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SSR-Based Genetic Diversity and Population Structure Analysis of Selected Okra (*Abelmoschus esculentus*)

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ABSTRACT: Okra (*Abelmoschus esculentus*) is the most important cultivated vegetable in Nigeria. It has a low caloric composition with myriads of essential nutrients and minerals needed for daily utilization by the human body. Understanding of genetic diversity and population structure can be used for okra breeding. In the present study, seventeen okra accessions consisting of 15 landraces and 2 commercial varieties were characterized using SSR markers to explore genetic diversity and population structure. In total, 8 SSR markers detected 50 SSR alleles with a mean of 6.25. The highest number of alleles was detected by three primers, namely AVRDC-Okra56 (14), AVRDC-Okra78 (9) and AVRDC-Okra89 (8). The average gene diversity was 0.61 with polymorphic information content ranging from 0.19 to 0.87 and a mean of 0.58. Dendrogram and principal component analysis based on SSR data showed three major groups of the studied okra accessions, revealing substantial genetic variation among the collection. The model-based population STRUCTURE analysis also grouped the accessions into three subpopulations (K = 3) with moderate levels of gene admixture. These findings provide information for future molecular identification of diverse okra varieties and can be used to perform genetic analyses, develop future varieties and implement conservation programs.

Keywords: Okra, Genetic diversity, Polymorphism, Population structure, Nigeria

Introduction

The plethora of value attached to using plants as sources of nutrients and medicine is enormous (Oppong-Sekyere *et al.,* 2012). Okra (*Abelmoschus esculentus*), an herbaceous and annually grown vegetable of the family Malvaceae (Naveed *et al.,* 2009), consists of over 244 genera and more than 4200 species with its origin in Ethiopia. It is abundantly present in tropical, sub-tropical regions and in some parts of Europe and America (Oyelade *et al.,* 2003; Andras *et al.,* 2005; Saifullah and Rabbani, 2009; Patil *et al.,* 2015). Okra cultivation has a high value for domestic consumption in most parts of West Africa. The diverse and distinct phenotypes such as height, flowering and fruiting duration and pod dimensions (length and weight) attributed to okra are functions of the synergistic action of two or more genes (Binalfew and Alemu, 2016). Okra plants can grow in soils with varying macro and micronutrient contents, with their pods and succulent leaves growing without irrigation. The transformation of the flowers into fruits takes 2-3 days and the fruits have arrays of green-colored and circular-shaped seeds (Moosavi *et al*., 2018). A chromosomal distribution anomaly during cell division leads to two forms of okra. They can be diploid (2n = 60 – 70) or tetraploid (2n = 120 – 140) (Nwangburuka *et al*., 2011).
Okra has a low caloric composition and is mostly harvested unripe, with myriads of essential nutrients and minerals needed for daily utilization by the human body. Diseases such as diabetes, cardiovascular diseases (Gemede *et al.,* 2015) and cancer in recent times have been treated via the consumption of okra, which is

endowed with a bulk of secondary metabolites of pharmacological relevance to human health. The highly tender pod is edible and serves as a tasty vegetable which contains bioactive compounds such as vitamins, folates, minerals, carbohydrates, dietary fibres, and antioxidants (anthocyanin, carotenoid, and flavonoids), unsaturated fats (Gemede *et al.,* 2016; Binalfew and Alemu, 2016; Petropoulos *et al*., 2018; Wu *et al*., 2020). Due to its bulky fibre content, its consumption can help manage weight and lower cholesterol. Peter *et al.* (2021) found that the flavonoids (myricetin and quercetin) in Okra have antidiabetic efficacy in managing insulin-independent diabetes.

Genetic diversity plays a pivotal role in the crop improvement process by the selection of desirable traits and harnessing heterosis to achieve offspring of better quality and optimal economic value. Diverse genotypes can be bred for the specific traits responsible for pod production in okra. Genetic diversity is a key component of biodiversity, form the basis of species diversity and most important in improving crops and maintaining germplasm (Zhang *et al*., 2015). High-throughput molecular markers help capture genetic diversity at the DNA level and provide information on the accession's relatedness and the shared phylogenetic relationships among plants (Glaszmann *et al.,* 2010; Luo *et al.,* 2020). Today, RNA sequencing remains one of the most revolutionary techniques. Based on transcriptome data, Schafleitner *et al*. (2013) developed SSR markers for genetic diversity assessment. Sixty-one SSR markers were obtained and can be used for okra improvement schemes.

Molecular markers include simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), intergenic simple sequence repeat (ISSR), and amplified fragment length polymorphism (*Sawadogo et al.,* 2009). Simple sequence repeats (PCR-based) markers are essential for plant breeding schemes due to their abundance, co-dominance, high polymorphism, uncomplicated technology, and multiple genomic alleles (Kachare *et al*., 2019, Tiwari *et al.,* 2019). Genetic diversity analysis using high throughput molecular markers have been widely used in vegetables (cabbage, broccoli, and others) for plant breeding improvement (Zhan *et al.,* 2014; Chu *et al.,* 2020; Yan *et al*., 2021).

There is limited genomic information on the use of DNA-based molecular marker technology in delineating the genetic diversity of okra (Gulsen *et al.,* 2007; Prakash *et al*., 2011; Kumar *et al.,* 2017). Other markers such as ISSR, RAPD, and AFLP have been used for analyzing genetic diversity of okra (Prakash *et al.,* 2011). This provides essential information for heterotic selection among broad collections of okra. Sun *et al.* (2023) analyzed the population structure of okra germplasm using SNP molecular markers. The results showed that several SNP loci are linked to genes for salt tolerance and has potential to be used in molecular marker assisted breeding. Molecular markers such as CAAT box-derived polymorphism (CBDP) and start codon targeted (SCoT) markers have been used to evaluate genetic diversity among wild and farmed okra (Puneeth *et al*., 2023). It showed that CBDP markers were highly effective compared to SCoT markers. Also, a previous study of genetic diversity of different okra populations using SSR markers showed genetic divergence among them (Kumar *et al*., 2017).

In Nigeria, there is minimal or no information on the utilization of molecular markers for genetic diversity evaluation among okra accessions grown in Nigeria, it is based on morphological characterization (Omonhinmin and Osawaru, 2005). Okoh *et al.* (2018) reported analysis using RAPD markers for estimating molecular diversity among Nigerian okra accessions, which showed the importance of molecular markers in detecting the genetic relationship and identifying plant germplasm. Simple sequence repeats (SSRs) are the preferred molecular markers for genetic diversity studies and germplasm analysis in efficient breeding processes to develop new and improved okra accessions. Other studies have shown that SSR markers effective in assessing genetic diversity among okra accessions of various germplasm collections (Mkhabela *et al*., 2022; Das *et al*., 2022). Currently, there are no reports on the application of SSRs for understanding the extent of genetic diversity and structure among okra accessions in Nigeria. Therefore, the study aimed to use SSR markers to evaluate the genetic diversity and population structure of okra collected from some major growing areas of Nigeria.

Materials and methods

Plant materials: A collection of seventeen (17) *A. esculentus* accessions, including local (15) and commercial (2) varieties were used in this study (Table 1). The local varieties were sourced from the market, farmers and the commercial varieties were obtained from the germplasm collection of the National Horticultural Research Institute (NIHORT), Ibadan, Oyo State. Those samples were collected from most of the southern areas of Nigeria. They were planted in the field during the 2022 rainy season to maturity (Figure 1 shows fruits collected from some of the okra accessions). The experiment was conducted at the Research Farm, Department of Cell Biology and Genetics, University of Lagos, Lagos State, Nigeria.

Table 1: Accessions and collection sources of *A. esculentus* used in genetic diversity assessment

Codes	State and Towns of Collection	Variety Name/Variety Type
AE01	Lagos, Lagos	Kuruso, local variety
AE02	Lagos, Lagos	African giant 2, local variety
AE03	Imo, Owerri	Local variety
AE04	Lagos, Lagos	African giant 1, local variety
AE05	Ogun, Abeokuta	Local variety
AE06	Imo, Owerri	Local variety
AE07	Long (Lagos)	Local variety
AE08	Oyo, Ogbomoso	Local variety
AE09	Oyo, Ibadan	NHAe47-4, commercial variety
AE10	Oyo, Ibadan	LD88, commercial variety
AE11	Ondo, Akure	Local variety
AE12	Lagos, Lagos	Local variety
AE13	Lagos, Lagos	Local variety
AE14	Lagos, Lagos	Local variety
AE15	Osun, Oshogbo	Local variety
AE16	Lagos, Lagos	Local variety
AE17	Lagos, Lagos	Local variety

Figure 1: Morphological features of *A. esculentus* mature fruits with different fruit shape, color, shape, length, surface between ridges, shape of apex, hairiness and diameter at mid length

DNA extraction and SSR genotyping: Fresh leaf tissue of *A. esculentus* was collected in dry envelopes and sealed. The envelopes were stored at −80°C until the tissue was lyophilized. DNA extraction from the lyophilized tissue was done using the CTAB method described by Doyle and Doyle (1990). Using Nanodrop and 1 % agarose gel electrophoresis, the concentration and quality of the extracted samples was determined. Six SSR markers were chosen from SSR mined from leaf and pod transcriptomes (gene sequences) of okra (Schafleitner *et al*., 2013). Additional six primers previously developed from *A. esculentus* were selected from later studies (Ravishankar *et al*., 2018; An *et al*., 2022) as shown in Table 2. For PCR amplification reactions for all SSR primers were optimized on 3 samples following the protocols previously reported by the authors (Schafleitner *et al*., 2013; Ravishankar *et al*., 2018; An *et al*., 2022). The nine (9) SSR primer pairs with clear amplification products were analysed across the 17 *A. esculentus* genotypes studied (Table 1). The PCR products were separated by 6 % polyacrylamide gel electrophoresis and a 50 bp DNA ladder. The amplified products were visualized in a UV gel documentation analyzer. The presence of a band was scored as '1' and its absence as '0' and used to analyze genetic diversity**.**

Genetic diversity indices and structure analysis: The number of alleles, major allele frequency (MAF), expected heterozygosity [also known as "gene diversity" (GD)] (Nei, 1973) and Polymorphic Information Content (PIC) value (Botstein *et al*., 1980), for each SSR locus, were estimated using Power Marker version 3.25 (Liu and Muse, 2005). The generated binary data for all the SSR loci were used to compute the distance matrix using Dice's distance in Darwin (Perrier and Jacquemoud-Collet, 2006). The dendrogram based on an unweighted pair group method with arithmetic mean (UPGMA) using Darwin was generated (Perrier and Jacquemoud-Collet, 2006) as follows:

$$D_c = 2n_{xy}/(n_x + n_y),$$

where n^x and n_y represent the putative SSR alleles for materials X and Y, respectively, and n_{xy} represents the putative SSR alleles shared between X and Y.

Principal coordinate analysis (PCoA) was performed, using the distance matrix from Dice's distance. The analysis was performed in population structure of 17 accessions and membership probability of assigning genotypes to subgroups (K = 3). The model-based software program STRUCTURE v2.3.4 (Pritchard *et al*., 2000) was used to assess the population genetic structure of the analyzed accessions using our SSR marker dataset. Five runs were performed for each number of population (k) set from 1 to 10. Burn-in time and Markov Chain Monte Carlo (MCMC) replication number were set to 100,000 for each run. The most probable K-value was obtained via Structure Selector (Li *et al*., 2018) and the Q plot at the most probable K-value was generated.

Table 2: Characteristics of 12 SSR primers evaluated in the study

Locus	Repeat motif	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Cluster-12086.22033	(CAG)7	GTTGGTTGGTGTTGTTGGACA	TCAAGCCATGGAGGAGCTTG
Cluster-12086.35646	(TCT)5	GCGGTGAGTGAGGCCTTATC	GCAATGGGCTTCCACCTACT
Cluster-1635.0	(GAC)5(GAA)8	TGAGATGCGTCAGCTTAGGC	CATGCGGTGGCTTCACAATA
AeKVR 102		TTCCGATGAGGAACTAGATGGAT	CCCAGTTGGACCTTGAGAGA
AeKVR-137		TAAAGGGTATAATGCAGCCATGT	TCGTGGTTAGTTTCGTTTTCCT
AeKVR-153		TGGAGTGTGCTGCCAAGTTTAT	TTGTTTATGCTGTGATGCTGAC
AVRDC-Okra1	(AAG)13	ATGGAGTGATTTTTGTGGAG	GACCCGAACTCACGTTACTA
AVRDC-Okra9	(AAT)12	ACCTTGAACACCAGGTACAG	TTGCTCTTATGAAGCAGTGA
AVRDC-Okra56	(GAA)44	GGCAACTTCGTAATTTCCTA	TGAGTAAAAGTGGGGTCTGT
AVRDC-Okra64	(TCT)22	AAGGAGGAGAAAGAGAAGGA	ATTTACTTGAGCAGCAGCAG
AVRDC-Okra78	(TAT)11-(TATTGT)4-(TATCGT)4	CTCCGACAATTCAAGAAAAG	CACCCAATCAAGCTATGTTA
AVRDC-Okra89	(AGC)8	TTTGAGTTCTTTCGTCCACT	GTATTTGGACATGGCGTTAT

Results

Diversity indices of SSR loci: Eight polymorphic *A. esculentus* SSR primers used for studying the 17 *A. esculentus* accessions were analysed in the present study. A total of 50 alleles were detected and the allele number ranged from 2 (Cluster-12086.35646, AVRDC-Okra1, AVRDC-Okra9) to 14 (AVRDC-Okra56), with a mean of 6.25 (Table 3). Moreover, the major allele frequency of SSR loci varied from 0.16 to 0.88, with an average of 0.48. The gene diversity of SSR loci ranged from 0.21 (Cluster-12086.35646) to 0.88 (AVRDC-Okra78), with a mean of 0.61. Polymorphic information content (PIC value) also varied from (0.19) to (0.87), with a mean of 0.58. Table 3 summarizes the results of eight SSR markers. Genetic distance ranged from 0.14 to 1. 00, with an average value of 0.66.

Table 3: Genetic diversity analysis of 17 *A. esculentus* at 8 polymorphic SSR loci

Locus	Allele number	Major allele frequency	Gene diversity	Polymorphic information content
Cluster-12086.22033	6	0.35	0.73	0.68
Cluster-12086.35646	2	0.88	0.21	0.19
Cluster-1635.0	7	0.22	0.84	0.81
AVRDC-Okra1	2	0.88	0.21	0.19
AVRDC-Okra9	2	0.79	0.34	0.28
AVRDC-Okra56	14	0.32	0.8	0.78
AVRDC-Okra78	9	0.16	0.88	0.87
AVRDC-Okra89	8	0.24	0.85	0.83
Mean	6.25	0.48	0.61	0.58

Cluster analysis and population structure: A Jaccard's genetic distance-based dendrogram of 17 *A. esculentus* accessions revealed genetic relationships. The dendrogram showed the accessions are clustered into three main clusters (Figure 2). The first cluster included ten accessions and the second included seven accessions. Grouping accessions into the same cluster may show possible genetic similarity among accessions. The PCoA analysis also divided the 17 accessions into three main groups (Figure 3), consistent with the results obtained from dendrogram analysis. Structure analysis revealed that the maximal ΔK value was observed at a K value of 4 (Figure 4a), grouping the 17 accessions studied into three distinct genetic clusters as shown in Figure 4b.

Clusters 1, 2, and 3 comprised 41 %, 29 % and 30 % of the total genotypes, respectively, and a substructure within the first main cluster was detected (yellow). Accessions from the same states were placed in the same cluster. Also, the two commercial varieties (AE09 and AE10) were grouped in Cluster 2 (blue cluster) and the remaining 15 local varieties were assigned in three main clusters. Admixtures were seen in this study, but AE11 and AE12 were considered the most admixed types. The model-based structure shows clear subpopulations, suggesting a population structure.

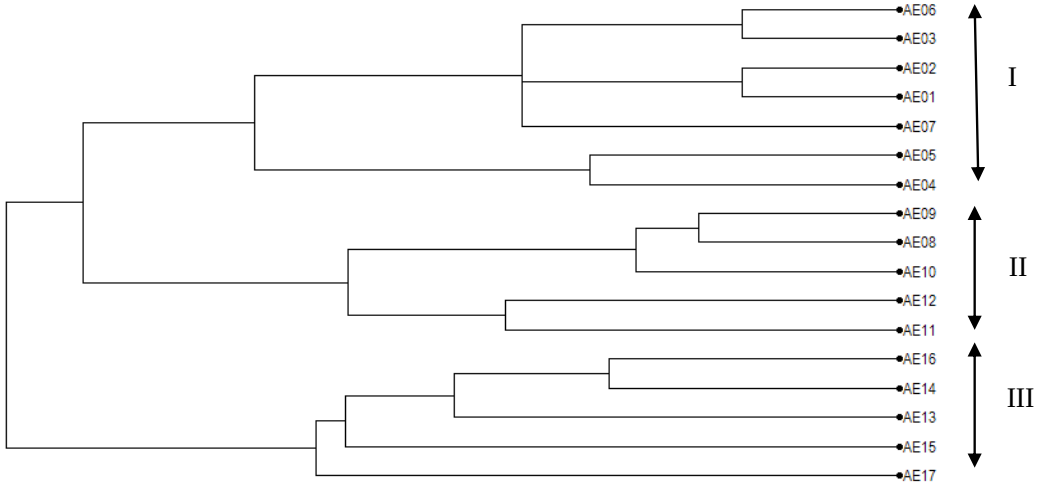

Figure 2: Dendrogram for 17 *Abelmoschus esculentus* accessions based on Dice's genetic distance

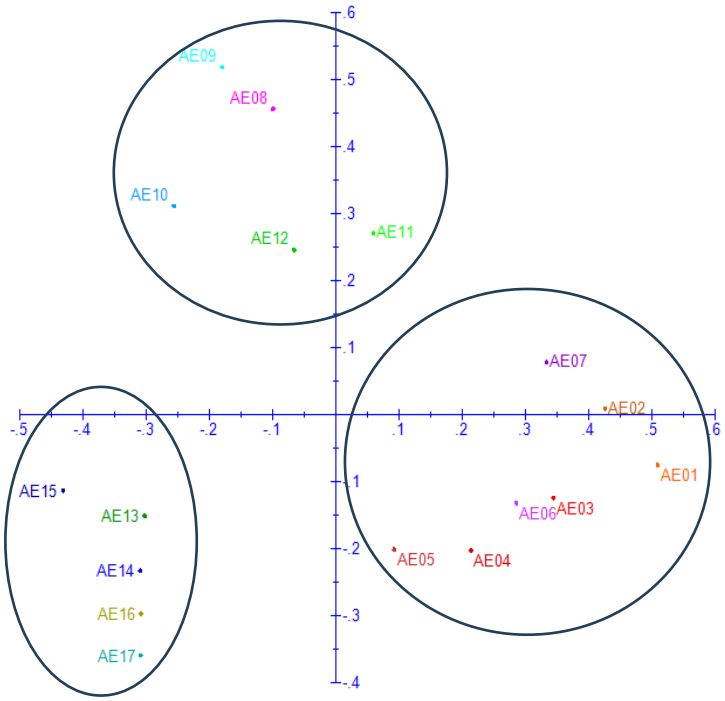

Figure 3: Principal coordinates of 17 *Abelmoschus esculentus* accessions based on 8 SSR loci

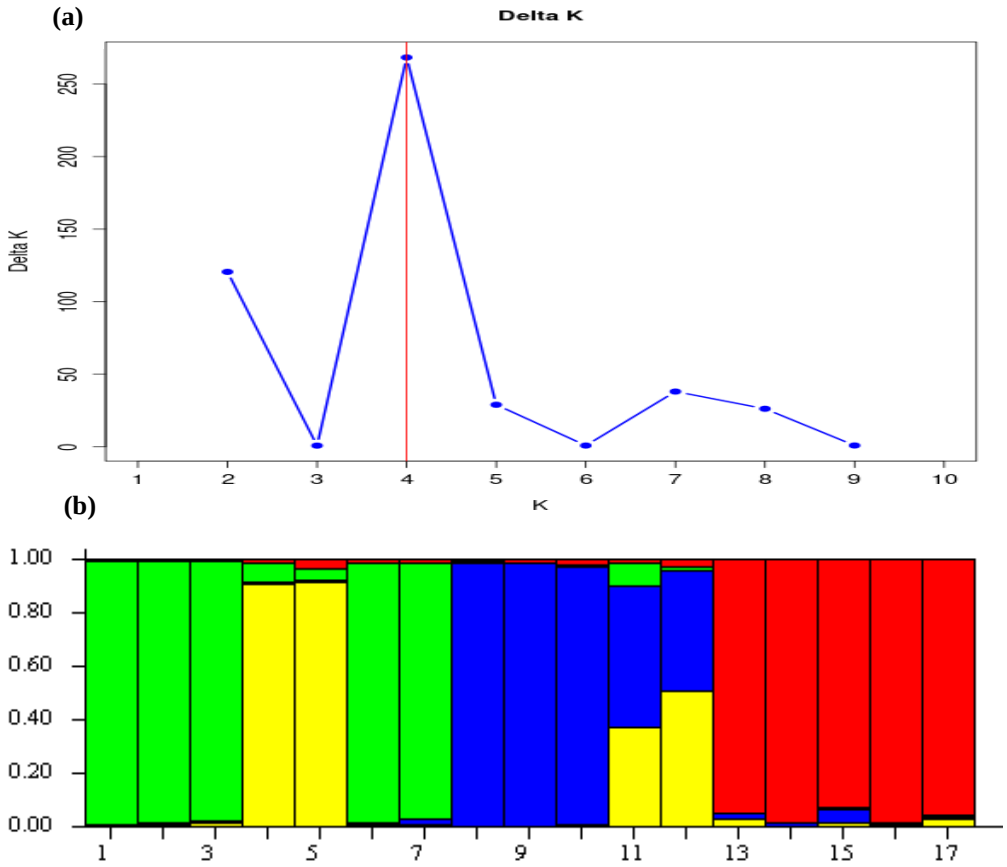

Figure 4: Population structure of 17 accessions membership probability of assigning genotypes (K = 4)

Discussion

Analysis of genetic diversity and population structure of okra genotypes are essential for breeding in the future and contribute to the conservation of diverse genotypes. Genetic diversity estimates the genetic variation of a set of varieties and this variation can be used for crop improvement. Molecular markers are well known techniques in the evaluation and assessment of genetic diversity and estimation of the structure of the population. The characterization of okra cultivars in Nigeria has been based on agromorphological traits (Alake, 2019; Olayiwola *et al*., 2021). Morphological characteristics are not sufficient to represent genetic distinction among plant varieties. However, molecular marker studies have also been conducted using RAPD (Okoh *et al*., 2018). In the present study, local and commercial okra varieties with unique fruit characteristics cultivated in various parts of Nigeria were evaluated with SSR markers developed by Schafleitner *et al*. (2013), Ravishankar *et al*. (2018) and An *et al.* (2022). SSR makers are highly polymorphic, co-dominant and reproducible with potential resources in breeding programs.

Among the amplified 9 SSR markers studied, 88 % (8) of them were found to be polymorphic among the okra varieties, which is greater than the 75 % reported by Kumar *et al*. (2017) but lower than the 91 % reported by Schafleitner *et al*. (2013). A total of 50 alleles were produced in the present study with a mean value of 6.25, which is twice the mean allele number reported by Das *et al*. (2022) and, but slightly lower than Kumar *et al*. (2017). The presence of more alleles reflected the existing genetic diversity of the analysed set of accessions and varietal identification. The polymorphic information content (PIC) measures the informativeness and effectiveness of each marker used in the genetic divergence study. According to this study, 62 % of the molecular markers were extremely informative while 27.5 % were informative. The PIC value ranged between 0.19 and 0.81 and a mean PIC value of 0.58, showing variability among the varieties examined. The polymorphism values suggest that the chosen markers are dependable for detecting genetic diversity. The value was higher than those obtained by Das *et al.* (2022), Kumar *et al*. (2017), and Schafleitner *et al.* (2017). Different genetic backgrounds of the varieties used for the genetic diversity study may cause varying research results.

Clustering based on dendrogram, principal component analysis (PCA) and STRUCTU RE of the studied varieties separated them into three major groups. Thus, the grouping of accession reveals genetic resemblance and divergence relationships between genotypes. Furthermore, the separation of the varieties into three distinct clusters based on the SSR markers may be a result of within-species variability and adaptation to environmental conditions. Principal component analysis simplifies data by distributing them into different components (Lever *et al*., 2017). The principal coordinate analysis also showed that clusters 1 and 2 are closer than cluster 3, which is further apart.

Population structure analyses quantify divergence and admixture within plant populations and species. The STRUCTURE analysis is often used to study the population structure of crops (Adedugba *et al*., 2023; Adeyemo *et al*., 2023; Bhadmus *et al*., 2022; Adeyemo *et al*., 2019). The STRUCTURE result obtained in this study is consistent with the clustering pattern of dendrogram and PCA. This suggests that the results of the model are dependable. The structure analysis also showed the presence of sub-groups in the first cluster, highlighting genetic variation and gene flow among varieties. These findings are consistent with those of Yildiz *et al*. (2015). In this study, cluster 2 has the commercial varieties, AE09 and AE10, this shows a close genetic relationship, resulting from artificial breeding. Local varieties are widely distributed in clusters 1 and 3, reflecting substantial genetic diversity among the varieties studied. The admixture in AE11 and AE12 indicates the blending of genetic materials among different varieties. It is therefore essential to increase the hybridization of parental stock selection to have a prominent level of genetic variability in the okra cultivars.

Conclusion

In the present study, SSR markers revealed the presence of moderately high genetic distance in the studied Okra varieties including 2 commercial varieties. The results showed that there are admixtures in the analysed varieties. Based on the SSR markers, the close genetic relationship of commercial varieties was established. These findings provide information for future molecular identification of diverse varieties of the okra and can be used to perform genetic analyses, develop future varieties and implement conservation programmes.

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