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Assessment of genetic diversity in tomato (*Solanum lycopersicum* L.) based on SSR Markers

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ABSTRACT

The use of densely bred cultivars for production has restricted genetic variety; on the other hand, genetic variation provided by germplasm accessions is regarded as the raw resources of plant genetics. Therefore, genetic diversity was evaluated in twenty-seven genotypes of tomatoes using SSR markers to study the population structure which provides information at the molecular level. Out of 44 primers, six primers showed polymorphism sizes varying between 160 and 400 bp. Dendrogram was constructed to investigate the genetic relationships among genotypes and cluster analysis was done and genotypes were grouped into two major clusters, which indicates a significant influence of environment on genetic diversity. Analysis of molecular variance revealed 96 percent of total variation within the population and 4 percent among the populations indicating the diversity of genotypes. Nine genotypes were found to be pure which was due to the common source used in developing the lines revealed through STRUCTURE analysis. The present research has shown that SSR markers are efficacious for acquiring unique fingerprint image of tomato relatives and trying to assess genetic diversity among them, and that they could be used for a wide range of practical specific purpose such as various data sources and which was before for distinctiveness of tomato genotypes. The presence of high genetic differentiation allows the selection of promising tomato genotypes to be used in hybridization, mapping, gene pyramiding, molecular breeding, and future exploration.

Keywords: *Cluster analysis, Dendrogram, Genetic diversity, Molecular variance, Primers, Polymorphism, SSR, STRUCTURE analysis, tomato,*

INTRODUCTION

Tomato (Solanum lycopersicum L.) is one of the important vegetable crops grown widely all over the world and is the most versatile garden vegetable grown [1]. Tomato fruits are used in different food preparations, preserved in different forms, consumed as a salad, and utilized in the preparation of processed products viz., puree, soup, powder, ketchup, paste, sauce, and canned whole fruits while pickles and chutney are prepared from unripe green fruits. Tomato fruits are rich in lycopene (an antioxidant), ascorbic acid, and ß-carotene and valued for their color and flavor [2]. Lycopene is treasured for its anticancer attribute, antiseptic properties, blood purifier, and antioxidant which is often colligated with carcinogenesis. Efficient study and assessment of germplasm are the most important for future agronomic and genetic crop improvement. Characterization is an essential method for the identification of genetic diversity between different genotypes. Different molecular markers viz., RFLP, AFLP, RAPD, and SSR were used to study genetic variation. Among all the markers, the SSR marker is widely used for variety identification and discrimination power for genotypes with limited genetic variation [3]. However, molecular markers are an efficient method to inspect the genetic basis of agronomic traits between breeding lines [4]. Additionally, molecular characteristic differences among the cultivars could be utilized for crop improvement in hybridization programs and a tool to link quantitative trait loci (QTLs) responsible for the variation in functional genes. Therefore, the present study was initiated to assess the molecular diversity of a different set of tomato genotypes.

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Materials and methods

Plant material

The experiment was carried out at the experimental farm of the Department of Vegetable Science and Floriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during the spring-summer of 2019-20. All 27 tomato genotypes (Table 1) were grown and maintained under a modified naturally ventilated poly house and most of the genotypes were collected from Himachal Pradesh, Taiwan, and the private sector.

Genomic DNA isolation and PCR amplification

Fresh leaves of each plant were collected for molecular characterization. All the genotypes used in this study were subjected to SSR assay. Genomic DNA was isolated from young leaf tissue (0.5-1g) of each entry using the CTAB method [5]. A total of 44 SSR primers were screened for their polymorphism. For amplification of genomic DNA, a reaction mixture of 12.5µl volume was prepared using 7.15µl of sterilized distilled water, 1.0µl template DNA (25ng/µl), 0.5µl of forward and 0.5µl of reverse primer (5µM), 1.0µl MgCl2 (25mM), 1.25µl 10 X PCR buffer (10mM Tris-HCl, 50mM KCl, pH 8.3), 1.0µl dNTP mix (0.2mM each of dATP, dGTP, dCTP, and dTTP) and 0.1µl Taq polymerase (5U/µl). The amplifications were carried out in S1000TM Thermal Cycler (BIO-RAD). The amplified PCR products were separated on 3 percent agarose (HIMEDIA) gel and stained with ethidium bromide (0.5µg/ml). The gels were visualized and photographed using the Gel Documentation Unit (BIO-RAD).

Table 1 List of tomato (Solanum lycopersicum L.) genotypes used in the study and their sources

Sr. No.	Genotypes	Source
1.	BWR5 (F/R)	CSKHPKV, Palampur
2.	CLN 1314G	CSKHPKV, Palampur
3.	Palam Pride	CSKHPKV, Palampur
4.	1-2	CSKHPKV, Palampur
5.	12-1	CSKHPKV, Palampur
6.	15-2(H/R)	CSKHPKV, Palampur
7.	16-B	CSKHPKV, Palampur
8.	BL333-1	CSKHPKV, Palampur
9.	Palam Pink	CSKHPKV, Palampur
10.	Hawaii 7998	AVRDC, Taiwan
11.	BBWR 11-1	CSKHPKV, Palampur
12.	BBWR 21-7-16	CSKHPKV, Palampur
13.	BT 20-3 Red Egg Shape	CSKHPKV, Palampur
14.	BBWR 10-7-18	CSKHPKV, Palampur
15.	BBWR 10-6	CSKHPKV, Palampur
16.	BBWR 13-7-9	CSKHPKV, Palampur
17.	BBWR 18-7	CSKHPKV, Palampur
18.	BBWR 10-7-17	CSKHPKV, Palampur
19.	7-2	CSKHPKV, Palampur
20.	BT 20-3 Yellow Egg Shape	CSKHPKV, Palampur
21.	BT 20-3 Yellow Round	CSKHPKV, Palampur
22.	Hawaii 7996	AVRDC, Taiwan
23.	CLN 2123	CSKHPKV, Palampur
24.	PTH-1	CSKHPKV, Palampur
25.	Avtar	Nunhems Company
26.	Rakshita	Pvt. Sector
27.	Naveen 2000+	Pvt. Sector

Statistical analysis

The amplified DNA of 27 genotypes of tomato generated SSR marker profiles. The presence or absence of each SSR band of a particular molecular weight was scored manually. A binary data matrix with '1' indicating the presence of a particular molecular weight and '0' its absence was generated separately for each primer. The binary data were used to generate a similarity matrix using Jaccard's coefficient. Genetic distances (GD) were calculated as GD = 1 – [Cij/(ni+nj- Cij)]. The data was subsequently used to construct a dendrogram using the unweighted pair group method with arithmetical averages (UPGMA) in the SAHN program of the NTSYS – PC package (version 2.02). The Polymorphic information content (PIC) values were calculated [5, 6]. and various parameters of genetic variation viz., observed the number of alleles, the effective number of alleles, [6] gene diversity, genetic diversity overall populations, genetic diversity within the population, coefficient of gene differentiation (proportion of genetic diversity between populations), polymorphic loci and gene flow were estimated using POPGENE [7]. The analysis of the molecular variance procedure in GenALEx 6.5 [8] was used for the generation of binary data. The Patterns of genetic relationship contained in the matrix were visualized by Principal Coordinates Analysis (PCoA) IN GenALEx 6.5.

Results

Genetic diversity through molecular markers

Out of 44 SSRs, six primers showed polymorphism and a total of 13 amplicons (size varying between 160 and 400 bp) were produced (Figures 1 and 2). An average of 2.17 polymorphic fragments per primer was observed (Table 2). The Polymorphic information content (PIC), a parameter associated with the discriminating power of markers, ranged from 0.258 (Tom 144-145) to 0.430 (SLM6 22) with a mean of 0.333 per primer (Table 2). [9] found 32 primers polymorphic indicating 63.3% of all the amplified loci. PIC has been used usually for evaluating the informative potential of markers in different germplasm and cultivated genotypes [10]. It was reported that the PIC value for primers varied from 0.153 to 0.30 [11] and 0.11 to 0.50 [12]. Eight Bulgarian tomato lines were characterized and found 299 amplified alleles with a mean of 1.869 alleles per locus and the average polymorphic information content (PIC) found was 0.196 [13].

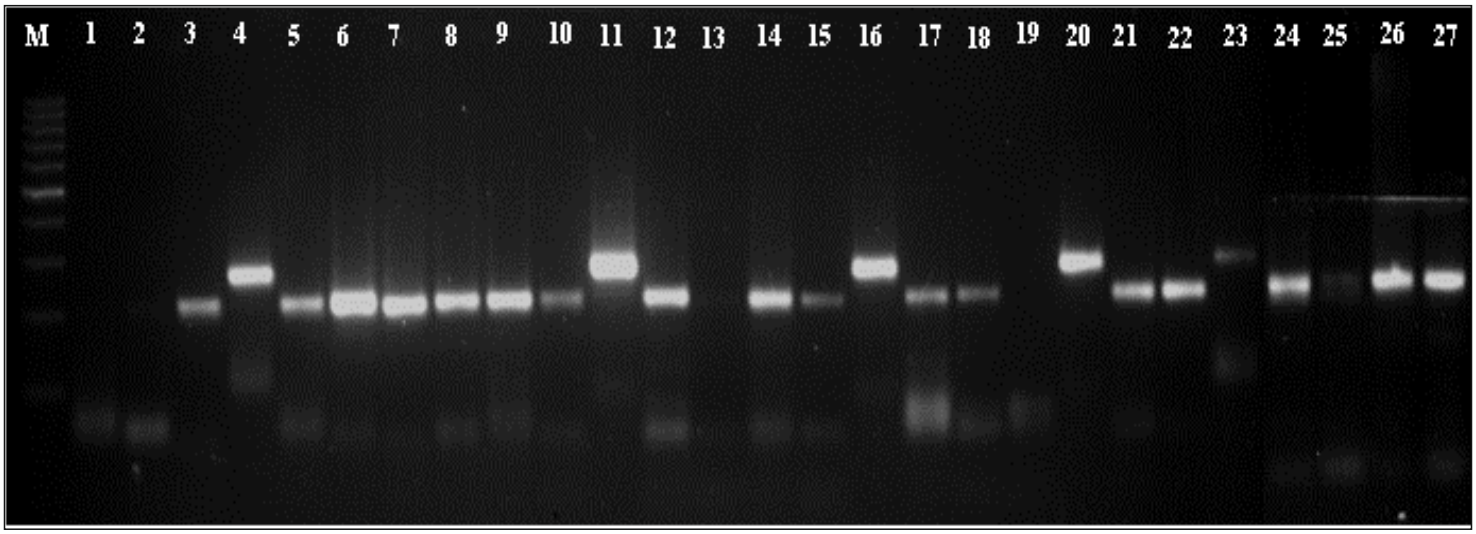


Figure 1 SSR profile of 27 tomato genotypes using primer Tom 39A-40A, M=100 bp DNA ladder

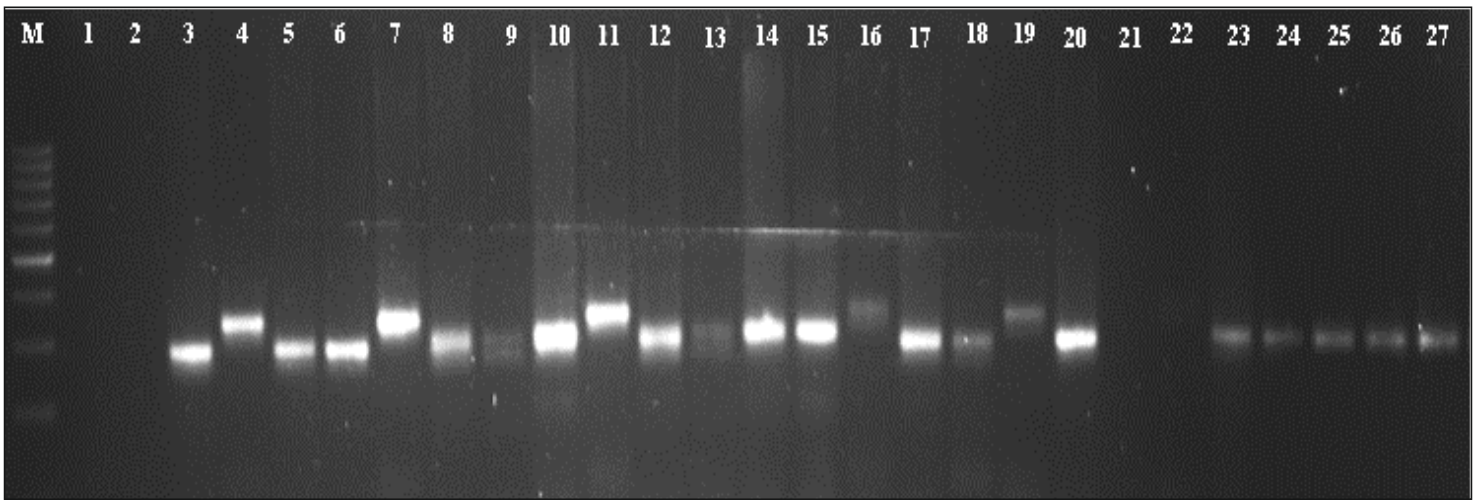
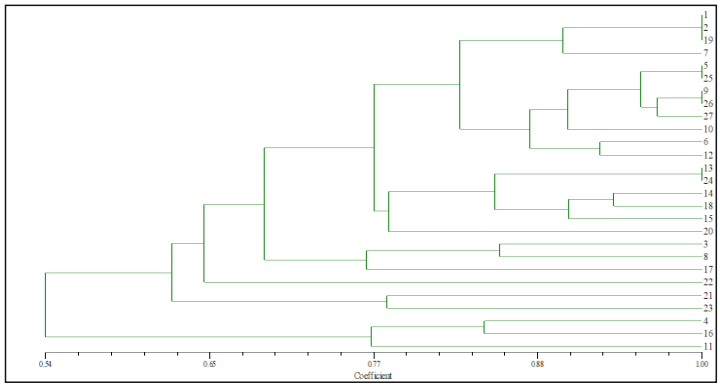


Figure 2 SSR profile of 27 tomato genotypes using primer Tom 144-145, M=100 bp DNA ladder

Table 2: Details of polymorphic SSR bands and their fragment size generated by six primers

Sr. No	Primers	Number of fragments	Number of polymorphic fragments	Polymorphic Bands (%)	PIC Value	Effective Multiplex Ratio (EMR)	Marker Index (MI)	Fragment Size (bp)
1	Tom 59-60	2	2	100	0.346	2	0.692	196-253
2	Tom 144-145	2	2	100	0.258	2	0.516	164-222
3	Tom 39A-40A	2	2	100	0.318	2	0.636	160-220
4	AW031453	2	2	100	0.326	2	0.652	. . . . .
5	SLM6-50	2	2	100	0.320	2	0.640	247
6	SLM6-22	3	3	100	0.430	3	1.290	284
	TOTAL	13	13	600	1.998	13	4.426	
	MEAN	2.17	2.17	100	0.333	2.17	0.738	

Effective multiplex ratio (EMR) ranged from 1 to 3 with an average of 2.17 per primer, while Marker Index (MI) ranged from 0.516 (Tom 144-145) to 1.290 (SLM6 22) with an average of 0.738 per primer. Based on the SSR markers, a dendrogram was constructed to investigate the genetic relationships among genotypes. The genotypes were grouped into two major clusters (Figure 3). Cluster A and cluster B comprised 24 and 3 genotypes, respectively. Cluster A further divided into various sub-clusters (Table 3). Maximum genotypes were found in sub-cluster A2. Sub-cluster A1 comprised four genotypes viz., BWR5 (F/R), CLN 1314G, 7-2, and 16-B. Sub-cluster A2 had eighteen genotypes viz., Palam Pride, 12-1, 15-2(H/R), BL333-1, Palam Pink, Hawaii 7998, BBWR 21-7-16, BT 20-3 Red Egg Shape, BBWR 10-7-18, BBWR 10-6, BBWR 18-7, BBWR 10-7-17, BT 20-3 Yellow Egg Shape, Hawaii 7996, PTH-1, Avtar, Rakshita and Naveen 2000+ while sub-cluster A3 contained only two genotypes namely BT 20-3 Yellow Round and CLN 2123. AMOVA was computed to assess the molecular variation among tomato genotypes for two main population groups. AMOVA depicts the high proportion of variability within the population i.e. 96 percent of total variation whereas, only 4 percent of genetic variation among the population (Table 4). The clustering of the population into two distinct groups represents the population diversity between the groups and indicates a significant influence of the environment on genetic diversity. Some earlier researchers also studied phylogenic relationships by cluster analysis and placed all studied tomato genotypes into 2, 6, and 9 clusters respectively [14, 9, 10]. Cluster analysis of 24 genotypes was grouped into two main distinct clusters with a dendrogram generated from NTSYS-pc software with cluster 1 having 8 genotypes and the main cluster 2 being sub-divided into three sub-clusters [3]. However, 99% and 1% variation within and among the population was reported with the use of AMOVA in the genetic diversity study of tomatoes, respectively [15]. In Italian tomato genotypes (landraces and cultivars), 25.5% and 74.5% variation among the populations and within the population were observed with the use of AMOVA, respectively [16].



1=BWR5 (F/R), 2=CLN 1314G, 3=Palam Pride, 4= 1-2, 5= 12-1, 6= 15-2 (H/R), 7= 16-B, 8=BL333-1, 9=Palam Pink, 10=Hawaii 7998, 11=BBWR 11-1, 12=BBWR 21-7-16, 13=BT 20-3 Red Egg Shape, 14=BBWR 10-7-18, 15=BBWR 10-6, 16=BBWR 13-7-9, 17=BBWR 18-7, 18=BBWR 10-7-17, 19= 7-2, 20=BT 20-3 Yellow Egg Shape, 21=BT 20-3 Yellow Round, 22=Hawaii 7996, 23=CLN 2123, 24=PTH-1, 25=Avtar, 26=Rakshita, 27=Naveen 2000+

Figure 3 Dendrogram depicting genetic relationships among the tomato genotypes constructed by NTSYS–PC (version 2.02) using the UPGMA method

Table 3: Grouping of 27 tomato genotypes into different clusters based on SSR data

Clusters	Sub-cluster	Number of genotypes	Genotypes
A	A_1	4	BWR5(F/R), CLN 1314G, 7-2, 16-B
			Palam Pride, 12-1, 15-2(H/R), BL333-1, Palam Pink, Hawaii 7998, BBWR 21-7-16, BT 20-3 Red Egg Shape,

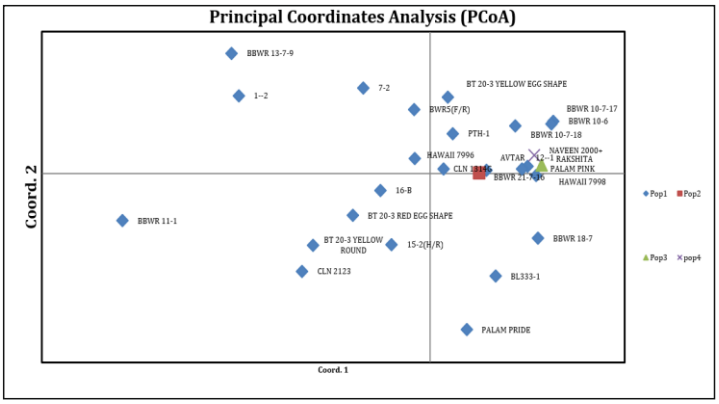
	A_2	18	BBWR 10-7-18, BBWR 10-6, BBWR 18-7, BBWR 10-7-17, BT 20-3 Yellow Egg Shape, , Hawaii 7996, PTH-1, Avtar, Rakshita, Naveen 2000+
	A_3	2	BT 20-3 Yellow Round, CLN 2123
B	-	3	1-2, BBWR 13-7-9, BBWR 11-1

Table 4: Analysis of molecular variance (AMOVA) in tomato genotypes

Marker System	Source	Df	SS	MSS	Variation	% of total Variation
SSR	Among populations	1	3.222	3.222	0.107	4%
	Within Population	25	66.333	2.653	2.653	96%
	Total	26	69.556		2.760	100%

Principal Coordinate Analysis (PCoA)

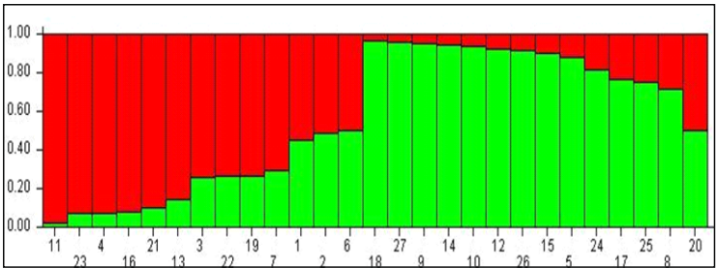
Principal Coordinate Analysis (PCoA) of the relationships between genetic resources of tomatoes and other plants provides a greater understanding of the complexity of the relationship of varieties, cultivars, accessions, and other genotypes in germplasm pools. Principal Coordinate Analysis (PCoA) was used to analyze the substructures of tomato genotypes (Figure 4). Using SSR markers, the percentage of variation is explained by the first 3 axes. Axis 1 contributes 23.03% variation, axis 2 contributes 14.54% variation and axis 3 contributes 13.88% variation. Population structure (Q matrix), estimated using STRUCTURE [17-18] and expressed as membership probabilities, is one way to correct spurious associations due to genetic relatedness. The population structure in the panel containing 27 genotypes was calculated using the 6 SSRs and a model-based approach of STRUCTURE. The STRUCTURE analysis divided the population into two main groups. (Figure 5), but the differentiations at K = 2 were almost consistent with pedigree knowledge with few exceptions. Thus, the pedigree information was used to guide the grouping of P1 and P2 groups. The P1 group consisted of 13 genotypes and P2 consisted of 14 genotypes (Table 5). Thus the grouping of the tomato genotypes generated by NTSYS software was further validated by STRUCTURE analysis at K = 2. STRUCTURE analysis revealed that nine of the line was found to be pure which may be due to the common source used in developing the lines. D2 analysis of the data divided the test population into twelve separate clusters, 11 genotypes under cluster I, and six genotypes under cluster IV while the rest of the clusters were having only one genotype each whereas SSR data analysis divided the population into two clusters: one cluster has 24 genotypes and other has only 3 genotypes (Table 6). A total of 10 genotypes were found common while comparing molecular clusters of D2 and SSR data analysis. A three-dimensional PCoA plot showed the distribution of tomato genotypes based on the morphological traits as cultivated tomatoes mainly clustered into two groups (S1 and S2) and the wild tomatoes except for S. habrochaites, clustered separately from the cultivated tomatoes [19]. PCoA analysis was used to confirm the results shown by the dendrogram and morphological traits in the study reported by [20]. Estimation of population structure in the landraces using a model-based Bayesian procedure implemented in the software Structure v2.3 [20] divided the genotypes into 4 clusters [19]. Genotypes were divided into two populations (at K=2) and four populations (at K=4) when estimating genetic diversity in genotypes with software Structure v2.3 [20].



Percentage of variation explained by the first axes

Axis	1	2	3
%	23.03	14.57	13.88

Figure 4: Principal Coordinate Analysis of 27 tomato genotypes



P1 ***P2***

Figure 5: Gene pool introgression based on the population structure analysis at K = 2

Table 5: Grouping of tomato genotypes using STRUCTURE software program

Group	Number of genotypes	Genotypes
Group-1	13	BBWR 11-1, 1-2, BT 20-3 Yellow Round, Palam Pride, 7-2, BWR5 (F/R), 15-2(H/R), CLN 2123, BBWR 13-7-9, BT 20-3 Red Egg Shape, Hawaii 7996, 16-B, CLN 1314G
Group-2	14	12-1, BL333-1, Palam Pink, Hawaii 7998, BBWR 21-7-16, BBWR 10-7-18, BBWR 10-6, BBWR 18-7, BBWR 10-7-17, BT 20-3 Yellow Egg Shape, PTH-1, Avtar, Rakshita, Naveen 2000+

Table 6: Comparison of clustering patterns using D2 static and SSR analysis data

Cluster No.	D^2	SSR	Common genotypes (NTSYS) and D^2
I	11	24	(9) 16-B, BBWR 18-7, BBWR 10-7-18, BBWR 10-6, CLN 1314G, 12-1, BBWR 21-7-16, 7-2, 15-2(H/R)
II	1	3	(1) BBWR 13-7-9

Future Scope of the Study

With the development of molecular biology, some DNA-based technologies have showed great potentiality in promoting the efficiency of crop breeding program, protecting germplasm resources, improving the quality and outputs of agricultural products, and protecting the Eco environment etc., making their roles in modern. Molecular markers allow detection of variations or polymorphisms that exist among individuals in the population for specific regions of DNA. Such type of study can be helpful in hybridization programmes, mapping, gene pyramiding, DNA fingerprinting, and conservation of tomato genotypes across India and other countries.

Conclusion

Genetic diversity and population structure were assessed in 27 genotypes of tomatoes by 44 SSR primers. An average of 2.17 polymorphic fragments per primer and an average of 0.738 Marker Index (MI) per primer were observed. The genotypes were grouped into two major clusters with 13 genotypes in P1 and 14 genotypes in P2.

Authors' contribution

Conceptualization of research (PS, VK); Designing of the experiment (PS,); Contribution of the experimental material (PS, VK); Execution of lab experiments (PS, VK, MK, S, AS); analysis of data and interpretations (VK, MK, MK, S); Preparation of manuscript (MK, S, VK); approval of manuscript (PS, AS).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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