International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 01-05-2026, Accepted: 01-06-2026, Published: 16-06-2026
Volume 11, Issue 2, 2026, Page No. 261-264

Genetic diversity analysis of tomato genotypes and varieties using RAPD markers

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DOI: https://doi.org/10.66856/ijfsn.2026.11.2.11094

Abstract
Assessment of genetic diversity is essential for the effective utilization of germplasm in crop improvement programmes. The present investigation was undertaken to evaluate the genetic diversity among twenty-two tomato genotypes using Random Amplified Polymorphic DNA (RAPD) markers. Genomic DNA isolated from all genotypes was amplified using twenty RAPD primers. A total of 231 amplified bands were generated, of which 206 were polymorphic, indicating a high level of genetic variation among the evaluated genotypes. The average polymorphism recorded was 86.94%. The number of amplified fragments per primer ranged from 4 to 20. Polymorphic information content (PIC) values ranged from 0.70 to 0.93 with an average value of 0.8665, demonstrating the high informativeness of the selected primers. Genetic similarity coefficients estimated using Jaccard's coefficient ranged from 0.29 to 0.85. Cluster analysis based on the UPGMA method grouped the genotypes into two major clusters, revealing substantial genetic divergence among the studied germplasm. The results demonstrated the effectiveness of RAPD markers in detecting genetic variability and identifying genetically diverse tomato genotypes that can be utilized in future breeding programmes.

Keywords: Tomato (*Solanum lycopersicum* L.), RAPD markers, genetic diversity, molecular characterization, germplasm, genetic relationship, UPGMA

Introduction
Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops in the world and is valued for its nutritional, economic and industrial importance. The crop is an important source of vitamins, minerals, carotenoids and antioxidants, particularly lycopene, which contribute significantly to human health. Continuous improvement of tomato cultivars requires the availability of genetically diverse germplasm resources. Assessment of genetic diversity among germplasm resources is a prerequisite for effective crop improvement programmes. Information on genetic relationships among genotypes facilitates the identification of diverse parental lines for hybridization and helps in the conservation and utilization of genetic resources (Govindaraj *et al*., 2015; Mohammadi and Prasanna, 2003) [10]. Morphological traits are often influenced by environmental factors and may not accurately reflect genetic relationships. Therefore, molecular markers have become valuable tools for the assessment of genetic diversity and characterization of germplasm. Among the various molecular marker systems, Random Amplified Polymorphic DNA (RAPD) markers are simple, rapid and cost-effective. RAPD markers require no prior sequence information and have been widely employed for genetic diversity studies, cultivar identification and phylogenetic analysis in several crop species. RAPD markers have been successfully used in tomato for evaluating genetic variability, cultivar identification and assessment of genetic relationships among breeding materials because of their simplicity, low cost and ability to detect DNA polymorphism without prior sequence information (Williams *et al.,* 1990; He *et al.,* 2003) [11]. The present study was therefore undertaken to assess the genetic diversity and relationships among twenty-two tomato genotypes using RAPD markers.

Materials and Methods
Plant Material
Twenty-two tomato genotypes and varieties were obtained from the Vegetable Research Station, Junagadh Agricultural University, Junagadh, Gujarat, India. Young healthy leaves were collected from each genotype for genomic DNA extraction.

DNA Isolation
Genomic DNA was isolated using the modified CTAB method described by Doyle and Doyle (1990) [3]. The quality and quantity of DNA samples were assessed prior to PCR amplification.

RAPD Analysis
Twenty RAPD primers showing clear and reproducible amplification patterns were selected for diversity analysis. PCR amplification was carried out following the procedure described by Williams *et al*. (1990) [11] with suitable modifications. Amplified products were separated on agarose gel, visualized under UV illumination and documented using a gel documentation system. Bands were scored as present (1) or absent (0) to generate a binary data matrix.

Data Analysis
Percentage polymorphism, polymorphic information content (PIC) and RAPD Primer Index (RPI) were calculated for

each primer. Genetic similarity coefficients among genotypes were estimated using Jaccard's similarity coefficient. Cluster analysis was performed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm implemented in NTSYSpc software.

Results and Discussion

RAPD Marker Polymorphism

Twenty RAPD primers produced a total of 231 amplified bands, of which 206 were polymorphic, indicating substantial genetic variability among the evaluated tomato genotypes (Table 1). The average polymorphism across primers was 86.94%. The number of amplified fragments ranged from 4 to 20 per primer. Primer OPN-04 generated the highest number of bands (20), followed by OPM-05 and OPN-01 with 18 bands each, whereas OPM-02 generated the fewest fragments (4 bands). The percentage polymorphism ranged from 36.36% to 100%. Ten primers exhibited complete polymorphism, indicating their high efficiency in discriminating among genotypes. PIC values ranged from 0.70 to 0.93, with an average value of 0.8665. The RAPD Primer Index (RPI) varied from 2.81 to 18.54, with primer OPN-04 recording the highest value. The high level of polymorphism observed in the present study demonstrates the effectiveness of RAPD markers in assessing genetic diversity among tomato genotypes. Similar levels of RAPD polymorphism in tomato germplasm have been reported by He *et al.* (2003) and Kaemmer *et al.* (1995) [6], who demonstrated that RAPD markers are effective tools for detecting genetic variation and distinguishing tomato cultivars.

Table 1: Size, number of amplified bands, percent polymorphism and PIC obtained by RAPD primers

Sr. No.	RAPD Primer	Band Size (bp)	Total No. of Amplicons (A)	Polymorphic Bands (B)			Mono- Mor phic Band	% Poly- Mor Phism (B/A)	PIC*	RPI
				S	U	T				
1	OPH-03	152-1739	15	10	1	11	4	73.33	0.91	13.62
2	OPI-02	222-1860	12	9	2	11	1	91.66	0.88	10.57
3	OPI-05	160-1742	15	11	4	15	0	100	0.88	13.23
4	OPI-08	235-1193	9	9	0	9	0	100	0.86	7.77
5	OPK-05	173-858	7	6	1	7	0	100	0.77	5.39
6	OPK-08	279-1295	10	10	0	10	0	100	0.88	8.79
7	OPK-10	191-792	9	9	0	9	0	100	0.85	7.65
8	OPM-01	172-729	11	11	0	11	0	100	0.90	9.85
9	OPM-02	256-1917	4	2	0	2	2	50	0.70	2.81
10	OPM-05	229-1408	18	17	1	18	0	100	0.93	16.73
11	OPN-01	143-924	18	11	6	17	1	94.44	0.90	16.18
12	OPN-04	199-1742	20	16	4	20	0	100	0.93	18.54
13	OPN-05	152-1169	10	8	1	9	1	90.0	0.87	8.68
14	OPO-01	173-1917	17	13	3	16	1	94.11	0.91	15.41
15	OPO-02	209-957	10	4	3	7	3	70.00	0.79	7.85
16	OPO-03	231-1216	11	9	0	9	2	81.81	0.89	9.74
17	OPO-04	221-1275	10	9	1	10	0	100	0.87	8.65
18	OPP-01	127-660	11	4	0	4	7	36.36	0.91	9.97
19	OPP-02	137-621	7	4	0	4	3	57.14	0.85	5.92
20	OPP-05	161-835	7	7	0	7	0	100	0.85	5.97
TOTAL			231	179	27	206	25			
AVERAGE			-	-	-	10.3	-	86.94	0.8665	10.166

S = Shared; U = Unique; T = Total Polymorphic Bands; PIC = Polymorphism information content; RPI = RAPD Primer Index = Number of Bands X PIC

Genetic Similarity Analysis

Jaccard's similarity coefficients among the twenty-two tomato genotypes ranged from 0.29 to 0.85 (Table 2). The lowest similarity coefficient (0.29) was observed between NTL-12-01 and Punjab Chhuhara, indicating maximum genetic divergence between these genotypes. Such divergent genotypes may be useful as potential parents for hybridization programmes aimed at generating broad genetic variability. The highest similarity coefficient (0.85) was recorded between GT-1 and JTL-15-71, suggesting a close genetic relationship between these genotypes. Overall, the similarity matrix revealed considerable variation among the tomato genotypes, reflecting a broad genetic base within the germplasm collection. Comparable ranges of genetic similarity among tomato accessions have also been reported by Hussein *et al.* (2011) [5], indicating that molecular markers are reliable tools for estimating genetic relationships and identifying diverse breeding materials.

Table 2: Jaccard's Similarity Coefficient of 22 tomato Genotypes Based on RAPD

varieties	Arkarakshak	Punjabchhuhara	H-24	Bhagya	AT-3	ATL-15-13	JTL-8-18	ATL-11-05	NTL-14-71	Selection22	NTL-12-07	NTL-12-06	NTL-12-01	JTL-12-02	JTL-12-11	JTL-15-02	JTL-15-05	JTL-15-71	GT-1	BSS-488	Arkavikas	JT-3
Arkarakshak	1																					
Punjabchhuhara	0.32	1																				
H-24	0.62	0.36	1																			
Bhagya	0.59	0.35	0.76	1																		
AT-3	0.59	0.35	0.74	0.75	1																	
ATL-15-13	0.57	0.43	0.64	0.59	0.70	1																
JTL-8-18	0.62	0.36	0.73	0.74	0.72	0.67	1															
ATL-11-05	0.63	0.30	0.67	0.65	0.64	0.62	0.76	1														
NTL-14-71	0.61	0.35	0.73	0.69	0.69	0.63	0.79	0.78	1													
Selection22	0.48	0.42	0.60	0.54	0.60	0.62	0.61	0.61	0.66	1												
NTL-12-07	0.60	0.34	0.65	0.62	0.61	0.60	0.71	0.69	0.69	0.60	1											
NTL-12-06	0.58	0.36	0.67	0.66	0.62	0.60	0.73	0.64	0.73	0.59	0.83	1										
NTL-12-01	0.59	0.29	0.55	0.58	0.55	0.55	0.65	0.63	0.58	0.50	0.56	0.59	1									
JTL-12-02	0.58	0.30	0.58	0.59	0.62	0.62	0.66	0.64	0.61	0.55	0.59	0.60	0.67	1								
JTL-12-11	0.62	0.32	0.61	0.64	0.61	0.61	0.67	0.64	0.61	0.53	0.61	0.61	0.69	0.73	1							
JTL-15-02	0.53	0.36	0.56	0.60	0.57	0.60	0.64	0.62	0.62	0.53	0.57	0.63	0.62	0.74	0.66	1						
JTL-15-05	0.57	0.36	0.66	0.63	0.65	0.65	0.66	0.66	0.68	0.61	0.66	0.67	0.56	0.71	0.62	0.70	1					
JTL-15-71	0.56	0.33	0.66	0.64	0.61	0.59	0.64	0.61	0.68	0.56	0.68	0.74	0.55	0.65	0.63	0.68	0.75	1				
GT-1	0.55	0.35	0.63	0.62	0.59	0.57	0.61	0.58	0.66	0.56	0.73	0.73	0.54	0.65	0.60	0.66	0.73	0.85	1			
BSS-488	0.61	0.32	0.66	0.62	0.63	0.65	0.66	0.66	0.69	0.59	0.68	0.68	0.64	0.77	0.69	0.74	0.74	0.76	0.78	1		
Arkavikas	0.63	0.33	0.60	0.59	0.62	0.65	0.60	0.60	0.63	0.52	0.62	0.63	0.57	0.68	0.64	0.69	0.71	0.72	0.71	0.83	1	
JT-3	0.48	0.34	0.57	0.52	0.54	0.54	0.52	0.53	0.59	0.54	0.58	0.59	0.44	0.56	0.51	0.58	0.67	0.71	0.71	0.68	0.65	1

Cluster Analysis

Cluster analysis based on Jaccard's similarity coefficients grouped the twenty-two tomato genotypes into two major clusters designated as Cluster A and Cluster B (Figure 1). Cluster A consisted solely of Punjab Chhuhara, indicating its distinct genetic background and divergence from all other genotypes. Cluster B contained the remaining twenty-one genotypes and was further subdivided into sub-clusters B1 and B2. Sub-cluster B1 was further divided into B1.1 and B1.2. Sub-cluster B1.1 contained Arka Rakshak alone, while B1.2 included H-24, Bhagya, AT-3, JTL-8-18, NTL-14-71, ATL-11-05, NTL-12-07, NTL-12-06, ATL-15-13, JTL-15-05, JTL-15-71, GT-1, BSS-488, Arka Vikas and JT-3. Cluster B2 comprised NTL-12-01, JTL-12-02, JTL-15-02, JTL-12-11 and Selection-22. The clustering pattern clearly demonstrated the existence of substantial genetic diversity among the studied tomato genotypes and confirmed the effectiveness of RAPD markers for germplasm characterization. Similar clustering patterns based on RAPD markers have been reported by Matin *et al.* (2010) [9], who observed that UPGMA analysis effectively grouped tomato genotypes according to their genetic relationships and diversity levels.

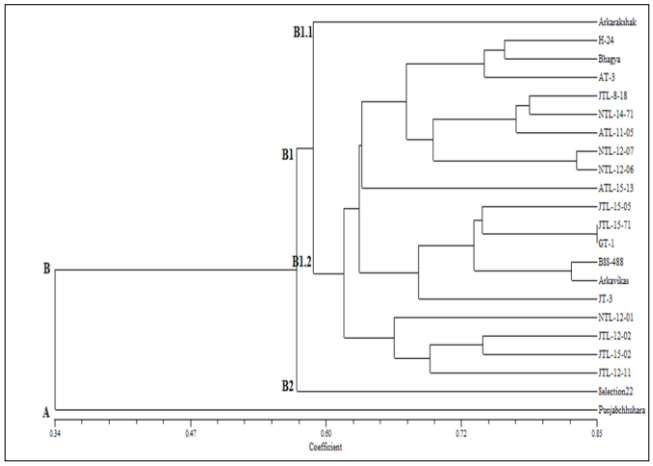

Fig 1: Dendrogram depicting the genetic relationship among 22 tomato genotypes based on the RAPD data from 20 Primers

Conclusion

The present investigation revealed substantial genetic variability among twenty-two tomato genotypes using RAPD markers. A high level of polymorphism (86.94%) and informative PIC values demonstrated the effectiveness of RAPD markers in detecting genetic variation. Genetic similarity and cluster analyses revealed both closely related and highly divergent genotypes within the germplasm collection. The identified genetically diverse genotypes may serve as valuable parental resources for future tomato improvement programmes. RAPD markers proved to be a useful and efficient tool for molecular characterization and genetic diversity assessment in tomato.

Acknowledgements

The authors are thankful to the Department of Biochemistry and Biotechnology, College of Agriculture, Junagadh Agricultural University, Junagadh, for providing laboratory and research facilities. The present study forms a part of the M.Sc. (Agricultural Biotechnology) dissertation research work of the first author, Ms. Jayashree M.J., submitted to Junagadh Agricultural University, Junagadh, Gujarat, India.

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