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# ASSESSMENT OF GENETIC DIVERSITY AMONG OKRA (*ABELMOSCHUS ESCULENTUS* L.) GENOTYPES USING SSR MARKERS

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**ABSTRACT**

The present investigation aimed to assess the genetic diversity among 28 Okra (*Abelmoschus esculentus*) genotypes collected from the NBPGR (New Delhi) using SSR markers. Genomic DNA was isolated from fresh leaves of all the genotypes and quantified. PCR amplification was performed with 15 SSR primers, and the resulting banding patterns were recorded and analyzed using Jaccard's similarity coefficient. The SSR analysis generated 452 bands from 15 primers, with 10 SSR primers showing 100% polymorphism. The genetic diversity estimated by the PIC value for the SSR loci ranged from 0.40 to 0.88. The cluster dendrogram revealed two major clusters, and SSR primers OKRA 109, OKRA 157, OKRA 120, OKRA 137 and OKRA 175 provided the maximum accession coverage in the Okra genome. The study demonstrated that the genotypes were clearly distinguishable from each other when grouping was carried out using SSR markers, and these SSR primers can be used for the characterization of Okra genotypes and future breeding programs for Okra improvement

***Key words :*** Okra, SSR Marker, Genetic Diversity, Crop improvement.

## Introduction

Okra (*Abelmoschus esculentus*) is an important vegetable crop that is widely cultivated in tropical and subtropical regions of the world. It is a member of the *Malvaceae* family and is commonly known as lady's finger, bhindi, or gumbo. Okra is a highly nutritious crop and is an excellent source of vitamins, minerals, and dietary fiber. It is used in various dishes and has medicinal properties as well. *A. esculentus*, chromosome numbers 2n=72, 108, 120, 132 and 144 are derived as part of a regular series of polyploids with a basis chromosome number of 12 (Datta and Naug, 1968). The availability of genetic diversity and its successful collection, maintenance, utilization and conservation is pre-requisitefor crop improvement program (Poehlman and Sleper, 1995) aimed at improving yield, quality and resistance to biotic and abiotic stresses. The variability among germplasm can be assessed by using different morphological and molecular markers. Genetic diversity provides the basis for the selection of superior genotypes and the development of new cultivars through hybridization and selection. In okra, morphological variability had been assessed by several researchers (Ariyo *et al.*, 1993; Aladele, 2009; Osawaru *et al.*, 2013). Despite, broad genetic base within *A. esculentus* (Saifullah *et al.*, 2010), it is still difficult to discriminate genotypes based on their phenotype. Moreover, phenotypic based selection and genetic analysis, which does not reliably reflect true genetic diversity, are highly affected by environment, quantitative inheritance of traits, partial and dominance trait expression (Saifullah *et al.*, 2010 and Akash *et al.*, 2013). Many of these

complications associated with phenotype-based assay can be easily overcome through DNA based molecular markers. With the advent of recent methods in molecular biology, different molecular markers have been applied for the study of molecular diversity in okra. Few of these are RAPD (Martinello *et al*., 2001), ISSR (Yuan *et al*., 2014); AFLP (Akash *et al*., 2013) and SRAP (Gulsen *et al*., 2007) and SSR markers (Sawadogo *et al*., 2009). SSR markers have emerged as a powerful tool for the assessment of genetic diversity in crop plants. SSR markers are highly polymorphic and reproducible, and they can be used to identify and differentiate genotypes at the molecular level. The use of SSR markers for the assessment of genetic diversity in okra has been limited, and there is a need to identify new sources of genetic diversity to enhance the breeding programs for this crop. In this study, we assessed the genetic diversity of 28 okra genotypes using 20 SSR markers. The results of this study will provide valuable information for the development of new okra cultivars with improved yield, quality and resistance to biotic and abiotic stresses.

## Materials and Methods

### Plant material and DNA extraction

Seed material of twenty-eight genotypes of Okra (*Abelmoschus esculentus*) was collected from ICAR-National Bureau of Plant Genetic Resources (Table 1). The genotypes were selected based on their diverse origin, growth habit, and other phenotypic characteristics. Seed material was grown under laboratory conditions. Genomic DNA isolation in okra is challenging due to the presence of mucilage (viscous glue-like textured polysaccharides) that interferes with nucleic acid purification and makes DNA unmanageable during downstream processes and molecular analysis. To overcome polysaccharide interference during DNA extraction, the yellow and etiolated fresh leaves were collected from 10 to 14-day-old seedlings raised in plastic cups under dark conditions.

**Table 1 :** List of selected twenty-eight genotypes of Okra used in present investigation.

| S. no. | Accession No. | Important Traits | Place of collection | Location | Biological status |
|---|---|---|---|---|---|
| 1. | IC 128070 | Single stem | Gujarat | Akola, NBPGR RS | - |
| 2. | IC 128071 | Medium tall spreading | Maharashtra | Akola, NBPGR RS | - |
| 3. | IC 128072 | - | Telangana | New Delhi, NBPGR-DGE | Landrace |
| 4. | IC 128080 | - | Telangana | New Delhi, NBPGR-DGE | Landrace |
| 5. | IC 128087 | - | Telangana | Akola, NBPGR RS | Landrace |
| 6. | IC 128089 | - | Telangana | New Delhi, NBPGR-DGE | Landrace |
| 7. | IC 128123 | Tall branch, pink stem | Chhattisgarh | Akola, NBPGR RS | Landrace |
| 8. | IC 128127 | Green bold seeds | Chhattisgarh | Akola, NBPGR RS | Landrace |
| 9. | IC 128142 | - | Telangana | New Delhi, NBPGR-DGE | Released Variety |
| 10. | IC 128143 | - | Maharashtra | Akola, NBPGR RS | Others/Unknown |
| 11. | IC 128146 | - | Jharkhand | New Delhi, NBPGR-DGE | Others/Unknown |
| 12. | IC 128891 | - | Maharashtra | Akola, NBPGR RS | Others/Unknown |
| 13. | IC 128892 | - | Maharashtra | Akola, NBPGR RS | - |
| 14. | IC 128893 | - | Maharashtra | Akola, NBPGR RS | - |
| 15. | EC 169504 | - | - | New Delhi, NBPGR-DGE | - |
| 16. | EC 169505 | - | - | New Delhi, NBPGR-DGE | - |
| 17. | EC 169511 | - | - | New Delhi, NBPGR-DGE | - |
| 18. | EC 169513 | - | - | New Delhi, NBPGR-DGE | - |
| 19. | EC 169948 | - | - | New Delhi, NBPGR-DGE | - |
| 20. | EC 177900 | - | - | New Delhi, NBPGR-DGE | - |
| 21. | EC 280756 | - | Bangladesh | Akola, NBPGR RS | - |
| 22. | EC 284327 | - | Bangladesh | Akola, NBPGR RS | - |
| 23. | EC 305609 | - | Bangladesh | Akola, NBPGR RS | - |
| 24. | EC 305610 | - | Bangladesh | Akola, NBPGR RS | - |
| 25. | EC 305611 | - | Bangladesh | Akola, NBPGR RS | - |
| 26. | EC 305618 | - | Bangladesh | Akola, NBPGR RS | - |
| 27. | EC 305631 | - | Bangladesh | Akola, NBPGR RS | - |
| 28. | EC 305632 | - | Bangladesh | Akola, NBPGR RS | - |

100mg of leaf material was taken for DNA extraction. DNA was extracted from the leaves using the SDS lysis buffer method. The DNA concentration and purity were determined using a Nanodrop spectrophotometer.

**SSR Marker selection and PCR amplification**

A set of 15 SSR markers was selected based on their polymorphism, reproducibility, and chromosomal distribution. The PCR amplification was carried out in a 15 µL reaction volume containing 6.25 µl Master mix (Takara), 0.25 µl of each forward and reverse primer, 5.25 µl of Nuclease free water, and 3 µl of 50 ng of genomic DNA template. The PCR conditions were as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at the specific temperature for each marker for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 min. The PCR products were separated on a 1.5% agarose gel and visualized under UV light after staining with ethidium bromide. The size of the amplified fragments was determined by comparing with a 100 bp DNA ladder.

**Molecular Data analysis**

In this study, 15 SSR markers were used to assess the genetic diversity among 28 okra genotypes. The complex genomic nature of okra, which includes the possibility of amphi-diploidy, can make the identification of alleles from homologous chromosomes on specific loci challenging. To overcome this challenge, the SSR bands were scored as dominant and considered polymorphic when absent in some samples. The percent polymorphism for each marker was calculated using the formula: (Number of polymorphic bands/Total number of scored bands) × 100. Additionally, the polymorphic information content (PIC) was calculated as a measure of the informativeness of each marker. The pair-wise genetic similarity was generated using Jaccard's co-efficient. A dendrogram was constructed using the unweighted pair group method average (UPGMA) clustering and multivariate principal coordinate analysis (PCoA) was performed with binary data to visualize the genetic relationships among the okra genotypes. NTSYSpc version 2.02 (Rohlf 2000) and XLSTAT 2022 (Addinsoft, 2007) software were used to generate the dendrogram and PCoA, respectively.

## Results and Discussion

The genomic DNA 28 Okra genotypes was extracted and concentration determined by measuring the optical density of DNA samples at 260/280 nm (Supplementary Table 1). The variation in the quantity of extracted DNA was obtained due to measuring error and differential

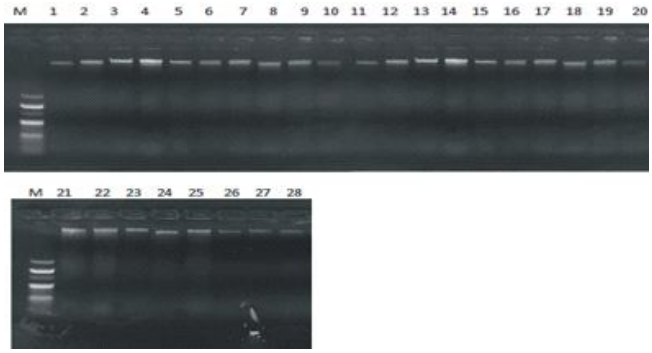


**Fig. 1 :** Genomic DNA extracted from the leaves at the seedling stage from 28 Okra Genotype.

grinding of leaf samples taken from different varieties. In most of the cases, the absorbance ratio (ratio of absorbance at 260 and 280 nm) was found to fall within the range of 1.80 to 1.95, reflecting the superior quality of DNA in the samples extracted from the leaves at the seedling stage of different varieties used in the present study (Fig. 1). PCR amplification of 15 SSR primers were used for the molecular diversity analysis (Fig. 2). Among these primers, 10 SSR primers showed 100% polymorphism. One SSR primer showed 50% polymorphism, four primers showed no polymorphism (Table 2). A total of 452 bands were scored from 15 primers, with an average of 18.04 bands per primer. The number of alleles per locus varied from 1 to 2. The overall size of amplified products ranged from 100 bp to 400 bp (Table 2). The highest number of alleles was observed with primers namely AVRDC OKRA 63, AVRDC OKRA 77, AVRDC OKRA 78, OKRA 86, OKRA 110, OKRA 109, OKRA 157, OKRA 175, OKRA 120, and OKRA 135 that provide the concise data regarding the number of unique alleles and their distribution in various genotype (Table 2). The PIC value was 0.55 per marker, and it varied from 0.4 (AVRDC OKRA 77) to 0.88 (OKRA 157). The genetic diversity of each SSR locus appeared to be associated with the number of alleles detected per locus. The higher the PIC value of a locus, the higher the number of alleles detected. Similar results were also reported by Fougat *et al.*, (2015) used 18 SSR markers to analyse the genetic diversity among 24 okra accessions, a total of 85 amplicons were found with a high amount of polymorphism (93.72 percent) and a mean polymorphic information content (PIC) of 0.53. Kumar *et al.* (2016) used 40 SSR (SSR) markers to evaluate the genetic diversity among 96 accessions of *Abelmoschus* from different continents, of which 92 accessions were *A. esculentus* and one accession each of *A. tuberculatus, A. moschatus, A. moschatus* subspecies *tuberosus* and *A. Manihot* with 30 SSRs produced a total of 213 bands, averaging 7.1 bands per SSR with

**Table 2 :** Characteristics of the amplification products obtained with 15 SSR primers used to analysis the analysis the Genetic diversity of okra accessions.

| Primer name | Forward Primer Sequence(5' to 3') | Reverse Primer Sequence(5' to 3') | Mol. wt. range (bp) | Total alleles | Polymorphic bands | Monomorphic bands | PIC | RP | Polymorphic percentage |
|---|---|---|---|---|---|---|---|---|---|
| AVRDC OKRA 63 | AACACATCCTCATCCTCATC | ACCGGAAGCTATTTACATGA | 150-200 | 2 | 2 | 0 | 0.66 | 0.33 | 100 |
| AVRDC OKRA 77 | CTGTTIGTTCGTCGTAATCA | AAAGTTTCTTCCTTTCCACC | 200-250 | 2 | 2 | 0 | 0.4 | 0.59 | 100 |
| AVRDC OKRA 78 | CTCCGACAATTCAAGAAAAG | CACCCAATCAAGCTATGTTA | 200-250 | 2 | 2 | 0 | 0.48 | 0.51 | 100 |
| OKRA 86 | TTTCCCTAATGAGTGGACC | GGGTCTGTTTTGTTGTTGTT | 250-300 | 2 | 2 | 0 | 0.53 | 0.46 | 100 |
| OKRA 109 | GGCAACAACAGTTCTCCTT | AATTGGGGTTAGTGACCATA | 250-300 | 2 | 2 | 0 | 0.75 | 0.24 | 100 |
| OKRA 110 | CCAAAGTCTGGTTCTCATTC | CAATCTCCCATGGTTTAGAA | 100-150 | 2 | 2 | 0 | 0.45 | 0.7 | 100 |
| OKRA 137 | TGAAGCACAGATCAAAGATG | ATACAGGCAAAGCACAGAGT | 150-200 | 1 | 1 | 0 | 0.71 | 0.28 | 100 |
| OKRA 144 | TGATTATGGTTGCCTGAAT | GCCCACTGACAGCTTATTGAA | 200-250 | 1 | 0 | 1 | 0.00 | 1.00 | 0 |
| OKRA 151 | ATCTCCTATCTGTCTCCGGT | ACTTGGCAACTAAGCAAAAG | 300-400 | 1 | 0 | 1 | 0.00 | 1.00 | 0 |
| OKRA 164 | AAAAGACTCATCGTGGACC | GTGATTCCCTAATCCAAACA | 150-200 | 1 | 0 | 1 | 0.00 | 1.00 | 0 |
| OKRA 157 | CAGAAACATCTCCAACATCA | ACACTTTTCAAGGGAAACC | 250-300 | 2 | 2 | 0 | 0.88 | 0.11 | 100 |
| OKRA 175 | CAGAAGGTCCCTTTATTCCT | TCACCCAACCATTTCTCTAC | 300-350 | 2 | 2 | 0 | 0.69 | 0.3 | 100 |
| OKRA 120 | CCATTTACTACCCCTTCTCC | CTCAGAATGTGTGATGATGC | 150-250 | 2 | 2 | 0 | 0.75 | 0.24 | 100 |
| OKRA 135 | GTCCACAGGCATATTAGCAT | AATTGTGCAGAGTATTTGGG | 100-150 | 2 | 1 | 1 | 0.49 | 0.50 | 50 |
| OKRA 126 | GAGAGAGATTGCTTCGACTG | TAAACTTTAAACTCAGCGGC | 250-300 | 1 | 0 | 1 | 0.00 | 1.00 | 0 |

60.66 percent were polymorphic. The average PIC of primers was 0.52. The coefficient of Jaccard ranged from 0.107 to 0.969. Kumar *et al.* (2017) evaluate the germplasm conservation of certain polymorphism information and genetic diversity of okra. A total of 248 different bands were identified polymorphic, with an average of 2.4 bands per primer, according to microsatellite markers. PIC values ranged from 0.42 to 0.99, with an average of 0.72, while resolving power (RP) values ranged from 0.14 to 3.97, with an average of 1.84. The average genetic similarity value among the okra germplasm was 0.77, ranging from 0.50 to 0.95 and Mohammed *et al.* (2020) used 32 okra accessions which were then genotyped using 16 chosen SSR markers from three geographic locations. SSR markers yielded a total of 71 loci, with 67 percent of them being polymorphic. The average genetic distance between accessions was 4.65, ranging from 2.2 to 7.1.

The input matrix for genetic analysis among the twenty-eight okra genotypes was prepared by scoring the main alleles obtained. A dendrogram was prepared to indetify the genetic relationship among different genotypes of Okra. The cluster analysis generated showed a significant genetic variation among the okra genotypes studied, with similarity coefficients ranging between 0.64 and 0.96 (Supplementary Table 2). The 28 okra genotypes were divided into two major clusters that was demarcated at a cutoff similarity coefficient level of 0.64, below which the similarity values narrowed conspicuously. Cluster B was the largest and included 21 genotypes. Jaccard's coefficient of similarity revealed a high degree of similarity to the extent of 96 % exists between genotype EC 284327 -EC305609 and genotype EC169511 - EC169513 also showed genetic similarity, whereas IC128142 keeps very low levels of similarity to the members of other clusters (Supplementary Table 2). Major cluster A contains seven genotype namely IC128071, EC305618, EC305631, EC169504, EC305611, EC280756 and EC305632 with

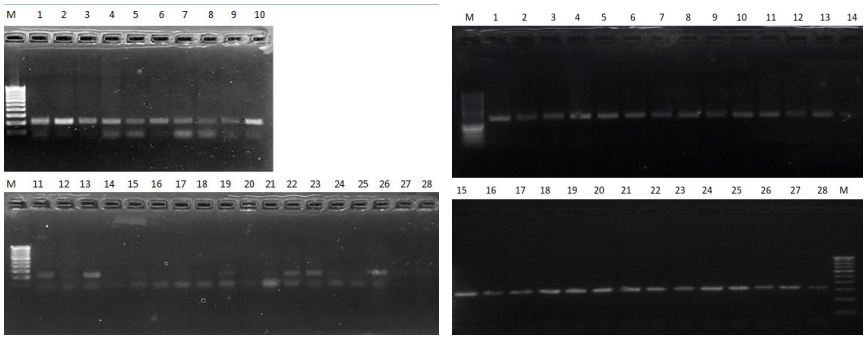


**Fig. 2 :** Representation of agarose gel electrophoresis of PCR product of primers AVRDC OKRA 77 and AVRDC OKRA 78 for twenty-eight Okra genotypes; M-DNA ladder (100 bp).

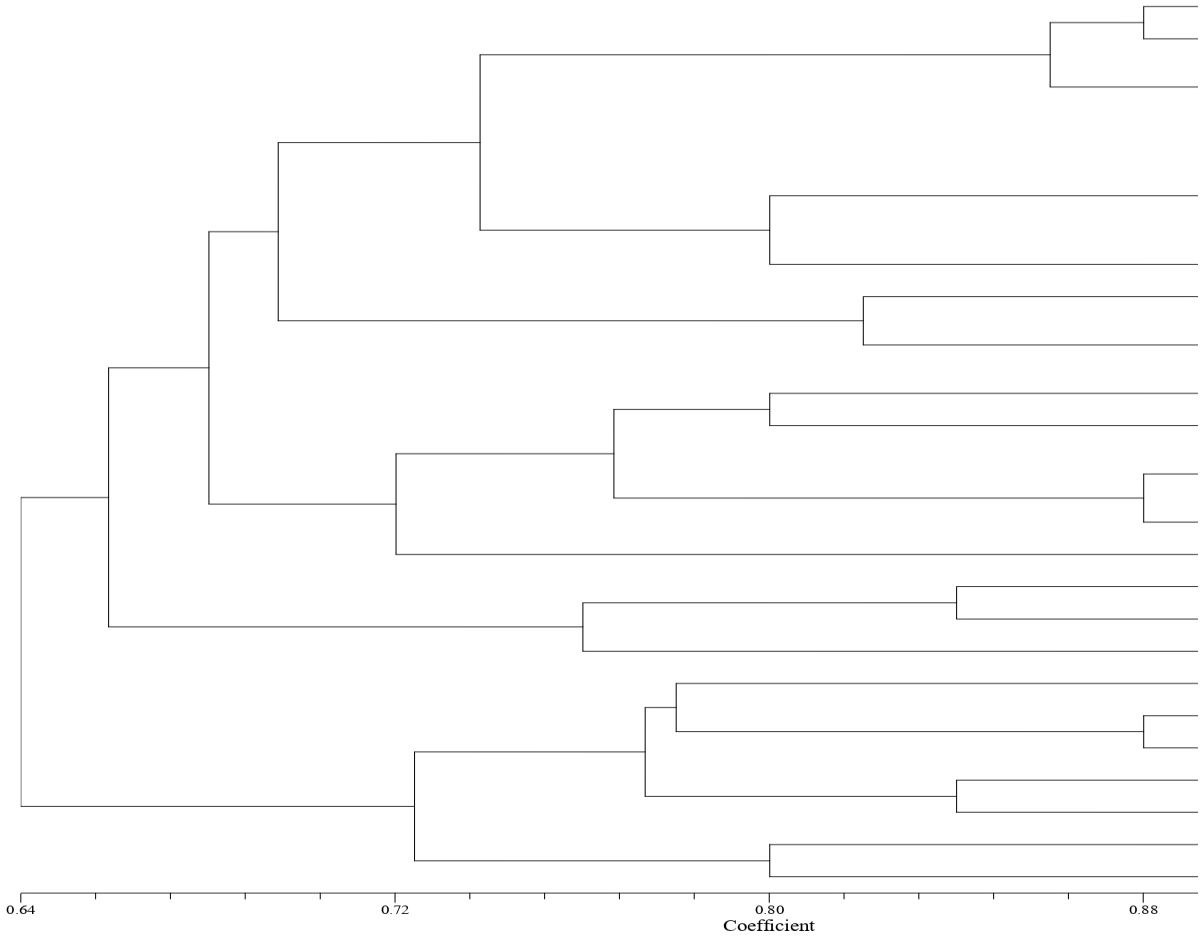


**Fig. 3 :** Dendrogram showing clustering of 28 genotypes of Okra constructed by using UPGMA cluster analysis of genetic similarity based on SSR data.

similarity coefficient (0.71-0.96.). Cluster B consist of 21 genotype IC128070, IC128072, IC128087, IC128089, EC169505, EC169511, EC169513, EC169948, EC177990, IC128080, EC284327, EC305609, IC128123, IC128892, IC128127, IC128146, IC128143, IC128142, IC128891, EC305609 and IC128893, with similarity coefficient ranges from 0.66 to 0.88. Dendrogram revealed that genotype IC128142 is distantly related to IC128143, IC128127 and IC128146, with a similarity coefficient of 0.72. genotype IC128893 is also distantly related to IC128891 and EC305609 with a similarity coefficient of 0.75 and EC169511 and EC169513 are very close to each other with a similarity coefficient of0.92. IC128127 and IC128146 are very close to each other, with a similarity coefficient of 0.93. in subgroup II of cluster B IC128087 and IC128089 are very close to each other with a similarity coefficient of 0.96. in subgroup III of cluster B, EC284327 and EC305609 are very close to each other with a similarity coefficient of 0.96. the genotypes IC128071, EC305618, EC305631, EC169504, EC30561, 1EC280756, and EC305632genotypes belong to different clusters and are genetically diverse. On the other hand, the best genotypes may further be improved on the basis of the above information based on genetic analysis by SSR markers of genotypes using molecular tools. Based on the study,

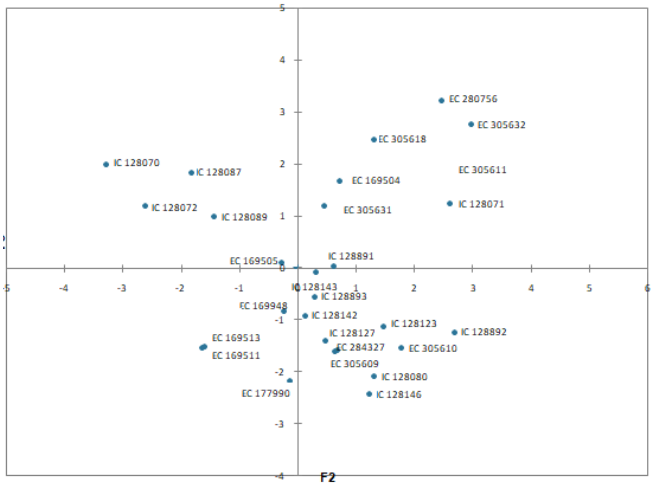


**Fig. 4 :** Principal coordinate analysis of twenty-eight genotypes of Okra.

the extensive range of similarity values for related genotypes using microsatellites provides greater confidence for the assessment of genetic diversity and relationships. The study revealed the existence of considerable genetic variation among the studied genotypes of okra. Low levels of genetic variation and high levels of genetic relatedness were found in each of the Okra genotypes. whereas significant levels of genetic variation were observed between Okra genotypes. Significant genetic variations at the maximum number of loci between genotypes indicate rich genetic resources in Okra.

The spatial distribution pattern of entry-wise genetic profiles (Fig. 4) was in complete agreement with the interrelationship displayed by hierarchical classification based on similarity coefficients. SSR primers-dependent genetic profiles of the genotypes along the two main axes demonstrated that the genotypes were conveniently accommodated into Four well-separated major genotypic groups. Therefore, hierarchical cluster analysis as well as principal coordinate analysis exposed that wide genetic variation existed among the evaluated Okra genotypes. perusal of the dendrogram and spatial distribution pattern exhibited that genetic polymorphism identified at the molecular level on the basis of variation in the size of simple sequence repeats was a practical resource for discriminating entries and analysing distinction and divergence. The panel of markers used in the current study showed a remarkably greater amount of genetic polymorphism, allowing for the distinctive genotyping of 28 entries under assessment. In addition, the markers used in this research appeared to be adequate to discriminate the 28 Okra entries under evaluation. Information on intra and inter-genotypic genetic variation from the present study might be useful for breeders in making decisions for the improvement of the Okra genotype through selective breeding and cross-breeding programs.

## Conclusion

The study aimed to estimate the genetic diversity among 28 different genotypes of okra through the analysis of genomic DNA and polymorphism of SSR markers. Superior quality of DNA extracted from the leaves at the seedling stage. PCR amplification of 15 SSR primers identified significant polymorphism, with 10 SSR primers showed 100% polymorphism, one SSR primer showed 50% polymorphism and four primers showed monomorphism among 28 Okra genotypes. The highest number of alleles was observed with primers, namely AVRDC OKRA 63, AVRDC OKRA 77, AVRDC OKRA 78, OKRA 86, OKRA 110, OKRA 109, OKRA 157, OKRA 175, OKRA 120 and OKRA 135 indicating genetic diversity among the genotypes. The PIC value ranged from 0.4 to 0.88, indicating varying degrees of genetic diversity among SSR loci. The dendrogram revealed that two major clusters were demarcated at a cut-off similarity coefficient level of 0.64, below which the similarity values narrowed conspicuously, cluster B was the largest and included 21 genotypes, while cluster A comprised 7 genotypes. The dendrogram and spatial distribution pattern confirmed the genetic polymorphism observed, facilitating discrimination and analysis of genotypic differences. The identified markers can serve as useful tools for genotyping Okra genotypes and making informed decisions in breeding programsand its implications for crop improvement.

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