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RESEARCH ARTICLE

Molecular and morphological characterization reveals high genetic diversity among okra (*Abelmoschus esculentus* (L.) Moench) genotypes

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Abstract

This study investigated the genetic diversity of 25 okra (*Abelmoschus esculentus* (L.) Moench) genotypes using phenotypic and molecular characterization methods. Phenotypic analysis of 11 quantitative traits revealed significant divergence among accessions, particularly for traits such as the coefficient of infection, average fruit weight, fruit yield and number of fruits per plant. High Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) values were observed for plant height, number of branches per plant, number of nodes per plant, average fruit weight, fruit yield and coefficient of infection. High heritability with genetic advance as a percentage of mean was observed for plant height, number of branches per plant, number of nodes per plant, internodal length, number of fruits per plant, fruit length, fruit girth, average fruit weight, fruit yield and coefficient of infection. The germplasm was categorised into six groups after Mahalanobis D^2 analysis. Molecular characterisation of the 50 SSR markers identified substantial genetic diversity. Twenty one markers were polymorphic, exhibiting 2-4 alleles per locus. Fifteen SSR markers showed high polymorphism information content values exceeding 0.5, with a range of 0.41 to 0.8. Jaccard's similarity coefficient ranged from 0.17 to 0.86 (average 0.63). Phenotypic and molecular analyses clustered the genotypes into six distinct groups, underscoring the considerable genetic variability within the studied okra germplasm. These findings provide valuable resources for future okra breeding programs.

Keywords: crop improvement; germplasm characterization; Mahalanobis; molecular markers; okra

Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) plays a vital role in global food security, particularly in tropical and subtropical regions of Africa and Asia. This warm-season, dicotyledonous annual thrives in diverse soil conditions, from sandy to clayey and has a growing season of 90-100 days. Okra is predominantly grown in Africa and Asia, with India being the world's second-largest producer, contributing to approximately 70 % of global production. Often cross -pollinated, okra exhibits a chromosome number of 2n = 130. This highly nutritious, low-fat vegetable is a rich source of protein, fiber, minerals, vitamin C, folate and vitamins B1, B6 and K. India, the world's second-largest producer, accounts for a substantial 73 % of the global okra area, with major production concentrated in states like West Bengal and Gujarat. However, despite its importance, comprehensive studies on okra's genetic diversity remain limited. Although okra cultivation spans diverse soil types and climates, okra production faces significant challenges, notably pests and diseases. Okra yellow vein mosaic virus (OYVMV), likely originating in India, poses a major threat, impacting plant growth, yield and fruit quality.

Developing resistant varieties through strategic breeding programs is crucial to mitigating OYVMV's impact (1). A diverse gene pool offers a high probability of identifying natural resistance genes against OYVMV. These genes can be incorporated into elite cultivars through breeding, thereby conferring resistance to the virus (2, 3). Relying on a single resistance gene can lead to the emergence of new viral strains that can overcome resistance. Genetic diversity allows breeders to pyramid multiple resistance genes, creating durable and long-lasting resistance against evolving OYVMV strains (4). Various resistant sources may confer protection against different OYVMV strains. Genetic diversity enables breeders to combine resistance genes that are effective against a wide range of viral strains, leading to broad-spectrum resistance (5). Genetic diversity

studies coupled with molecular markers have facilitated marker-assisted selection. Genetic diversity is the cornerstone of crop improvement. Understanding the variation within a species, such as okra, is crucial for the development of resilient and productive varieties. The Mahalanobis D^2 statistic, a powerful multivariate analysis technique, allows us to quantify this diversity based on key traits, providing a robust measure of genetic divergence. This complements molecular analysis, which offers a precise view of genetic diversity that is unaffected by environmental fluctuations (6). By exploring and utilizing the genetic diversity of okra, breeders can develop improved varieties with enhanced OYVMV resistance, contributing to sustainable okra production and global food security (7).

Molecular marker-based analysis, particularly using simple sequence repeat (SSR) markers, offers valuable insights into the genetic architecture of this crop. Understanding and preserving the genetic diversity of okra is essential for sustainable crop improvement and the development of resilient varieties. Okra's genetic diversity has not been extensively studied, warranting further research to elucidate the genetic architecture of this important vegetable crop.

Simple sequence repeats, also known as microsatellites, are highly effective molecular markers. These short repeating DNA sequences are highly polymorphic, making them ideal for distinguishing between different okra genotypes. SSRs have been widely used to assess genetic variation and relationships in okra and other crops (8, 9).They offer a rapid and reliable method for identifying unique genotypes and linking genetic markers to valuable traits, thereby accelerating the breeding process.

Okra shows extensive phenotypic, genotypic and genomic diversity that can be exploited for breeding, conservation and resilience. Recent chromosome-scale genome sequencing (≈1.19 Gb) revealed multiple whole-genome duplication events and an expanded gene repertoire for secondary metabolism and stress responses, genomic features that likely underlie okra's nutritional richness and adaptive potential (10).

Phenotypically, okra germplasm displays wide variation in vegetative and reproductive traits (plant height, days to flowering, number of branches, pod length/width, pod weight, number of pods per plant and yield components). Multivariate field studies and Mahalanobis D^2 analyses repeatedly group accessions into distinct divergence clusters, indicating clear, exploitable morphological divergence among cultivars, landraces and advanced lines. This morphological divergence provides breeders with trait combinations for heterosis breeding, trait-specific selection and parental choice (11).

At the molecular level, SSR (microsatellite) marker surveys across regional collections detect moderate to high allelic richness, gene diversity and population structure, often separating accessions by agro-ecological origin or by landrace vs. improved cultivar status. SSR studies (and transcriptome-derived EST-SSRs) have proven effective for fingerprinting, genetic structure analysis, germplasm management and marker-assisted selection for target traits. Combining SSR results with phenotypic data often reveals concordant groupings and helps identify genetically distinct, agronomically promising genotypes (12).

Okra production is geographically concentrated, with India as a dominant producer (a large share of global production commonly reported around ≈60-70 % depending on the data source and year), followed by major contributions from countries in West Africa and parts of Asia. This concentration means large reservoirs of landrace diversity exist within the Indian subcontinent and neighbouring regions; but it also signals risk, as reliance on a narrow set of improved cultivars can reduce effective diversity and increase vulnerability to pests and diseases (13).

Biotic constraints notably Yellow Vein Mosaic Virus (YVMV) and whitefly vectors, cause serious yield losses in okra and are the subject of intense breeding research. Developing durable YVMV resistance benefits directly from broad germplasm sampling: (1) diverse resistance sources reduce the chance of a single pathogen strain breaking resistance, (2) combining (pyramiding) multiple resistance loci can give broader and longer-lasting protection and (3) combining molecular markers with phenotyping accelerates introgression of resistance into elite backgrounds. Recent breeding and molecular pathology studies emphasize integrated strategies (germplasm screening, marker development and wide hybridization) to broaden the resistance base (14).

Finally, integrating Mahalanobis D^2 (multivariate phenotypic divergence), SSR marker-based diversity and genome resources provides a robust multi-tiered strategy to (a) quantify diversity, (b) rationally select parents with complementary divergence for crosses, (c) identify marker-trait associations for MAS and (d) conserve genetically unique accessions. The synergy between field-based multivariate analyses and molecular/genomic tools is therefore central to unlocking okra's breeding potential and building durable, resilient cultivars (15).

This study aimed to leverage both phenotypic and molecular approaches, using the Mahalanobis D^2 statistic and SSR markers, to comprehensively assess the genetic diversity within a collection of okra germplasms. Our objectives were to characterize morphological diversity, analyse molecular diversity using SSR markers and identify superior genotypes based on both phenotypic and molecular data. The central hypothesis of this research is that significant genetic variability exists among the okra germplasms and that integrating phenotypic and molecular analyses will more accurately reveal distinct diversity patterns and help identify genetically superior lines for future breeding programmes. This integrated approach provides valuable insights for the improvement and conservation of okra germplasm.

Materials and Methods

A total of 25 genotypes of okra (*Abelmoschus esculentus* (L.) Moench), originated from different agro-climatic zones, were obtained (Table 1). Field and laboratory experiments were conducted at the Department of Genetics and Plant Breeding Farm, Annamalai University, during April 2023. Molecular analysis was performed at the Molecular Biology Laboratory of Annamalai University, Chidambaram.

Field evaluation and data collection

The experiment was conducted in a randomised complete block design with three replications, with a spacing of 30 cm between the plants and a row–to-row distance of 45 cm for phenotypic evaluation. The recommended plant protection measures were followed to raise healthy crops. The data were recorded for 11 quantitative characteristics: days to first flowering, plant height, number of branches per plant, number of nodes per plant,

Table 1. List of okra genotypes used in the study

S No.	Genotypes	Source
1	Kashi Lalima	(IIVR), Varanasi
2	Solar 600	Solar seeds, Bengaluru
3	Arka Abhay	IIHR, Bengaluru
4	Pusa Sawani	IARI, New Delhi
5	Ashoka	Ashoka seeds, Bangalore urban
6	Ankur 41	Ankur Seeds, Aurangabad
7	Summer gold	Released by private company
8	Thorn okra	Obtained Namakkal local
9	Red okra	Aadhiyagi seeds, Oddanchatram
10	Arka Anamika	IIHR, Bengaluru
11	Elephant trunk long	Obtained Namakkal local
12	Green long	Obtained Namakkal local
13	Green paruman long	Obtained Namakkal local
14	Villupuram local	Local cultivar from Tamil Nau
15	Salem local	Local cultivar from Tamil Nau
16	Red round okra	Aadhiyagi seeds, oddanchatram
17	Malai okra	Aadhiyagi seeds, oddanchatram
18	Tree okra	PDK garden, Trichy
19	Chidambaram local	Local cultivar from Tamil Nau
20	White okra	PDK garden, Trichy
21	Double colour okra	Aadhiyagi seeds, oddanchatram
22	Elephant husk okra	Aadhiyagi seeds, oddanchatram
23	Green okra	Aadhiyagi seeds, oddanchatram
24	Seven-line okra	PDK garden, Trichy
25	Kasturi okra	PDK garden, Trichy

internodal length, number of fruits per plant, fruit length, fruit girth, average fruit weight, fruit yield per plant and coefficient of infection.

Analysis of variance

Mean values of the genotypes in each replication were subjected to analysis of variance (11). Coefficient of variation was calculated using the standard formula (12). The broad sense heritability for each trait was computed using the commonly used formula (13). Genetic advances were classified according to a previously established method (14).

DNA extraction and SSR marker amplification

Genomic DNA was extracted from young okra leaves using a modified CTAB method (16). Briefly, 2 g of leaf tissue was ground in 1 mL of CTAB buffer containing 5 µL of β-mercaptoethanol and polyvinylpyrrolidone (PVP). After incubation at 60 °C for 40 min, the mixture was extracted using chloroform: isoamyl alcohol (24:1). DNA was precipitated using isopropanol, washed with 70 % ethanol and resuspended in TE buffer. DNA quality was assessed using 0.8 % agarose gel electrophoresis.

A set of 50 SSR primers was used for PCR amplification. Each 15 µL reaction contained 10X Taq buffer with magnesium chloride ($MgCl_2$), deoxynucleoside triphosphates (dNTPs), Taq DNA polymerase, forward and reverse primers, template DNA and sterile distilled water. The PCR cycling conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 ° C for 2 min, with a final extension at 72 °C for 7 min. The amplified products were separated on a 3 % agarose gel and visualized using ethidium bromide staining. A 100 bp DNA ladder was used for size estimation.

Statistical analysis

Statistical analysis was performed using TNAU STAT. Cluster analysis was performed using the conventional formula (14). A binary matrix representing SSR marker amplification profiles was used to calculate Jaccard's similarity coefficient between all okra accessions. This analysis, performed using DARwin 6.0 software (17), provided a measure of pairwise relatedness based on shared alleles. A dendrogram illustrating the relationships among accessions was constructed using the unweighted neighbour joining method with 1000 bootstrap replicates.

Results

Performance of genotypes based on quantitative traits

The significant variation observed across all 11 quantitative traits indicates the presence of substantial genetic diversity among the evaluated okra genotypes. The superior fruit yield recorded in Arka Anamika and the lower YVMV incidence in Arka Abhay and Ankur 41 demonstrate the coexistence of yield potential and disease tolerance within the germplasm. Such variations in earliness, fruiting behavior and disease response are consistent with recent studies reporting strong genotypic differences for yield and YVMV resistance in okra (18-20). These findings support the use of these genotypes as parents in improvement programmes, especially where both high productivity and virus tolerance are required.

Genetic variability

Phenotypic and genotypic coefficient of variation were categorised as low (below 10 %), medium (10-20 %) and high (above 20 %) to draw conclusions about these parameters. Higher estimates of the GCV and PCV coefficients of variation were observed for several traits. Plant height (23.57, 25.19), number of branches per plant (23.57, 25.19), number of nodes per plant (22.6, 23.21), number of fruits per plant (26.47, 26.97), average fruit weight (22.89, 23.43), fruit yield per plant (27.10, 27.60) and coefficient of infection (64.63, 65.36) all had high intensities of PCV and GCV. The moderate values of PCV and GCV were observed for internodal length (19.19,

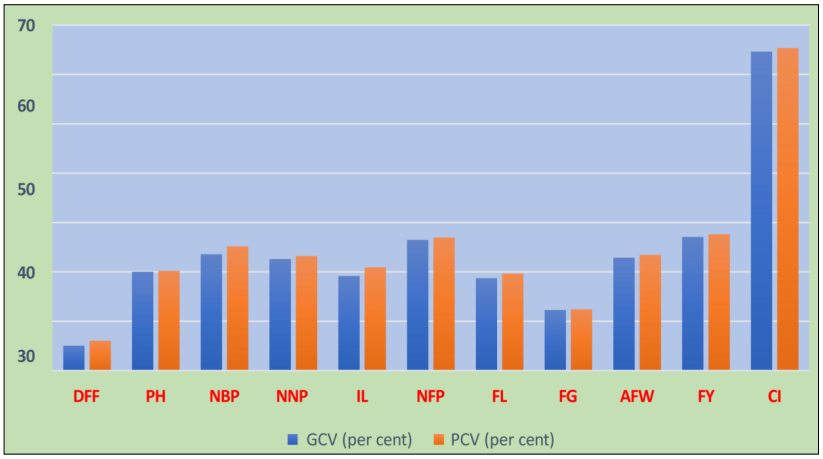

Fig. 1. Phenotypic and genotypic coefficient of variation in per cent for 11 quantitative traits.

20.90), fruit length (18.74, 19.66) and fruit girth (12.28, 12.44). Low values of GCV and PCV were observed for days to first flower (5.08, 6.07) (Fig. 1).

Heritability is a measure of the extent of phenotypic variation caused by additive gene action. Broad-sense heritability was estimated for all the characters under study and ranged from 70.16 to 97.84 %. High heritability (>60 %) was observed for the following traits: days to first flower (DFF), 70.16 %; plant height (PH), 97.84 %; number of branches per plant (NBP), 87.55 %; number of nodes per plant (NNP), 94.83 %; internodal length (IL), 84.25 %; number of fruits per plant (NFP), 96.32 %; fruit length (FL), 90.84 %; fruit girth (FG), 97.46 %; average fruit weight (AFW), 95.46 %; fruit yield per plant (FY), 96.39 %; coefficient of infection (97.78 %) (Fig. 2). The genetic advance (% of the mean at 5 % selection intensity) ranged from 8.77 to 131.65 %. Plant height (40.75 %), number of branches per plant (45.44 %), number of nodes per plant (45.34 %), internodal length (36.28 %), number of fruits per plant (53.52 %), average fruit weight (46.07 %), fruit length (36.79 %), fruit girth (24.97 %), fruit yield per plant (54.81 %) and coefficient of infection (132.91 %) exhibited high (>20 %) genetic advance as a percentage of the mean. Low genetic advance (% of the mean <10 %) was observed for days to first flower (8.77 %) (Fig. 2).

Clustering based on quantitative characters

Clustering analysis of 25 genotypes based on 11 quantitative traits was performed using TNAU STAT and a dendrogram was constructed, as shown in Fig. 3. The studied genotypes were broadly divided into six clusters. Cluster I was the largest with 13 genotypes; cluster II with eight genotypes; and clusters III, IV, V and VI with one genotype each (Table 2). The separation of genotypes into separate clusters indicates the existence of a significant level of genetic diversity in the investigated material. The presence of significant genetic diversity among the parental materials evaluated in this study suggests that this material may be a suitable source for choosing diverse parents for a hybridization program. Similar findings were reported by previous researchers in studies involving 13 genotypes (9), 25 genotypes (22) and 46 genotypes (23)of okra crop.

Intra and inter cluster average distances

The average D^2 values of intra and inter-cluster distances are shown in Fig. 3. Genotypes that were grouped in the same cluster were less divergent than those that were placed in different clusters. The intra-cluster (same cluster) distance ranged from 0.00 and 7942.14. The maximum differences among the genotypes were shown by cluster II (7942.14), whereas clusters III, IV, V and VI showed zero intra-cluster distance.

The diversity among the clusters varied from 10651.34 to 36802.59. The maximum inter-cluster distance was observed between clusters IV and VI (36802.59), followed by clusters V and VI (31648.61) and clusters I and VI (28247.72). The minimum inter-cluster distance was observed between clusters I and III (10651.34), followed by clusters II and III (12028.58) and clusters II and IV (12805.68).

Percent contribution of characters towards divergence

The percent contribution of each character towards divergence is presented in Fig. 4. Coefficient of infection (51.33 %) was found to be the most significant contributor to divergence, followed by average fruit weight (20.66 %), fruit yield (12.33 %), number of fruits per plant (4.66 %), fruit length (4.33 %), plant height (3 %), internodal length (2.33 %), number of nodes per plant (1 %), fruit girth (0.33 %) and days to first flower. The number of branches per plant did not contribute to total divergence.

SSR polymorphism

Fifty SSR primers were used to determine genetic diversity in a set of 25 okra genotypes. SSR primers that produced reproducible and clear scorable amplification products were selected in the present study for genetic diversity analysis. Out of the 50 tested, 21 polymorphic SSR markers provided valuable insights into the genetic diversity of the okra collection. The number of alleles per locus ranged from 17 to 64. The alleles identified in the 25 genotypes were classified into two categories, shared alleles and unique alleles.

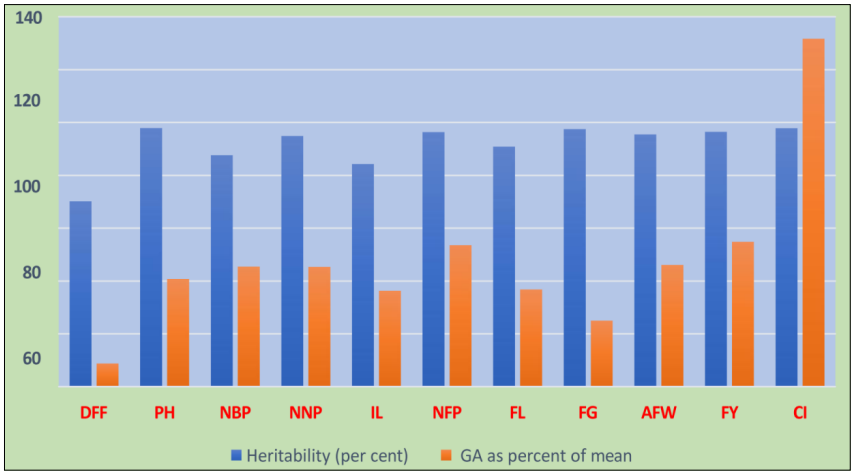

Fig. 2. Heritability and genetic advance as per cent of the mean for 11 quantitative traits.

Table 2. Distribution of 25 okra genotypes among different clusters based on Mahalanobis D^2 analysis

Clusters	Number of genotypes	Genotypes
1	13	Arka Abhay, Ankur 41, Solar 600, Red round okra, Arka Anamika, Malai okra, Green paruman, Thorn okra, Kashi Lalima, Elephant husk okra, Chidambaram local, Summer gold, Elephant trunk long
2	8	Pusa Sawani, Ashoka, Salem local, White okra, Double colour okra, Kasturi okra, Red okra and Villupuram okra
3	1	Green long
4	1	Green okra
5	1	Seven-line okra
6	1	Tree okra

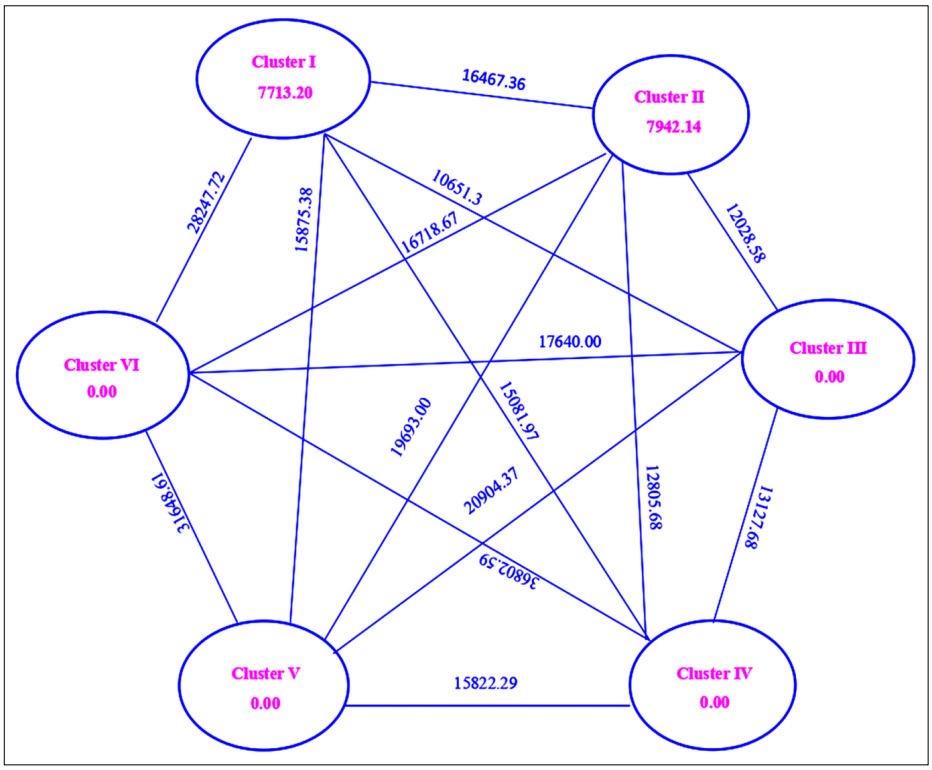

Fig. 3. Cluster diagram showing average intra- and inter-cluster D^2 values of okra genotypes.

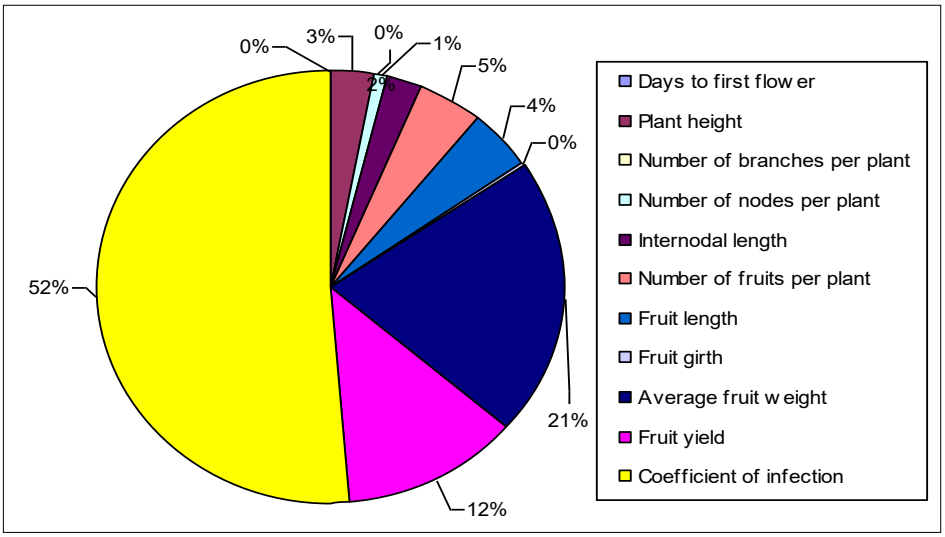

Fig. 4. Contribution of various quantitative traits to the divergence of genotypes.

A total of 534 shared alleles and 27 unique alleles were generated in the form of amplified products, using 21 primer pairs. The overall size of the PCR products amplified using the 21 SSR primer pairs ranged from 95 to 350 bp. The PIC value ranged from 0.41 in the case of okra 141 and 0.80 in the case of okra 105, with an average value of 0.62. Fifteen SSR markers exhibited high PIC values (> 0.5), indicating their informativeness in distinguishing between genotypes and assessing genetic diversity (Table 3). Jaccard's similarity coefficient, calculated based on the SSR data, ranged from 0.17 to 0.86, with an average of 0.63 (Table 4), further demonstrating the genetic variability within the studied genotypes. This information can be leveraged for targeted selection and breeding strategies aimed at improving yield, disease resistance and other important traits of okra.

Cluster analysis and genetic distance

A dendrogram constructed based on the SSR analysis using DARwin is shown in Fig. 5. The 25 genotypes studied were broadly divided into six clusters and 16 sub-clusters. These clusters included Cluster I with six genotypes, Cluster II with three genotypes, Cluster III with one genotype, Cluster IV with six genotypes, Cluster V with six genotypes and Cluster VI with three genotypes. The genetic relationships among okra genotypes were assessed using UPGMA cluster analysis of the similarity coefficient.

Discussion

The significant variation observed across all 11 quantitative traits indicates the presence of substantial genetic diversity among the evaluated okra genotypes. The superior fruit yield recorded in Arka Anamika and the lower YVMV incidence in Arka Abhay and Ankur 41 demonstrate the coexistence of yield potential and disease tolerance within the germplasm. Such variations in earliness, fruiting behavior and disease response are consistent with recent studies reporting strong genotypic differences for yield and YVMV resistance in okra (21-23). These findings support the use of these genotypes as parents in improvement programmes, especially where both high productivity and virus tolerance are required.

Table 3. Summary of SSR markers showing polymorphism

Primer	No of locus	Size of alleles (bp)	No of alleles	PIC
Okra 77	3	171 - 223	18	0.76
Okra 12	2	166 -204	17	0.73
Okra 39	3	125 -160	25	0.65
Okra 63	2	128-152	25	0.5
Okra 125	2	115-167	18	0.72
Okra 137	3	102-183	24	0.60
Okra 14	3	178-205	18	0.77
Okra 124	3	150-254	25	0.51
Okra 109	2	144-196	21	0.65
Okra 103	2	138- 164	25	0.48
Okra 141	3	95-120	29	0.41
Okra 111	2	146-189	24	0.49
Okra 166	3	100-135	26	0.43
Okra 148	3	211-289	41	0.66
Okra 89	2	190-220	17	0.76
Okra 54	3	203-250	25	0.64
Okra 1	3	133- 176	24	0.65
Okra 52	3	110-148	26	0.61
Okra 64	4	150-350	69	0.72
Okra 110	2	275-300	31	0.47
Okra 105	2	230-290	33	0.8
Average	55		561	0.62

Table 4. Similarity coefficient analysis

Unit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
e	0.5																							
3	0.52	0.4																						
4	0.59	0.81	0.76																					
5	0.55	0.67	0.67	0.28																				
6	0.52	0.39	0.25	0.71	0.59																			
7	0.72	0.66	0.70	0.64	0.78	0.67																		
8	0.67	0.56	0.48	0.68	0.61	0.53	0.48																	
9	0.63	0.61	0.57	0.71	0.61	0.58	0.63	0.45																
10	0.53	0.52	0.39	0.68	0.61	0.43	0.63	0.4	0.55															
11	0.57	0.55	0.52	0.43	0.5	0.47	0.52	0.62	0.58	0.48														
12	0.76	0.81	0.67	0.61	0.63	0.75	0.74	0.69	0.61	0.79	0.71													
13	0.68	0.61	0.48	0.69	0.61	0.53	0.64	0.55	0.39	0.64	0.58	0.46												
14	0.83	0.78	0.70	0.70	0.67	0.75	0.7	0.61	0.61	0.76	0.71	0.43	0.52											
15	0.72	0.74	0.66	0.76	0.70	0.71	0.73	0.68	0.59	0.75	0.81	0.35	0.44	0.46										
16	0.74	0.64	0.52	0.74	0.64	0.61	0.67	0.53	0.62	0.62	0.69	0.55	0.44	0.5	0.53									
17	0.78	0.72	0.68	0.68	0.61	0.73	0.63	0.59	0.55	0.74	0.62	0.52	0.55	0.47	0.5	0.43								
18	0.82	0.66	0.69	0.72	0.66	0.70	0.65	0.56	0.64	0.68	0.74	0.62	0.61	0.38	0.52	0.59	0.52							
19	0.78	0.69	0.72	0.72	0.69	0.79	0.68	0.67	0.67	0.73	0.66	0.65	0.71	0.56	0.64	0.53	0.34	0.52						
20	0.72	0.66	0.73	0.73	0.74	0.78	0.69	0.68	0.68	0.71	0.71	0.74	0.65	0.70	0.65	0.58	0.59	0.56	0.45					
21	0.76	0.84	0.83	0.65	0.66	0.84	0.81	0.78	0.68	0.78	0.71	0.57	0.6	0.51	0.55	0.71	0.55	0.56	0.55	0.56				
22	0.71	0.60	0.53	0.82	0.72	0.66	0.76	0.59	0.63	0.59	0.76	0.69	0.59	0.65	0.59	0.53	0.59	0.51	0.54	0.55	0.59			
23	0.72	0.84	0.76	0.6	0.61	0.81	0.85	0.85	0.75	0.78	0.67	0.61	0.69	0.61	0.65	0.71	0.64	0.65	0.59	0.65	0.32	0.68		
24	0.73	0.63	0.86	0.81	0.71	0.68	0.78	0.65	0.60	0.72	0.75	0.71	0.66	0.63	0.70	0.64	0.65	0.48	0.51	0.57	0.61	0.41	0.61	
25	0.74	0.85	0.86	0.67	0.68	0.85	0.82	0.83	0.69	0.83	0.72	0.64	0.67	0.59	0.63	0.76	0.58	0.59	0.62	0.63	0.17	0.66	0.37	0.59

(1) Kashi Lalima (2) Solar 600 (3) Arka Abhay (4) Pusa Sawani (5) Ashoka (6) Ankur 41 (7) Summer gold (8) Thorn okra (9) Red okra (10) Arka Anamika (11) Elephant trunk long (12) Green long (13) Green paruman long (14) Villupuram local (15) Salem local (16) Red round okra (17) Malai okra (18) Tree okra (19) Chidambaram local (20) White okra (21) Double colour okra (22) Elephant husk okra (23) Green okra (24) Seven-line okra

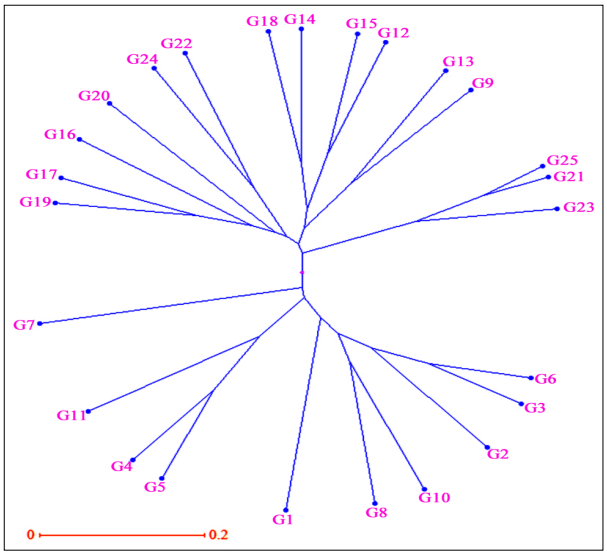

Fig. 5. Cluster diagram for molecular assessment.

High PCV and GCV values for traits such as plant height, number of branches, number of nodes, number of fruits, average fruit weight and fruit yield indicate strong inherent variability and less environmental influence on expression. High heritability combined with high genetic advance, as observed for most yield contributing traits, indicates the predominance of additive gene action. This implies that simple phenotypic selection would be effective in improving these traits. Similar conclusions have been documented in recent okra diversity studies, where yield traits consistently showed high heritability and high genetic gain (24, 25).

The formation of six distinct clusters indicates a wide range of genetic diversity among the 25 genotypes. The large clusters (I and II) reflect the presence of related or moderately similar genotypes, whereas clusters III - VI, each containing a single genotype, represent highly unique lines. Similar clustering patterns were recently reported in okra germplasm evaluations involving 13, 25 and 46 genotypes (26, 27), confirming that okra breeding populations often show structured yet distinct diversity blocks.

High inter-cluster distances, particularly between clusters IV and VI, reflect substantial genetic divergence, indicating that crossing parents from these groups would likely produce superior segregants with greater variability. Conversely, the smaller distances observed between clusters I and III as well as II and III indicate closer relatedness. Such distance-based patterns align with Mahalanobis D^2 studies in okra showing that wide inter-cluster separation is a reliable predictor of heterotic performance and transgressive segregation (28, 29).

The coefficient of infection contributed the most to total divergence, followed by average fruit weight and fruit yield. This highlights that disease reaction, fruit size and yield traits are the major drivers of genetic differentiation in okra. Recent reports also confirm that disease related traits and fruit size parameters contribute substantially to overall diversity and are key discriminators for genotype classification (22, 23).

The 21 polymorphic SSR markers used in the study revealed substantial allelic richness, with 17-64 alleles per locus and an average PIC of 0.62. The high PIC values in 15 markers indicate their suitability for detecting diversity and differentiating genotypes. The Jaccard similarity range (0.17-0.86) further supports the presence of both closely related and genetically distant genotypes.

These results agree with recent molecular studies in okra showing that SSR markers provide high polymorphism and reliably reflect true genetic diversity across germplasm collections (30, 31). The presence of unique alleles is especially important for identifying donor parents in breeding for YVMV resistance and yield improvement.

The UPGMA dendrogram separated the 25 genotypes into six major clusters and 16 sub-clusters, indicating strong molecular differentiation. The partial correspondence between molecular and morphological clustering emphasizes that both marker-based and trait-based analyses capture complementary aspects of diversity. Similar SSR-based clustering patterns have been documented in recent okra genetic diversity reports (32).

Genetic variability

The level of genetic variation for each characteristic and the heritability of the desired features are the two main factors influencing crop development programs. Plant height, number of branches per plant, number of nodes per plant, number of fruits per plant, average fruit weight, fruit yield and coefficient of infection all had high PCV and GCV intensities. These results are in consonance with previous findings in okra (33).

Moderate PCV and GCV were observed for the following traits: internodal length, fruit length and fruit girth. Previous studies have reported moderate PCV and GCV for internodal length (34), fruit length and fruit girth (35). Low (<10 %) PCV and GCV values were recorded for days to first flower as documented earlier (36). Low PCV and GCV values for traits suggest a greater influence of the environment on these traits, implying that phenotypic selection would be ineffective for breeding programs.

Heritability

The degree of phenotypic diversity caused by additive gene action is determined by heritability. Heritability is a useful indicator of how successfully features are transmitted from the parents to their progeny (37). Plant height, number of branches per plant, number of nodes per plant, internodal length, number of fruits per plant, fruit length, fruit girth, average fruit weight, fruit yield and coefficient of infection all had high heritability coupled with high genetic advance as a percentage of the mean, indicating that additive genes control these parameters and that selection would be beneficial for improving such parameters. This suggests that such traits may be generated through direct selection. Similar findings of coupled high heritability and genetic advance as per cent of the mean were also observed earlier (38). The trait days to first flowering exhibited high heritability with low genetic advance as per cent of the mean.

Morphological diversity (Mahalanobis D^2)

The Mahalanobis D^2 statistic is a valuable tool for quantifying the genetic divergence between genotypes and relating clustering patterns to geographic origin. Previous researchers have proven the efficacy and use of multivariate methods to quantify genetic diversity (39). The genetic divergence evaluation based on Mahalanobis D^2 statistics resulted in the classification of the 25 genotypes into six clusters (40). Cluster I had the most genotypes of the six clusters (13 genotypes), followed by Cluster II (8 genotypes) and then Clusters III, IV, V and VI (1 genotype each). The presence of significant genetic diversity among the parental materials evaluated in this study suggests that this material may be a suitable source for choosing diversified parents for hybridization programs. Similar findings were previously reported in 46 okra genotypes (22).

The average D^2 values of intra and inter-cluster distances are shown in Fig. 3. The diversity within clusters ranges from 0.00 to 7942.14, while inter-cluster distances range from 10651.34 to 36802.59. The maximum difference within the same cluster (intra-cluster) was observed in Cluster II, whereas the greatest difference among clusters (inter-cluster) was found between Clusters IV and VI. The above-mentioned data demonstrates a high degree of genetic variation among genotypes within different clusters and even within clusters, suggesting that the genetic bases of the genotypes are diverse. Similar to the current study, prior studies also observed adequate genetic diversity across various okra genotypes (22, 41).

Percent contribution of characters towards divergence

In the present study, the coefficient of infection was found to be the most significant contributor to divergence among the 25 okra genotypes, followed by average fruit weight and fruit yield. Previous evidence also supports that the coefficient of infection contributed the most to the total genetic divergence (42).

Genotyping studies based on SSR markers

One popular technique for quickly and easily analysing a large number of loci spread throughout the plant genome that does not consider environmental impacts is the use of molecular markers. It is also an effective method for evaluating genetic variations. Knowledge of molecular genetic variability may be applied for the identification of genetically distinct germplasms that enhance known genotypes. In the present study, the size of the amplified products varied from 95 to 350 bp. The PIC values ranged from 0.41 to 0.80. The highest PIC value was recorded for the primer okra 105 (0.8) and the minimum PIC value was recorded for the primer okra 141 (0.41). It was observed that the marker detecting a lower number of alleles showed lower gene diversity than those which detected a higher number of alleles which revealed higher gene diversity. Similar work was performed on okra and the reported PIC across all 50 loci values ranged from 0.00 to 0.865 with a mean value of 0.519. Alleles per locus ranged from 1 to 27 (43). The eight SSR markers used for studying genetic diversity revealed that the PIC value ranged from 0.37 to 0.86, with a mean value of 0.76 (44). Similarly, a study to evaluate genetic diversity using 82 SSR markers, out of which 37 SSR markers produced polymorphic bands, was reported. The PIC value ranged from 0.14 to 0.74 with an average of 0.54 (45).

The genetic relationship among okra was assessed using a UPGMA cluster analysis of the similarity coefficient. A dendrogram was constructed using six clusters and 16 subclusters. Similar findings were obtained for genetic variation in the seven okra landraces. DNA was extracted and subjected to PCR using 27 RAPD markers. The dendrogram derived from RAPD data categorized the genotypes into two primary clusters (46). The genotypes were divided into two major clusters based on cluster analysis using the UPGMA algorithm (47). The main goal is to find genetically distant genotypes with YVM-resistant traits which in turn are utilized to develop YVMV-resistant and high-yielding varieties. In the present study, the similarity coefficient for the five genotypes was computed based on the presence and absence of the amplified products using 21 pairs of primers. The analysis revealed genetic dissimilarity which clearly distinguished between the resistant and susceptible genotypes. The similarity coefficient values were highest between the genotypes Seven-line okra and Arka Abhay (86 %) followed by Kasthuri okra and Arka Abhay (86 %) and the similarity coefficient was the minimum between Kasthuri okra and Double colour okra (17 %). It was observed that genotypes that express less genetic similarity are highly diverse. The genetic similarity ranged from 14 to 86 %. A study evaluated the genetic similarity for ten okra genotypes using ISSRs and showed that the similarity values ranged from 0.714 to 1.00, with an average of 0.857 (48).

Comparison of morphological and molecular data

Both morphological (Mahalanobis D^2 analysis) and molecular analyses revealed six distinct clusters among the 25 okra genotypes. The congruence between phenotypic and genotypic clustering suggests that SSR markers can effectively reflect the phenotypic variation observed in this study (Fig. 6). However, it is important to note that the correlation between molecular markers and phenotypic traits can vary depending on the specific traits and the populations under investigation (49).

High-yielding and YVMV-resistant genotypes, including Arka Abhay, Ankur 41, Arka Anamika and Solar 600, clustered together in both the morphological and molecular analyses (Clusters I and V in D^2 analysis and Cluster I in SSR analysis). This colocalisation highlights the potential of using SSR markers to identify superior genotypes that combine desirable yield and disease resistance traits. Other genotypes exhibiting moderate resistance may be valuable resources for future breeding programs(50).

Out of the 50 SSR markers tested, 21 polymorphic SSR markers provided valuable insights into the genetic diversity of the okra collection. These markers revealed a range of 2-4 alleles per locus, with PIC values ranging from 0.41 to 0.80. Fifteen SSR markers exhibited high PIC values (> 0.5), indicating their informativeness in distinguishing between genotypes and assessing genetic diversity. Jaccard's similarity coefficient, calculated based on the SSR data, ranged from 0.17 to 0.86, with an average of 0.63, further demonstrating the genetic variability within the studied genotypes. This information can be leveraged for targeted selection and breeding strategies aimed at improving yield, disease resistance and other important traits of okra.

Conclusion

This study revealed substantial genetic diversity among 25 okra genotypes through both phenotypic evaluation and SSR analysis. High - performing and disease - resistant genotypes such as Arka Anamika, Arka Abhay, Ankur 41 and Solar 600 showed clear superiority for key agronomic traits. D^2 clustering and SSR markers (21 polymorphic loci; PIC 0.41-0.80) confirmed strong genetic divergence, with markers like Okra 105 and Okra 141 proving highly informative for YVMV resistance. The diversity identified provides valuable parents for breeding programs aimed at improving yield and disease resistance. These findings support sustainable okra cultivation by enabling the development and adoption of resilient, high-yielding varieties suited to current and future production challenges.

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Authors' contributions

PS and MS performed the study. PS wrote the manuscript. TS, SS and BR conceived the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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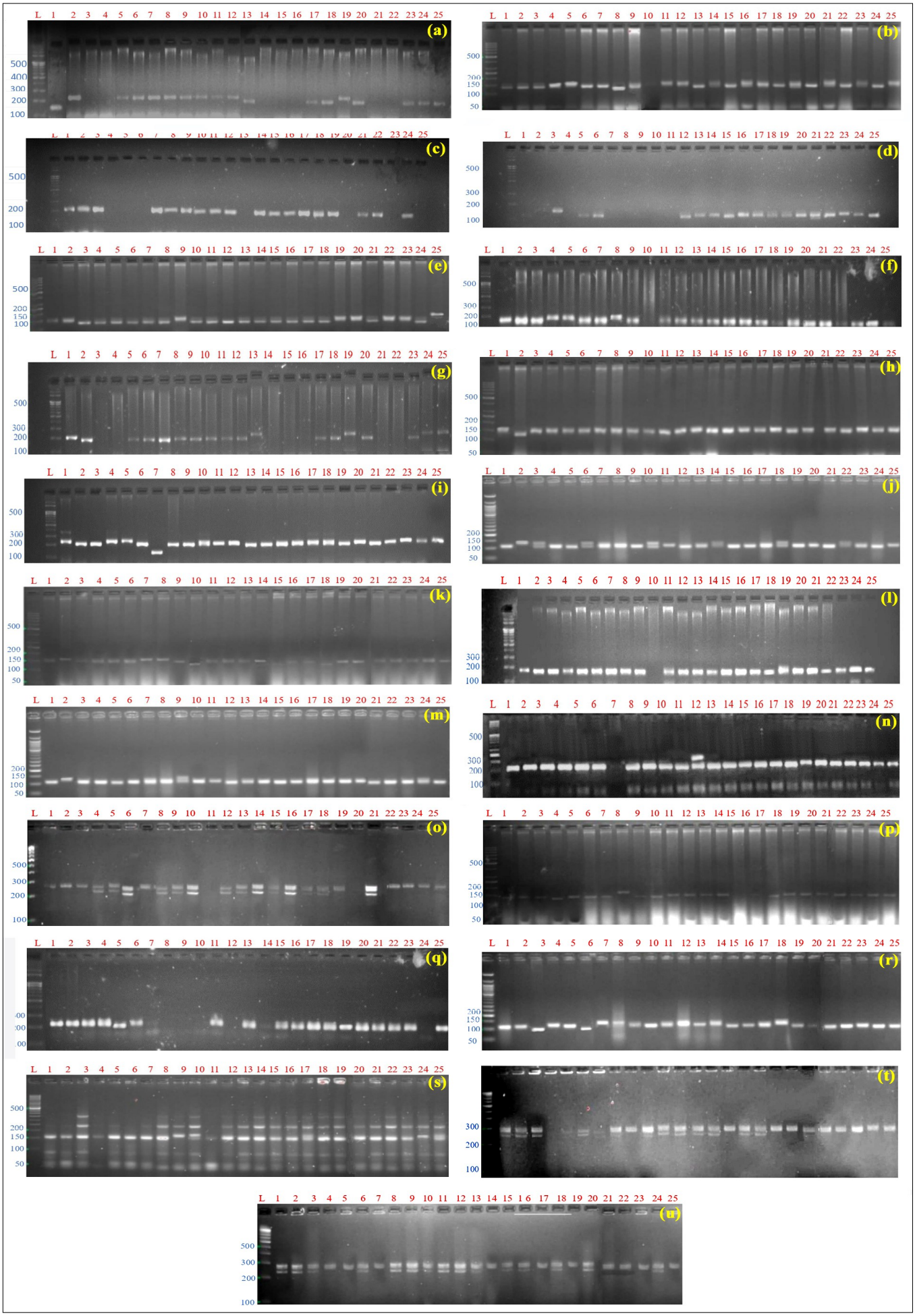

Fig. 6. Gel profiles showing the amplification of SSR primers with 25 okra genotypes. (a) 77, (b) 63, (c) 12, (d) 125, (e) 39, (f) 137, (g) 14, (h) 103, (i) 124, (j) 141, (k) 109, (l) 111, (m) 166, (n) 54, (o) 148, (p) 1, (q) 89, (r) 52, (s) 64, (t) 110, (u) 105.

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