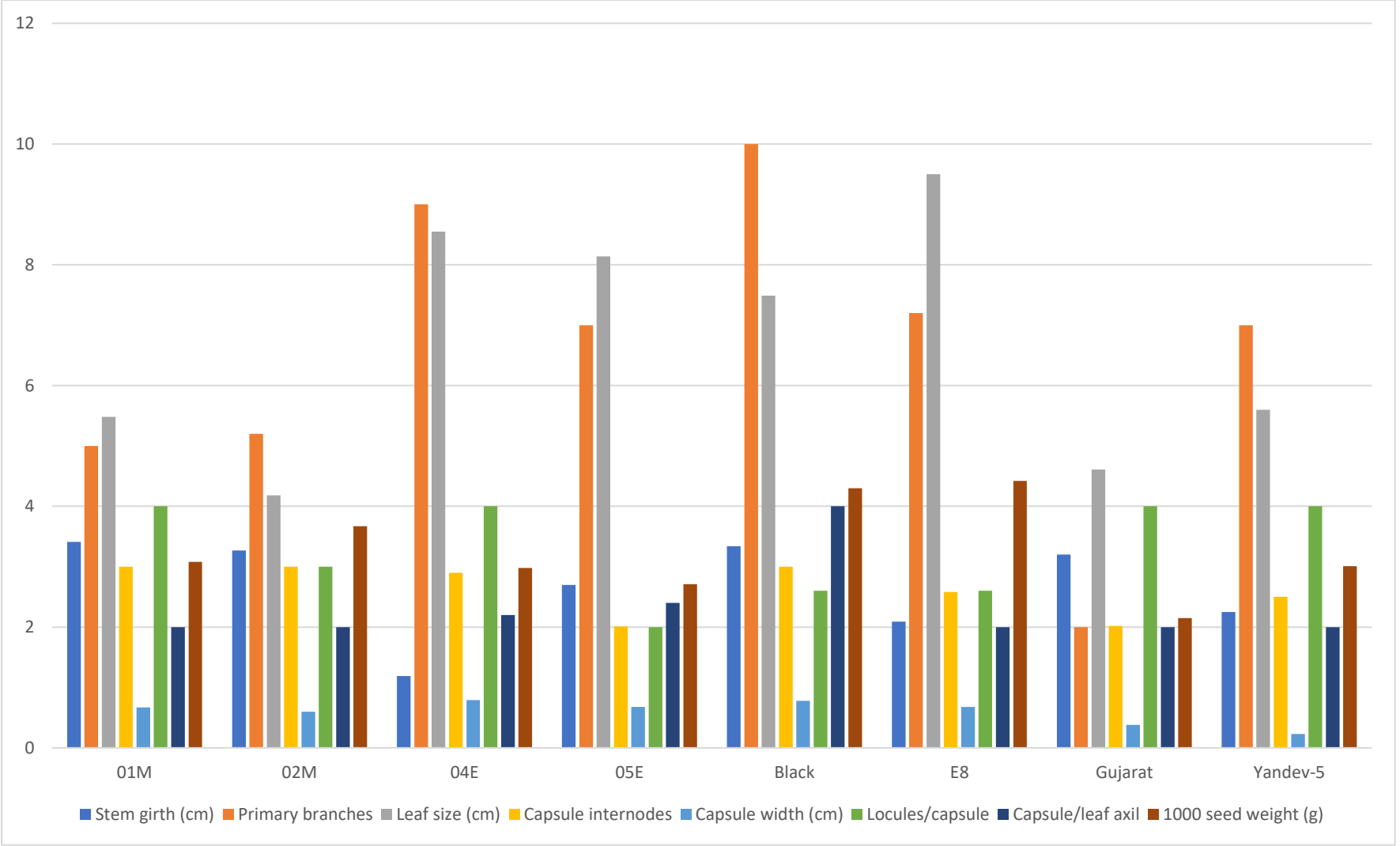

Figure 2. Some Agronomic traits of eight varieties of sesame means calculated from five samples representative plants of each genotype

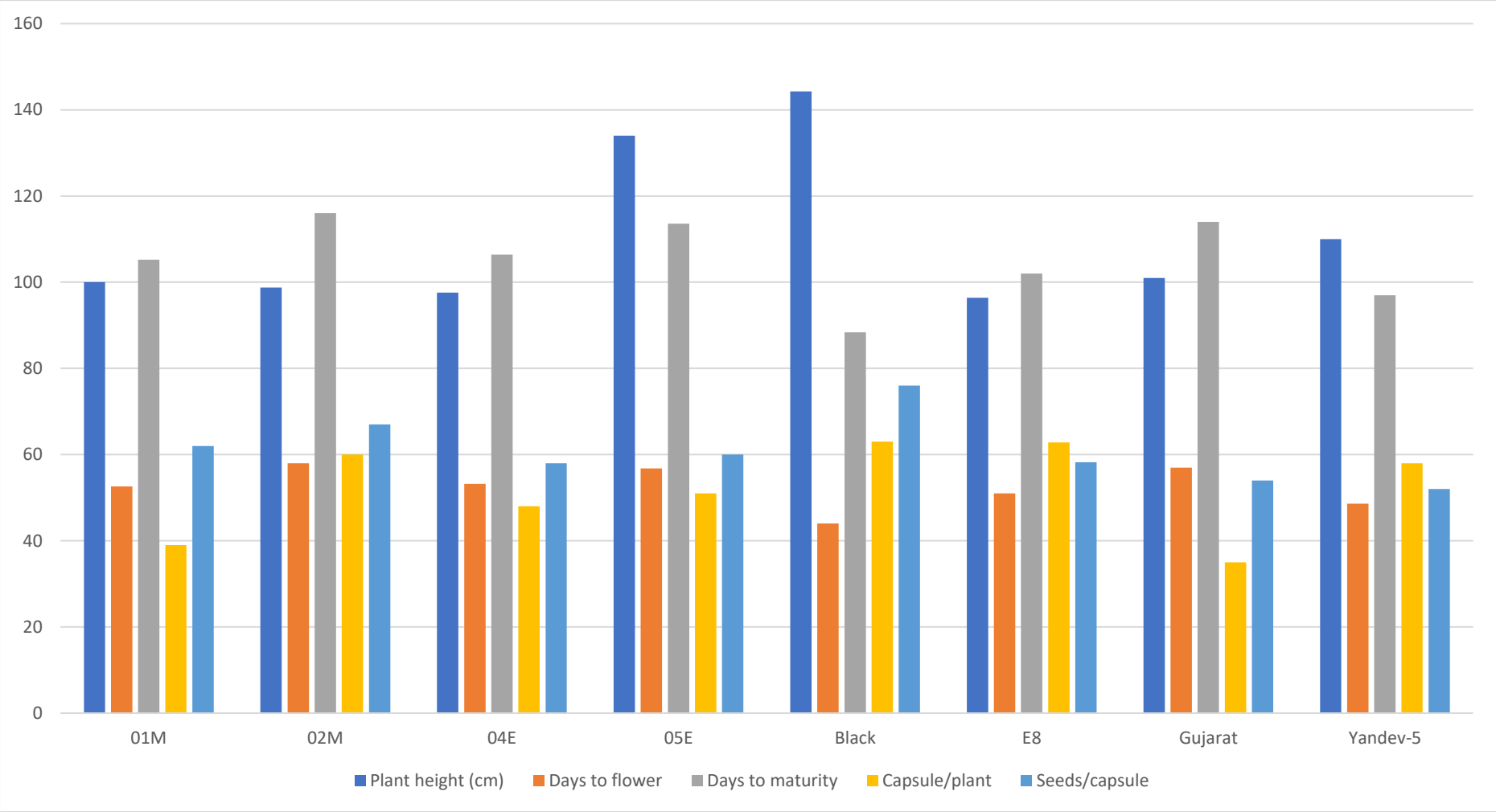

Figure 3. Plant height, physiological, capsules per plant and seeds per capsule traits of eight varieties of sesame means calculated from five samples representative plants of each genotype

Variations revealed by the quantitative traits of the genotypes studied, showed existing genetic diversity among the studied genotypes (Figures 2 and 3). This observed diversity could provide useful on identification and selection of superior traits useful in sesame crop improvement. Significant dissimilarities in growth parameters such as plant height, number of branches, stem girth and yield traits have been previously reported by Pham *et al*. (2010); Animasaum *et al*. (2017) are crucial for assessment of genetic divergence in sesame (Adu-Gyamfi *et al*., 2019; Animasaum *et al*., 2022). The early maturing Black and Yendev-55 varieties could be potential female parents for breeding early maturing sesame varieties. Besides, the black seeded sesame variety significantly better performance in yield attributes of capsule/plant, capsule internodes, seeds/capsule and 1000 seed weight make it an even better parent material for sesame improvement programs. Parameshwarappa *et al*. (2010) and Animasaum *et al*. (2022) have in their separate reports correlated better yield in sesame to earliness in maturity. Though, such correlation was not shown to be significant in this study.

Correlation analysis (Figure 4) showed positive and significant correlation days to flowering and days to maturity. Capsule per leaf axil was correlated with plant height and seeds per capsule. While capsule per plant had a positive association with 1000 seed weight. This is interesting giving that both are components of yield as plants with a greater number of capsules tend to have higher seed weights. Traits correlated together are said to be linked, and simultaneous improvement feasible (Azeez *et al*., 2017; Olorunmaya *et al*., 2019). This is why the concept of correlation is very important.

Multivariate principal component analysis (PCA) helps the plant breeder to select promising varieties through the estimation of individual traits contributions to the plant features (Iqbal *et al*., 2018). The PCA results show that 76.47 % of diversity among the genotypes studied was accounted for by the first three components (PC 1 – PC 3) (Table 5). The PC 1 accounted for 45.79% with major contributors as number of primary branches, capsule leaf axil, 1000 seed weight and seeds per capsule. The Black seed, 02M, E8 and 04E genotypes are major contributors to this component (Figure 6) with significantly higher 1000 seeds weight and number of seeds per capsule as important traits distinguishing these genotypes. Tanwar and Bisen (2017) while studying the variability among 96 sesame germplasm revealed that the PC 1

accounting for the highest variability related mostly to yield contributing traits. Stem girth and leaf size which is mostly growth traits are major contributors of the 16.85 % of diversity accounted for in PC 2 with the leaf size having the highest negative loading. While PC 3 accounting for 13.82% of the diversity had days to flowering, capsule interlocus and locules per capsule as major contributors.

Dissimilarity dendrogram of the studied sesame genotypes, established using Agglomerative clustering at Euclidean distance of 37 revealed three groups (Figure 8): two genotypes, E8 and Gujarat formed Group A. The second group, B has four members: Yandev-55, 04E, 01m and 02m. This group further has two sub-groups. Genotypes 05E and black occupy group C. These genotypes were characterized by high numbers of seeds per capsule and capsule per leaf axil.

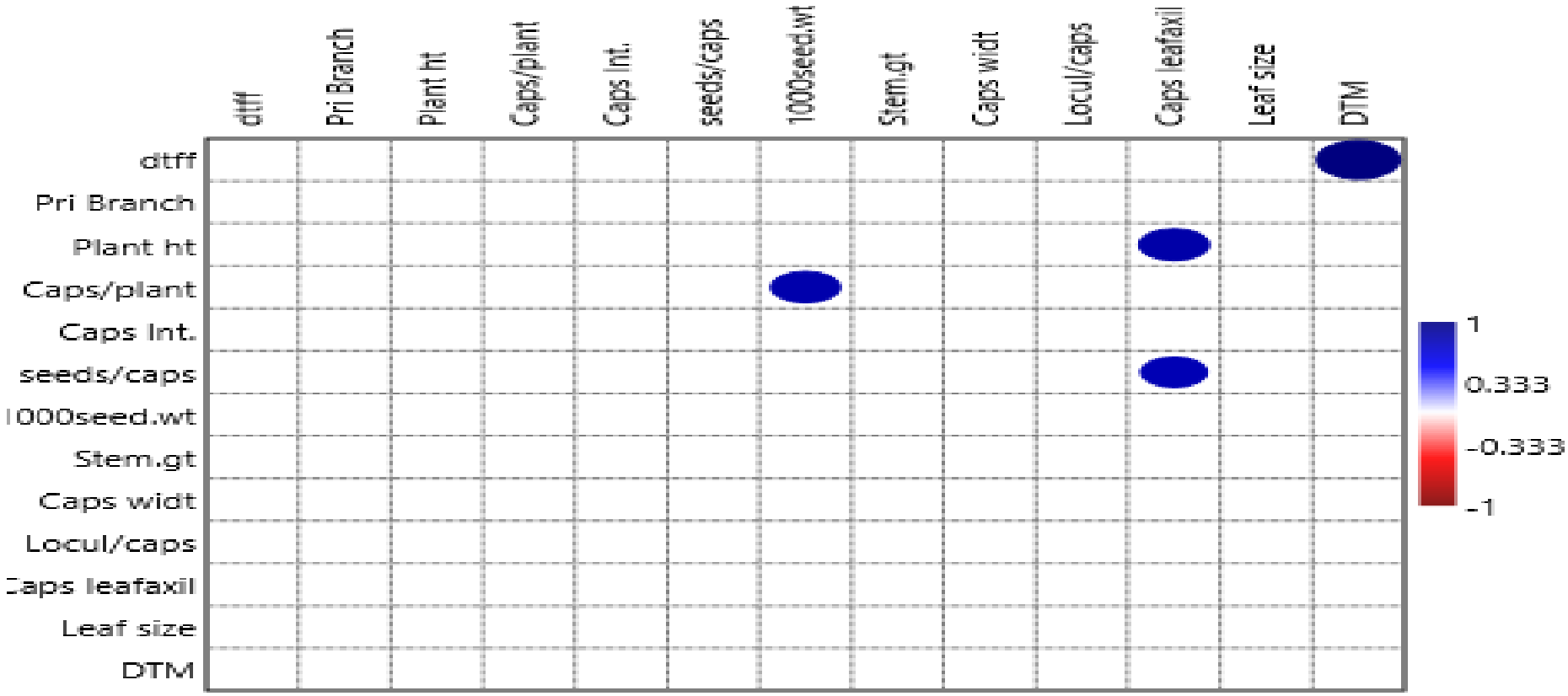

Figure 4. Plot of Pearson's 13 agronomic traits using correlation coefficients among eight sesame genotypes computed using Past 4.03

Table 5. Eigenvalues, cumulative variances and factor loadings with major morpho-agronomic traits for seven principal components derived from the PCA.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Eigenvalue	5.953	2.191	1.797	1.547	1.020	0.393	0.098
Variance	45.79	16.85	13.82	11.90	7.85	3.026	0.758
Cumulative variance (%)	45.79	62.64	76.47	88.37	96.22	99.24	100.00
				Loadings			
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Days to flower	-0.31018	0.077514	0.36974	0.30149	-0.02312	0.25853	0.099821
Primary Branches	0.35601	-0.25733	0.015456	-0.05051	0.13749	0.41961	-0.27612
Plant height	0.2584	0.28462	0.22579	-0.42117	0.14085	0.26942	-0.32254
Capsule/plant	0.2999	-0.10812	0.028464	0.041272	-0.61835	0.33221	0.11717
Capsule lnt.	0.20531	0.004692	-0.41804	0.50519	0.10086	0.22265	-0.32251
seeds/capsule	0.30813	0.37152	0.009304	0.26661	0.10741	0.11494	0.23352
1000 seed wt	0.33193	-0.05171	-0.07699	0.27066	-0.3959	-0.36174	0.17101
Stem girth	-0.01021	0.64918	-0.06641	0.028628	-0.10168	-0.33975	-0.33353
Capsule width	0.24525	-0.03772	0.26262	0.43463	0.45457	-0.17126	-0.0537
Locules/capsule	-0.22661	-0.13187	-0.55438	-0.00815	0.30592	0.091683	0.2239
Caps leaf axil	0.33974	0.2512	-0.00114	-0.20549	0.25156	0.082243	0.65406
Leaf size	0.22704	-0.42898	0.3319	-0.0546	0.16048	-0.39358	-0.04436
Days to maturity	-0.30739	0.083575	0.37365	0.30483	-0.01768	0.25626	0.11928

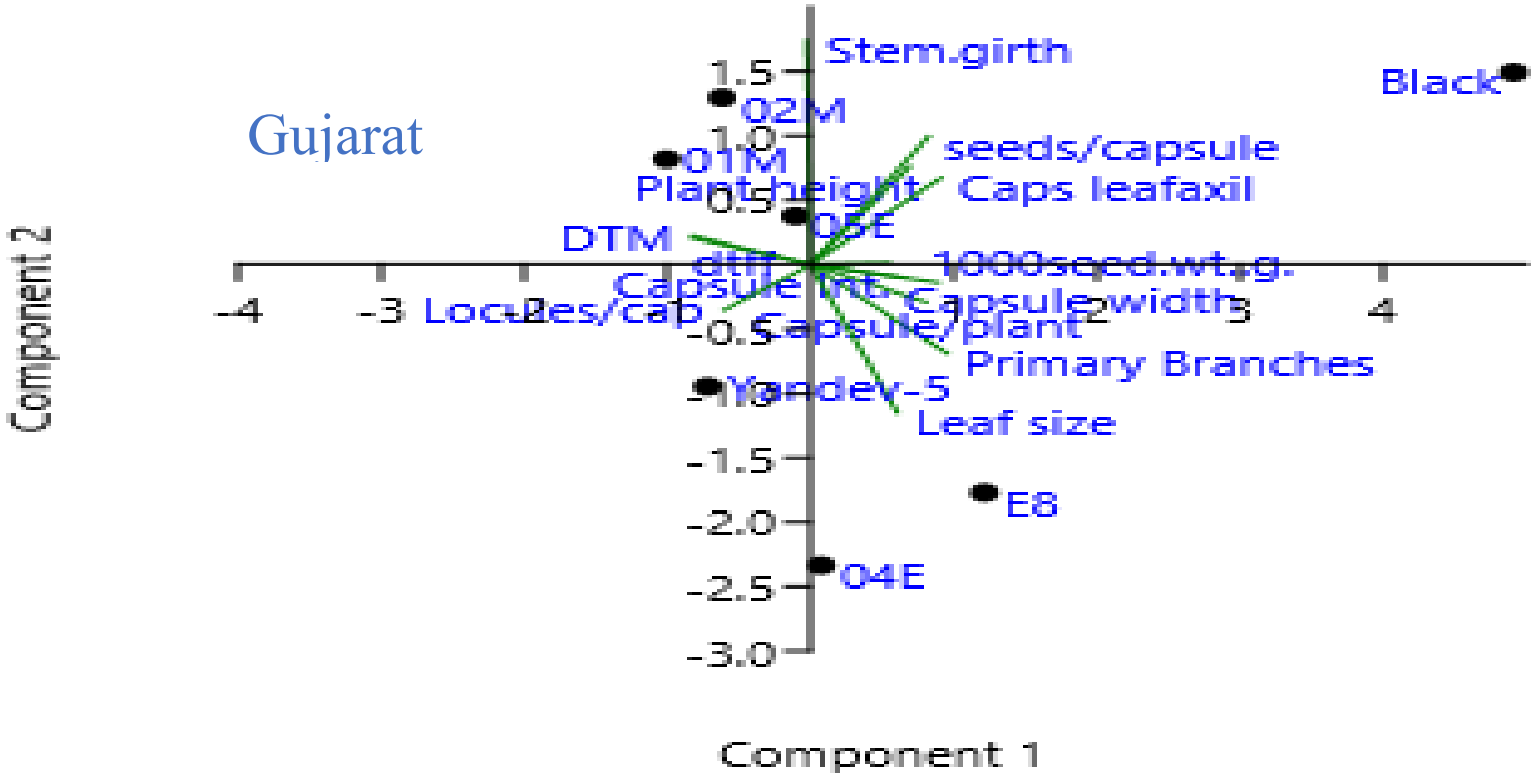

Figure **5**. Biplot showing similarity and dissimilarities of traits studied and corresponding parameters in PC1 and PC 2

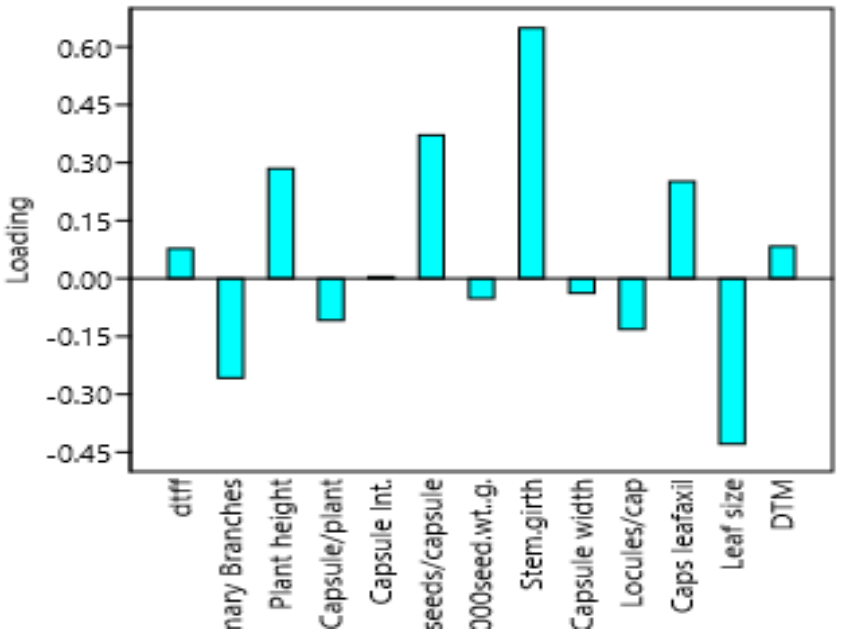

Figure 6. Traits principal coordinate 2 loading for 13 traits studied

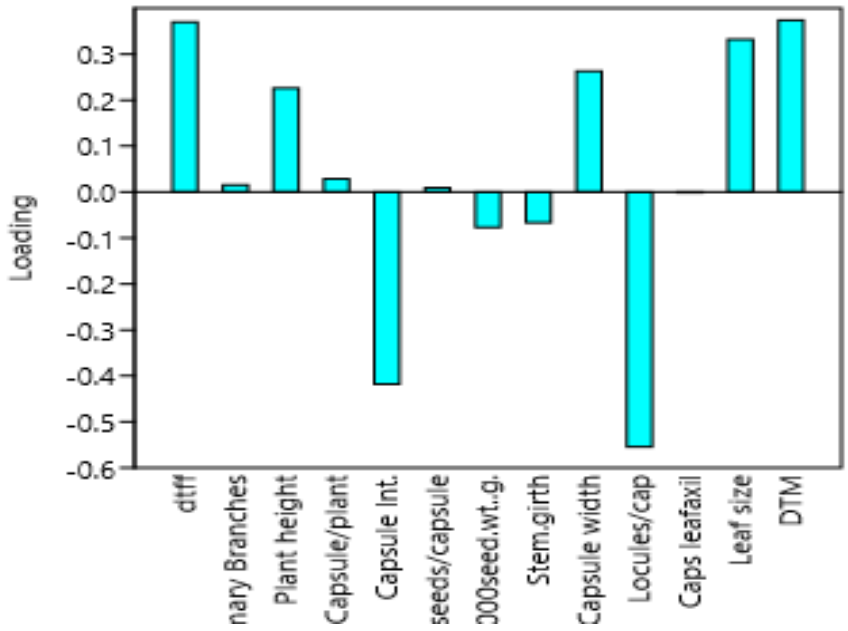

Figure 7. Principal coordinate 3 loading for 13 traits studied

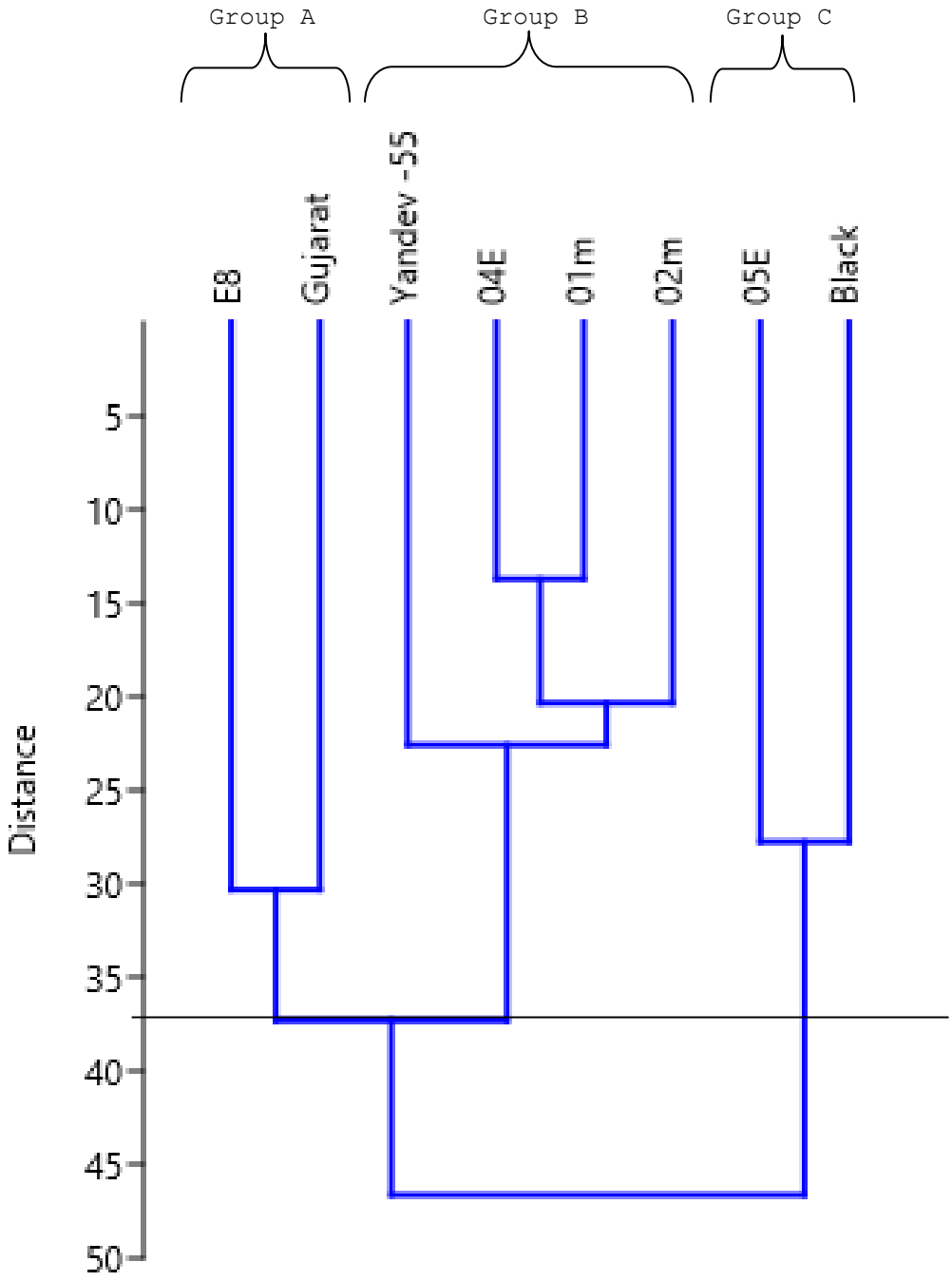

Figure 8. Dissimilarity dendrogram of 8 sesame genotypes, established from agromorphological traits using Agglomerative clustering method (UPGMA)

Molecular Analysis

Among the 16 ISSR primers tested, 11 (73 %) were used because of their clear and reproducible profile (Table 6). Other four primers (27 %) generated non readable and unclear amplification. The 11 primers amplified 472 bands of which 228 (48.3 %) were polymorphic. The polymorphism percentage ranged from 29.2 % in Primer 811 to 62.5 % in Primer 836. Polymorphic information content (PIC) value is the discriminatory power of primers used as a relative measure of polymorphism. The informativeness of a genetic marker in a study such as this is determined using the PIC values. PIC values in plants often range from zero to 0.5 for monomorphic ISSR-markers and polymorphic ISSR-markers present in 50 % of the plants and absent in the other 50 % (Roldan-Ruiz *et al*., 2010; El Harfi *et al*., 2021). PIC in this study with 11 sesame genotypes varied from 0.330 to 0.50 an indication of the informativeness of ISSR markers as well as their relevance in discriminating the populations studied. The variable and significant PIC values obtained by the primers used in this study (Table 6) confirmed the genetic variability of these 11 primers and reveals the difference in the degree of their ability to differentiate the genotypes. A primer is judged to be more polymorphic as its PIC value tends towards one and less polymorphic if the reverse is the case. The lowest PIC (0.33) observed in Primer 844 indicates less ability of this primer to detect diversity among the tested genotypes while the highest PIC value of 0.5 recorded for Primers 814 and 835 confirms their better ability to distinguish between genotypes. A representation of ISSR electrophoresis is presented in Figure 11.

Though, the PIC values observed in this study are lower than values reported by Anitha *et al*. (2010) in 10 Indian sesame populations (0.496 to 0.854 and Singh *et al*. (2015) in 44 Indian sesame (0.675); they are higher than PIC ranges of 0.002 to 0.35 reported by El Harfi *et al*. (2021) in 31 Moroccan sesame. The differences may be as a result of the different populations used in conjunction with the nature and number of molecular makers. The average PIC of 0.47 obtained in this study is slightly higher than 0.43 PIC value reported by Abate *et al*. (2015) in his study of Moroccan sesame using 7 ISSR markers and indication of high informativeness of the primer.

Table 6: Eleven (11) ISSR primers, sequences, total bands, polymorphic bands and polymorphic information content observed in 8 sesame genotypes used in this study

Primer code	Sequence (5'-3')	AT(°c)	TB	PB	%P	PIC
811	5'-GAG AGA GAG AGA GAG AC -3'	47	72	21	29.2	0.4132
812	5' GAG AGA GAG AGA GAG AA -3'	47	80	43	53.8	0.4972
814	5' CTC TCT CTC TCT CTC TA -3'	47	48	24	50.0	0.50
815	5' CTC TCT CTC TCT CTC TG -3'	47	24	13	54.2	0.4965
816	5' CAC ACA CAC ACA CAC AT -3'	47	40	21	52.5	0.4988
834	5' AGA GAG AGA GAG AGA AYT -3'	47	48	28	58.3	0.4861
835	5' AGA GAG AGA GAG AGA GYC -3'	47	24	12	50.0	0.50
836	5' AGA GAG AGA GAG AGA GYA -3'	47	56	35	62.5	0.4688
840	5' GAG AGA GAG AGA GAG AYT -3'	47	16	9	56.3	0.4922
844	5' CTC TCT CTC TCT CTC TRC -3'	47	24	5	20.8	0.3299
847	5' CAC ACA CAC ACA CAC ARC -3'	47	40	17	42.5	0.4888
Mean			42.91	20.73	48.3	0.47

Note: AT= annealing temp., TB= total bands, PB= polymorphic bands, %P= percent polymorphism

b

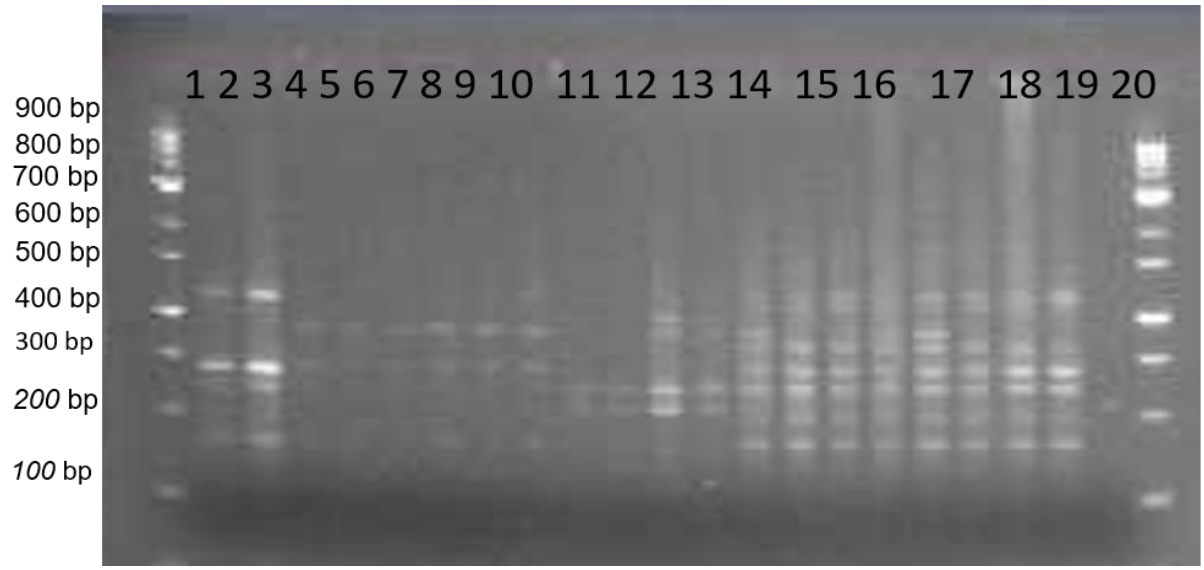

Figure 11: Electrophoresis gel of the ISSR primer 813 on 01M 02M 04E 05E

The results of the dendrogram of the studied sesame genotypes, established using Agglomerative clustering based on molecular evaluation reveals that at 0.04 genetic distance, the genotypes are discriminated (Figure 10). At the highest distance, only two groups are identified. The first group with a singular member (01M) showing the highest distance from the other group comprising all other genotypes. The similarities of diversity can be explained further along the tree; with the highest similarity occurring at the tip end. At a second distance, four groups are distinguished group I comprises the singular 01M genotype; group II has 02M genotype while the group III comprises other genotypes (E8, Black sesame and Gujarat) while the group IV has 04E and 05E genotypes. It is noteworthy to observe that all the genotypes were distinct in the genetically based on this assay. This result was similar to that from the dendrogram of the agro-morphological evaluation (Figure 9). Though, three groups were discriminated from the dendrogram of the agro-morphological data where E8 an Gujarat were also in one group. This work relates to a similar one by Mekonen *et al. (2019)* who studied 10 Ethiopian sesame cultivars using 11 ISSR markers generating four groups tow of which had single cultivars and the other two groups had five and three cultivars respectively.

The greatest genetic distance (0.63) was between genotypes 01M and 05E populations while the most closely related (0.16) were 04E and 05E populations. The genetic distance range of 0.16 to 0.63 differ from 0.02 - 0.46 range reported by Kurt and Arioglu (2018) in their study of genetic relationship among 24 Turkish sesame cultivars using 14 ISSR primers and 0.44 - 0.82 range reported by Mekonen *et al.* (2019). The differences observed among the various studies might be attributed to the populations used, and or the nature and number of molecular markers as well as the annealing temperatures.

Estimates of genetic diversity among the studied genotypes were calculated using AFLPsurv and the results are summarized in Table 7. Number and percentage (in parenthesis) of polymorphic loci ranged from 13 (23.2%) in Yandev-55 to 45 (77.6%) in 01M. Nei's (1973) gene diversity within the studied populations ranged between 0.12 and 0.41, indicating a substantial amount of variation within studied genotypes. The variance in diversity due to the sampling loci was generally higher (67% - 90%) than within individuals (10.0% - 33.0%).

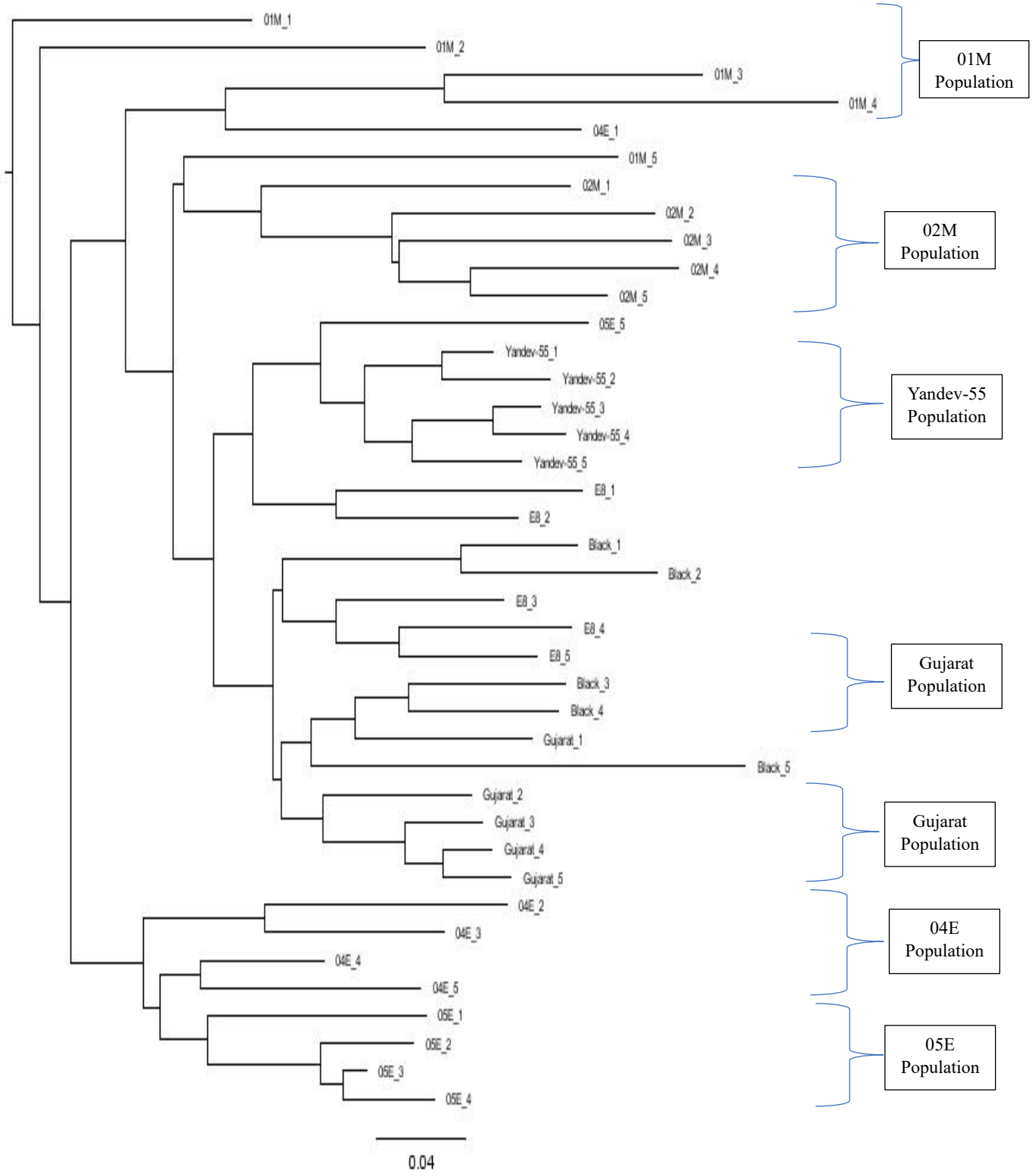

Figure 10. Dissimilarity dendrogram of 8 sesame genotypes, established from polymorphism of 11 ISSR Markers using Agglomerative clustering method

Table 7: Genetic diversity and differentiation of sesame genotypes as calculated with the Bayesian method using AFLPsurv 1.0

Genotypes	N	loc	PL	P (%)	Hj ± SE	VarI (%)	VarL (%)
01M	5	58	45	77.6	0.42 ± 0 .03	25.5	74.5
02M	4	58	32	55.2	0.25 ± 0 .03	28.6	71.4
04E	5	58	32	55.2	0.26 ± 0 .03	25.1	74.9
05E	4	58	22	37.9	0.16 ± 0 .028	33.0	67.0
Black	4	58	32	55.2	0.27 ± 0 .034	20.3	79.7
E8	4	58	26	44.8	0.22 ± 0 .033	17.7	82.3
Gujarat	4	58	16	27.6	0.14 ± 0 .03	13.0	87.0
Yandev-55	4	56	13	23.2	0.12 ± 0 .03	10.0	90.0

PL = number of polymorphic loci, P (%) = Proportion of polymorphic loci, Hj = Nie's genetic diversity, VarI % (Hj) = variance within individuals, VarL = variance due to sampling loci, VarL (%) =

Analysis of population structure (Table 8) showed that total genetic diversity in the overall samples is 0.36 was high and that the genotypes were differentiated from each other at genetic level. Fst values < 0.05 are low, 0.05 - 0.15 are medium and values greater than 0.15 are said to be high. The Fst value of 0.36 observed among the genotypes used for this study was high showing high genetic diversity of the populations studied (Table 8). This was higher than 0.29 recorded by Anggraeni *et al*. (2022) in his work to determine the genetic relationship between 81 accessions of sesame plants using ISSR markers.

Pair-wise distance analysis (Nei unbiased genetic distance) between populations (Table 9) ranged from 0.0461 (Gujarat and Black) to 0.30 (Gujarat and 02M). These values show that Gujarat is more closely related to black but greatly unrelated to 02M. Much lower genetic distance indicates the genetic relatedness of the populations studied due to similar geographic location of these populations which may be caused by the high rates of gene flow due to exchange of germplasm among farmers. Similar results have been reported by Abate *et al*. (2015) in his study of genetic diversity among 128 Ethiopian sesame populations and with earlier results by Dagmawi (2011) in evaluation of 82 Ethiopian and 38 exotic sesame accessions.

Table 8: Population genetic structure of eight sesame genotypes using 11 ISSR markers

N	Total gene diversity (Ht)	Average gene diversity within the population (Hw)	Nei's DST (Hb)	Fst
8	0.3603	0.2289 ± 0.033	0.1315 ± 0.008	0.362 ± 0.109
Variation		0.001092	0.000073	0.011914

Table 9. Nei's unbiased genetic distance below diagonal among sesame genotypes tested by 11 ISSR markers

	01M	02M	04E	05E	Black	E8	Gujarat
01M							
02M	0.2097						
04E	0.1141	0.1480					
05E	0.2183	0.2372	0.0933				
Black	0.1251	0.2682	0.1948	0.2852			
E8	0.2119	0.1497	0.2663	0.2359	0.0775		
Gujarat	0.1435	0.3005	0.2112	0.2317	0.0461	0.0836	
Yandev-55	0.1970	0.2561	0.2371	0.1871	0.1953	0.1226	0.1321

Conclusion

This current study evaluated the genetic diversity of eight sesame genotypes using their agromorphological performance and eleven ISSR markers. The results showed significant variations among the genotypes especially in the yield related traits but less variation in the morphology. The black sesame and E8 performed better in number of capsules per plant. The black sesame also had significantly highest number of seeds per capsule and was early in maturity This is earliness is a good triat for draught escape especially giving the current issues with climate change in its attendant negative impacts of draught. The studied genotypes also were diverse based on the use of molecular markers. The ISSR markers used for the first time on these Nigerian genotypes were very informative and efficient on detecting the diversity of the studies genotypes. Further studies on genetic diversity of Nigerian sesame accessions using ISSR markers using greater numbers of markers and genotypes will further unravel their degree of diversity is recommended.

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