

Original Research Article

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## Evaluation of Genetic Variability in Tomato (*Solanum lycopersicum* L. Mill) Genotypes using Microsatellite Markers

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### ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the most important economically plants in the family *Solanaceae*. Information about genetic diversity of any crop is important for successful employment of breeding programme and is of great significance to attain sustainability in crop production. The objective of this study was to evaluate the genetic diversity of 24 genotypes of tomato collected from ICAR-IIVR, Varanasi, India, using 25 simple-sequence repeats (SSRs). A total of 64 alleles were detected at an average of 2.720 alleles per SSR locus. The average major allele frequency and polymorphic information content were 0.6622 and 0.3875, respectively. The UPGMA cluster analysis induced by SSRs data grouped 24 tomato genotypes into 3 main clusters. Cluster I and II comprised 15 and 8 genotypes respectively, and a single tomato genotype CO-3 was grouped in cluster III. As per our expectation genotypes from same genetic base showed similarity among each other. The information obtained from SSR markers may well assist tomato breeders in identifying a limited number of highly differentiated genotypes to be selected for further use in developing suitable variety/genotypes with good quality potential.

#### Keywords

Genetic diversity,  
SSRs, PIC, PCA

#### Article Info

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### Introduction

Tomato, a popular garden vegetable, is an herbaceous plant belonging to family *Solanaceae*. It is a second most cultivated vegetable crop across the globe (Foolad, 2007; Rai *et al.*, 2012). Out of the total vegetable production in the world, approximately 14% accounts for tomato (Osei *et al.*, 2014). Being a vegetable of multipurpose utility in food and processing industries, tomato is also a rich source of phytonutrients for human consumption. At present, tomato breeders aim

at improving yield, fruit quality characteristics as well as tolerance towards biotic and abiotic stresses for which a prior apprehension of the existing genetic diversity within the tomato cultivars is a prerequisite (Foolad, 2007; Rai *et al.*, 2016). A precise evaluation of the variability in the tomato germplasm is of immense importance for the ongoing as well as subsequent crop improvement programmes for agronomic and genetic improvement of the crop (Reddy *et al.*, 2013). The availability of the completely sequenced genome and its enormous agricultural and economic

importance has made tomato a pre-eminent model for genetics, genomics and breeding studies (Henarehet.al. 2015). For the identification and estimation of genetic diversity various morphological, biochemical and molecular markers can be used. One of the important diagnostic features for differentiating genotypes are the morphological traits (Osei *et al.*, 2014), however, their efficiency is primarily influenced by environmental factors. With the advent of marker technology, the use of methods for diversity estimation has been extended from unstable morphological traits to stable biochemical and molecular traits. Several molecular marker viz. RFLP, RAPD, SSRs, ISSRs, AFLP and SNPs are presently available to assess the variability and diversity at molecular level, among which SSR markers are the most widely used (Frary *et al.*, 2005). Earlier studies have reported the use of morphological and molecular markers viz. SSRs for unraveling the genetic diversity in tomato germplasm (Agong *et al.*, 2001; Bredemeijer and Cooke, 2002; He *et al.*, 2003; Song *et al.*, 2006; Mazzucato *et al.*, 2008; Al-Aysh *et al.*, 2012). Dissection of the genetic diversity within the tomato germplasm will be useful in varietal identification, in deciphering the extent of genetic variation, in the construction of genetic linkage maps and in hybridization programmes (Naz *et al.*, 2013). The present study aimed at unraveling the existing genetic diversity within the tomato genotypes using microsatellite markers.

## **Materials and Methods**

### **Plant material**

A total of 28 tomato genotypes (Table 1, were used in this study. These genotypes were procured from Indian Institute of Vegetable Research (IIVR) Varanasi. All the genotypes were grown in a randomized complete block design (RCBD) with three replicates.

## **Molecular analysis**

### **DNA extraction**

The young leaves of common bean genotypes were powdered in liquid nitrogen and the genomic DNA was extracted based on the procedure of Doyle and Doyle with little modifications. The DNA quantity as well as quality was checked by Nanodrop (mySPEC, Wilmington, USA). Isolated high quality DNA was diluted to concentration of 25 ng/ $\mu$ l for further use.

### **SSR analysis**

SSR primers synthesized at IDT (Integrated DNA Technologies, USA) were used for studying polymorphism among 28 tomato genotypes. Details of SSR markers are given in Table 2. PCR amplification was carried out in 96 well Universal Gradient Thermal Cycler (Eppendorf, Germany) in a 25  $\mu$ l reaction mixture. The reaction mixture contained 2.5  $\mu$ l of each forward and reverse primers, 1 U of Taq polymerase (D1806- Sigma Aldrich, USA), 5  $\mu$ l of 10X PCR buffer with MgCl<sub>2</sub>, 2.5 mM of each dNTP (dTTPs, dGTPs, dCTPs, dATPs). Amplifications were performed as follows: Initial denaturation of 4 min at 94 °C, followed by 35 cycles of 94 °C 1 min, 50–55 °C 1 min, 72 °C 2 min and a final extension of 7 min at 72 °C. PCR products were mixed with loading dye (3–4  $\mu$ l). PCR products resolved on 3% agarose gel were visually examined under UV and documented using gel documentation system (MiniLumi, Sigma-Svi Bio Solutions Pvt. Ltd. New Delhi, India). Gels were visually scored and scanned for records. The clear and reproducible alleles amplified by each SSR among 28 genotypes were scored according to their fragment size (bp) corresponding to the 100 bp molecular weight marker (Sigma Aldrich, USA). Amplified fragments were scored as present (1) and absent (0) generating the 0 and 1

matrix and Polymorphism Information Content (PIC value) was calculated by using the following formula.

$$PIC = 2fi(1-fi)$$

Where,

fi = frequency of bands present

1-fi = frequency of bands absent

Pair-wise similarity between the genotypes was estimated using Jaccard's similarity coefficient (Jaccard, 1908). Cluster analysis based on unweighted pair-group method with arithmetic averages (UPGMA) clustering algorithm was performed using NTSYS-pc version 2.02e (Rohlf, 1998) to obtain a dendrogram. The major allele frequency, numbers of alleles, gene diversity, and Polymorphism Information Content (PIC) was analyzed using the Power Marker software ver. 3.23 (Liu and Muse 2005).

## Results and Discussion

### Allelic variation based on microsatellite markers

SSR polymorphisms in these 24 tomato genotypes were measured in terms of major allele frequency, numbers of alleles, gene diversity, and PIC (Table 3) using the Power Marker software ver. 3.23 (Liu and Muse 2005). The 25 SSR markers revealed the major allele frequency per locus varied from 0.4091 (SSR66) to 0.9091 (SSR96) with an average value of 0.6622 per marker. Above SSR markers revealed 64 alleles among these tomato genotypes. Allelic richness per locus varied widely among the markers, ranging from 2 to 5 (SSR139, SSR66 and SSR 47), with an average of 2.720 alleles per locus. The SSR loci (gene) diversity and heterozygosity per locus generated from amplification data and summarized in Table 2. The gene

diversity per locus ranged from 0.1694 (SSR96) to 0.7025 (SSR66), with an average value i.e. 0.4548 per marker. The polymorphic information content revealed by each primer are given in same table. The polymorphic Information content (PIC) ranged from 0.16-0.65 with an average value of 0.38.

The highest Polymorphic Information content (PIC-0.65) was estimated for primer SSR 139 and SSR66, followed by SSR 47 which had a PIC value of 0.62, whereas primer SSR 96 showed least PIC value i.e. 0.16.

### Cluster analysis

The dendrogram generated from SSR data, the tomato genotypes grouped in three main clusters as represented in Figure 1, in which CO-3 was totally distinguished from other genotypes that had grouped together. Cluster I and II were further divided into two sub clusters and comprised of 15 and 8 genotypes, respectively whereas, Cluster III comprised of single genotype i.e. CO-3. Principal component analysis (PCA) of 24 tomato genotypes using 25 SSR markers revealed similar results as observed by UPGMA based clustering (Figure 2).

Molecular markers have great potential to characterization of crop genotypes (Rai *et al.*, 2016). Molecular characterization is a convenient tool for assessing genetic diversity and characterization of various plant genetic resources with molecular markers offers a unique opportunity to define significant marker-trait associations of biological and agronomic interest (Park *et al.*, 2004; Semagn *et al.*, 2006; Mondini *et al.*, 2009; Parmar 2010). In this study, 25 SSR markers produced 64 alleles and the number of detected alleles over all loci across the genotypes ranged from 2 to 5 (SSR139, SSR66 and SSR 47), with an average of 2.720 alleles per locus (Table 3). Similar findings were also reported earlier.

**Table.1** List of Tomato genotypes

S. No.	Genotypes	Source	S. No.	Genotypes	Source
1.	C0-3	IIVR, Varanasi	13.	HisarArun	IIVR, Varanasi
2.	KashiSharad	IIVR, Varanasi	14.	Azad T-5	IIVR, Varanasi
3.	WIR-13706	IIVR, Varanasi	15.	Pusa Ruby	IIVR, Varanasi
4.	KashiVishesh	IIVR, Varanasi	16.	Pant –T-3	IIVR, Varanasi
5.	F-7028	IIVR, Varanasi	17.	EC-529083	IIVR, Varanasi
6.	Roma	IIVR, Varanasi	18.	S. Vaibhav	IIVR, Varanasi
7.	VRT-32	IIVR, Varanasi	19.	EC-501574	IIVR, Varanasi
8.	Money maker	IIVR, Varanasi	20.	EC-501575	IIVR, Varanasi
9.	Flora Dade	IIVR, Varanasi	21.	Pant- T-7	IIVR, Varanasi
10.	EC-520071	IIVR, Varanasi	22.	VRT-32-792	IIVR, Varanasi
11.	EC-317-6-1	IIVR, Varanasi	23.	F-7012	IIVR, Varanasi
12.	C-26-1	IIVR, Varanasi	24.	ArkaVikash	IIVR, Varanasi

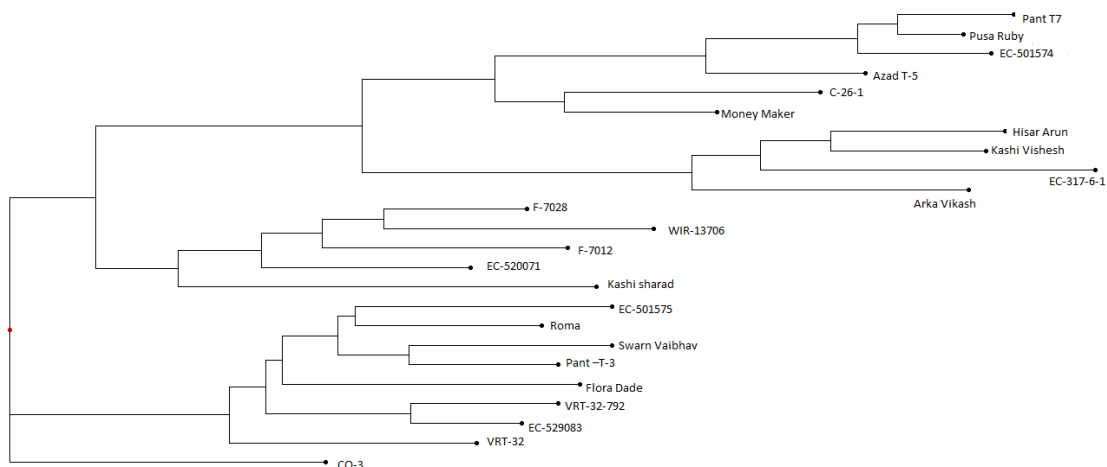
**Table.2** List of primers used in the study

S. No	Primer	Forward Sequence	Reverse Sequence
1	A1773078	5’GATGGACACCCTTCAATTTATGGT3’	3’TCCAAGTATCAGGCACACCAGC5’
2	A1778183	5’GCCAAGAAGATGAGTCTAGAGCATAG3’	3’CTCTCTCCCATGAGTTCTCCTCTTC5’
3	A1895126	5’GCTCTGTCCTTACAAAGTATACCTCC3’	3’CAATGCTGGGACAGAAGATTTAATG5’
4	A1486387	5’ACGCTTGGCTGCCTCGGA3’	3’AACTTTATTATTGCCACGTAGTCATGA5’
5	Y09371	5’TGAGAACAACGTTTAGAGGAGCTG3’	3’CGGGCAGAATCTCGAACTC5’
6	Y08306	5’AACGGTGGAAACTATTGAAAGG3’	3’CACCACCAAACCCCATCGTC5’
7	X90937	5’TGCCCATGACGTTCCATC3’	3’GACAGACAGAGAGACAGACTTAGAG5’
8	X90770	5’TG TAGATAACTTCTAGCGACAATC3’	3’ACGGACGGATGGACAAATG5’
9	LEttc002	5’TTCTCACACCTGCACACACC3’	3’AGCGGGATGATTACAGAAATG5’
10	TMS 37	5’CCTTGCAGTTGAGGTGAATT3’	3’TCAAGCACCTACAATCAATCA5’
11	TMS 48	5’ATTGCTCATAATAACCC3’	3’GGGACAAAATGGTAATCCAT5’
12	SSR 601	5’TCTGCATCTGGTGAAGCAAG3’	3’CTGGATTGCCTGGTTGATTT5’
13	SSR 139	5’TGGGTATGGGATTTACACCAA3’	3’AAACGAAGGCAACAACGAAG5’
14	SSR 9	5’CCCTTTGCAAGTTCTTCTTCA3’	3’TTCATGAGCCAACATAGGAGG5’
15	SSR 50	5’CCGTGACCCTCTTTACAAGC3’	3’TTGCTTTCTTCTCGCCATT5’
16	SSR 75	5’CCATCTATTATCTTCTCTCCAACAC3’	3’GGTCCCAACTCGGTACACAC5’
17	SSR 146	5’TATGGCCATGGCTGAACC3’	3’CGAACGCCACCACTATACT5’
18	SSR 66	5’TGCAACAACCTGGATAGGTCG3’	3’TGGATGAAACGGATGTTGAA5’
19	SSR 96	5’GGGTTATCAATGATGCAATGG3’	3’CCTTTATGTCAGCCGGTGTT5’
20	SSR 111	5’TTCTTCCCTTCCATCAGTTCT3’	3’TTTGCTGCTATACTGCTGACA5’
21	SSR 241	5’TCAACAGCATAGTGGAGGAGG3’	3’ TCCTCGGTAATTGATCCACC 5’
22	SSR 43	5’CTCCAAATTGGGCAATAACA3’	3’ TTAGGAAGTTGCATTAGGCCA 5’
23	SSR 110	5’TGTAACGTCAAACCTCAGGTG3’	3’ CTCCGCAATGTGTTGTATGG 5’
24	SSR 80	5’GGCAAATGTCAAAGGATTGG3’	3’AGGG TGTTCTTGATTGTCA 5’
25	SSR 47	5’ TCCTCAAGAAATGAAGCTCTGA3’	3’ CCTTGAGATAACAACCACAA 5’

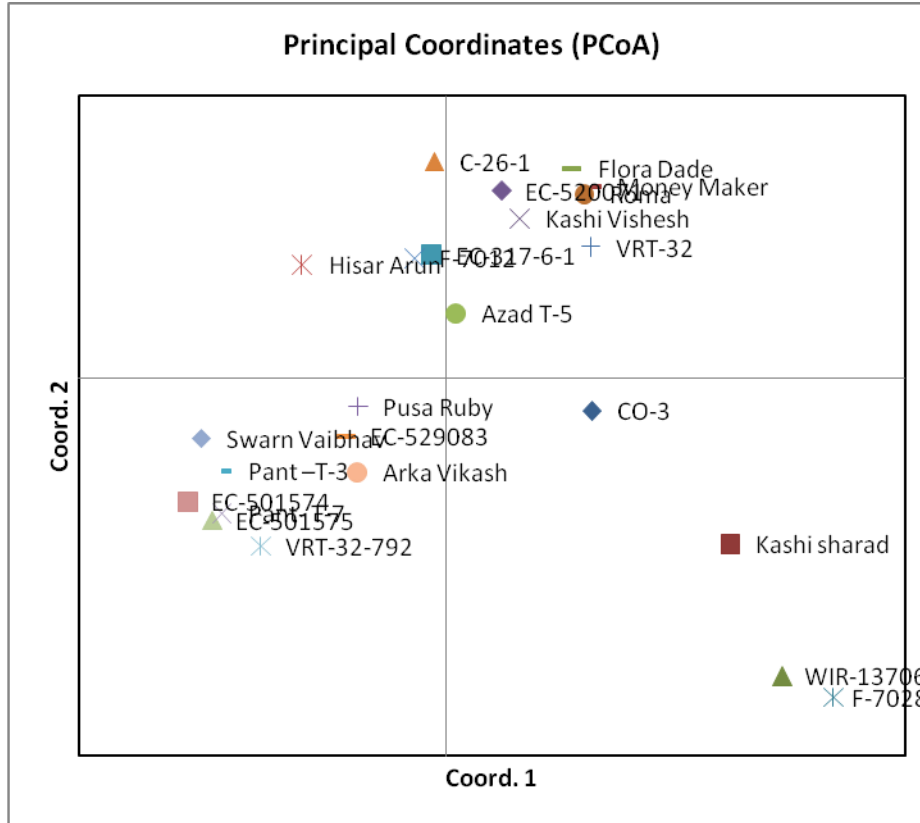
**Table.3** Major allele frequency, number of alleles, gene diversity, heterozygosity and PIC values of primers

S. No.	Marker	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
1	A1773078	0.8250	2.0000	0.2888	0.2500	0.2471
2	A1778183	0.8478	2.0000	0.2580	0.3043	0.2247
3	A1895126	0.7308	2.0000	0.3935	0.5385	0.3161
4	A1486387	0.7353	3.0000	0.4135	0.4118	0.3636
5	Y09371	0.7273	2.0000	0.3967	0.5455	0.3180
6	Y08306	0.5750	2.0000	0.4888	0.8500	0.3693
7	X90937	0.6071	2.0000	0.4770	0.7857	0.3633
8	X90770	0.8636	2.0000	0.2355	0.2727	0.2078
9	LEttc002	0.7750	2.0000	0.3488	0.1500	0.2879
10	TMS 37	0.5952	2.0000	0.4819	0.8095	0.3658
11	TMS 48	0.7368	2.0000	0.3878	0.0000	0.3126
12	SSR601	0.5357	2.0000	0.4974	0.9286	0.3737
13	SSR 139	0.4706	5.0000	0.6990	0.0000	0.6595
14	SSR 9	0.6190	2.0000	0.4717	0.0000	0.3604
15	SSR 50	0.7500	3.0000	0.4063	0.0000	0.3706
16	SSR 75	0.5714	3.0000	0.5533	0.0000	0.4728
17	SSR 146	0.6000	3.0000	0.5588	0.1500	0.4971
18	SSR 66	0.4091	5.0000	0.7025	0.0000	0.6526
19	SSR 96	0.9091	3.0000	0.1694	0.0909	0.1626
20	SSR 111	0.8182	2.0000	0.2975	0.3636	0.2533
21	SSR 241	0.5833	3.0000	0.5590	0.2500	0.4878
22	SSR 43	0.6957	2.0000	0.4234	0.4348	0.3338
23	SSR 110	0.5714	4.0000	0.5975	0.1429	0.5456
24	SSR 80	0.5500	3.0000	0.5850	0.0000	0.5129
25	SSR 47	0.4524	5.0000	0.6791	0.6190	0.6274
	Mean	0.6622	2.7200	0.4548	0.3159	0.3875

**Fig.1** Dendrogram of 24 tomato genotypes produced by UPGMA clustering method based on the genetic similarity



**Fig.2** Principal Component Analysis (PCoA) of 24 tomato genotypes



For example, Raveendar *et al.*, (2016) were reported that, total of 176 alleles were detected at an average of ten alleles per SSR locus. Bredemeijer *et al.*, (2002) have reported a mean number of 4.7 (range, 2-8) alleles per locus in 521 tomato accessions. Todorovska *et al.*, (2014) have reported a mean number of 1.8 (range, 1-6) alleles per locus in eight Bulgarian tomato accessions. Benor *et al.*, (2008) have reported an average of 4.3 alleles per locus in 39 determinant and in determinant tomato inbred lines. Smulders *et al.*, (1997) have detected 3 alleles per locus on average for 7 inbred lines of tomato. He *et al.*, (2003) have identified 2.7 alleles per locus on average among 17 varieties and 2 parental lines of tomatoes. Major allele frequency per locus varied from 0.4091 to 0.9091 (SSR96) with an average value of 0.6622 per marker. Gene diversity and heterozygosity per locus generated from

amplification data and summarized in Table 3. The gene diversity per locus ranged from 0.1694 (SSR96) to 0.7025 (SSR66), with an average value i.e. 0.4548 per marker. Raveendar *et al.*, (2016) were reported that the average major allele frequency i.e. 0.69 in 355 accession of tomato. The PIC value of each SSR locus was used to assess their level of in-formativeness (high, PIC greater than 0.5; moderate, 0.5 greater than PIC greater than 0.25; low, PIC less than 0.25) (Botstein *et al.*, 1980). To compare the genetic variabilities among the genotypes, we calculated PIC and heterozygosity of SSR primers used. The average PIC and heterozygosity were 0.3875 and 0.3159, respectively. Raveendar *et al.*, (2016) was reported that polymorphic information content were 0.39 for 355 Asian tomato accessions. Todorovska *et al.*, (2014) have reported an average PIC and GD value of

0.196 and 0.22, respectively, for Bulgarian tomato accessions. Compared to these reports, our result revealed higher level of mean PIC (0.03875) and heterozygosity value (0.03159). Therefore, our results will be useful for further genetic studies on tomato species. The genetic distance-based results observed in the dendrogram revealed a similar trend to the genetic similarity analysis, revealing genotypes were grouped into three clusters i.e., I, II and III, but it was not possible to distinguish among genotypes with different species (Figure 1).

Cluster I and II were further divided into two sub clusters and comprised of 15 and 8 genotypes, respectively. Whereas, Cluster III comprised of single genotype i.e. CO-3.PCoA analysis was also consistent with the clustering results (Figure 2).

It is concluded that SSR markers are effective in unravelling the genetic diversity within the tomato lines. The genetic diversity observed within the tomato genotypes will prove to be of utmost importance for germplasm classification, management, and further utilization in tomato breeding programmes.

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