



Genetic diversity in tomato (*Solanum lycopersicum* L.) genotypes revealed by simple sequence repeats (SSR) markers

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Abstract: Twenty five tomato (*Solanum lycopersicum* L.) genotypes were subjected to genetic diversity analysis using twenty SSR markers. Out of 20 markers used, 14 SSRs were polymorphic and a total numbers of 22 SSR alleles were generated by 14 SSR markers, out of which 19 were polymorphic and 3 were monomorphic, with an average of 1.57 alleles per locus. The range of amplified products was 100-400bp approximately. Jaccard's similarity coefficient varied from 0.65 between germplasm EC519821 and CO-3 to a maximum of 1.0 between genotypes EC519769 and DARL-66, with an average value of 0.83. Cluster analysis based on Jaccard's similarity coefficient using the unweighted pair-group method with arithmetic mean (UPGMA) revealed 2 distinct clusters, A and B, comprising 1 and 24 genotypes respectively and at 75 and 78 per cent similarity, respectively. The genotypes which showed similar morphological and genetic trends were grouped more or less together in both these cases were a few. Cluster A comprised most diverse germplasm (EC519821) belongs to *pimpinellifolium* wild species with similarity coefficient 0.65% and differentiated with other cultivated species. Cherry Tomato and Cherry-2 were trends in similar cluster similar with approximately 96% similarity. SSR markers were able in in differentiating the genotypes based on morphologically and genotypically. However, the grouping of 25 genotypes were independently of geographic distribution. The genetic distance information found in this study might be helpful to breeder for planning among these genotypes.

Keywords: Genetic diversity, Jaccard's similarity coefficient, SSR, Tomato genotypes

INTRODUCTION

Tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Mill.) is the fourth most economically important crop in the world: after rice, wheat, and soybean and widely grown plants in *Solanaceae* family. Recently, tomato is a preeminent model system for genetic studies in plants having a small genome size (about 950 Mb/haploid genome), availability of a wide range of high density molecular maps, DNA libraries (EST-expressed sequence tag and BAC-bacteria artificial chromosome) and microarrays (Barone *et al.* 2008)

Diverse germplasm including specific genotype are the most valuable basic materials for crop breeder to meet the current and future needs. Sometimes, Morphological difference can not be interpreted to provide accurate estimates of genetic differences because morphological characters are highly influenced by the environments and G x E interaction (Shirasawa *et al.*, 2013). In that situation, Molecular markers are powerful tools in the characterization and evaluation of genetic diversity within and between genetic populations (Russel *et al.*, 1997). They have the advantage of

providing thorough genome assessments' that are not influenced by the environmental factors, and directly reveals the polymorphism at DNA level (Ansari, 2015). Characterization of genotypes and varieties is mandatory for the purpose of registration with the competent authority and for granting plant breeder's rights under the criteria of Distinctness', Uniformity and stability (DUS). With the development of different molecular markers such as AFLPs (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphism) (Zhou *et al.*, 2015), SSRs (Simple Sequence Repeats) are powerful tool for genetic diversity analysis and characterization of germplasm because of their reproducibility, co-dominant in nature and good genome coverage (Caguait *et al.*, 2014; Edris *et al.*, 2014; Zhou *et al.*, 2015) and SNPs (Single Nucleotide Polymorphism) are being used for diversity analysis, characterization, marker assisted selection and gene cloning (Corrado *et al.*, 2013). Now a day, several molecular markers are developed, of which simple sequence repeats (SSRs) or microsatellites are the most widely used types. SSR markers have been successfully used in tomato for diversity analysis and

characterization of tomato germplasms (Dhaliwal *et al.*, 2011; El-Awady *et al.*, 2012; Sanghani *et al.*, 2014; Zhou *et al.*, 2015). SSR marker is a PCR-based technique, which involves amplification of DNA fragments between adjacent and inversely oriented microsatellites. This technique uses microsatellites, usually 16–25 bp long, as primers. These primers can be di-, tri-, tetra- or penta-nucleotides (Zhang *et al.*, 2014).

SSR marker fits worthy for a species like tomato, which has low level of variation as revealed by other markers (Bredemeijer *et al.*, 2002). Improvement of crop depends on the magnitude of genetic variability in economic characters, therefore, the evaluation and utilization of genetic variability in desired direction becomes extremely important in any yield improvement program.

Thus, keeping the above fact in mind, the objective of this study was to assess the genetic diversity of tomato (*S. lycopersicum* L.) within the genotype using SSR markers.

MATERIALS AND METHODS

Plant materials: Out of 25 tomato (*S. lycopersicum* L.) germplasm lines, 20 germplasm collected from core collection maintained at PCPGR (Pantnagar Centre for Plant Genetic Resources), Pantnagar, and 5 germplasm lines received from D.R.D.O., Pithoragarh, Uttarakhand. List of genotypes along with their source are given in Table 1.

SSR primers: Twenty SSR primer pairs were selected and synthesized from IDT (Integrated DNA Technologies, India). The detail of primer name, sequences, and corresponding annealing temperature and the amplified fragments are listed in Table 2.

Genomic DNA extraction: Healthy and mature leaf tissue (2 g) was ground in liquid nitrogen to a fine powder, and genomic DNA was extracted by using modified CTAB (hexa decyltrimethylammonium bromide) method (DOYLE and DOYLE, 1990) and quality of the DNA was estimated by separating it in 0.8% agarose gel stained with ethidium bromide.

DNA quantification and polymerase chain reaction: Purified DNA was quantified using GeneQuantU-Vspectrophotometer (GE Health Care Bio-sciences Ltd, U.K.) and diluted accordingly for further PCR analysis. The genotypes were screened for polymorphism with twenty microsatellite markers by slight modified method (Ollitrault *et al.* 2010). PCR amplification profile was carried out in 15ul volume containing 2μL 10x PCR reaction buffer, 25 mM MgCl₂, 1 mM dNTP mix, 5 pmol of each (Forward & Reverse) SSR primer and 20 ng of template DNA, 1 U of Taq DNA polymerase (3B Black Bio, Spain). PCR reactions were carried out in an Eppendorf mastercycler gradient thermocycler (Eppendorf, Hamburg, Germany) using the following temperature profile: initial step of 5 min at 94°C, 35 cycles of 30 sec at 94°C, 1 min at 50-63°C

Table 1. List of genotypes and their sources used for diversity study.

S. No.	Genotypes	Source
1	AC-576	PCPGR, Pantnagar
2	Sel-816-06	PCPGR, Pantnagar
3	ARTH-3	PCPGR, Pantnagar
4	EC-519821	PCPGR, Pantnagar
5	Cherry Sutton	PCPGR, Pantnagar
6	EC-519812	PCPGR, Pantnagar
7	Cherry Tomato	PCPGR, Pantnagar
8	EC-519977	PCPGR, Pantnagar
9	EC-519769	PCPGR, Pantnagar
10	CLN-2413	PCPGR, Pantnagar
11	PT-09-06	PCPGR, Pantnagar
12	Cherry-2	PCPGR, Pantnagar
13	EC-519818	PCPGR, Pantnagar
14	PT-19	PCPGR, Pantnagar
15	PT-8	PCPGR, Pantnagar
16	EC-519724	PCPGR, Pantnagar
17	DARL-67	DRDO, Pithoragarh
18	ArkaVikas	PCPGR, Pantnagar
19	DARL-69	DRDO, Pithoragarh
20	DARL-68	DRDO, Pithoragarh
21	DARL-66	DRDO, Pithoragarh
22	DARL-62	DRDO, Pithoragarh
23	Shalimar	PCPGR, Pantnagar
24	PantT-3	PCPGR, Pantnagar
25	CO-3	PCPGR, Pantnagar

(depending upon the primer sequence) and 1 min at 72°C and a final step of 7 min at 72°C. The amplification of PCR products were separated by using 3% agarose gel electrophoresis stained with ethidium bromide and documented by using UV-Pro gel documentation system.

Band scoring, estimation of diversity/ similarity and cluster analysis: The amplified bands were visually scored for the presence (1) or absence (0) in all the genotypes and the binary data were used for cluster statistical analysis by using NTSYS-pc version 2.11s software (Rohlf, 2002). The SIMQUAL program was used to calculate the Jaccard's similarity coefficients. Polymorphic information content (PIC) values were calculated for each SSR primers according to the formula given by (Smith *et al.*, 1997).

$$PIC = 1 - \sum P_i^2$$

where P_i^2 is the frequency of the i^{th} allele.

The similarity matrix value based on coefficient of similarity was used to generate dendrogram. Clustering was done by UPGMA using NTSYS-pc version 2.11s software (Rohlf, 2002).

RESULTS AND DISCUSSION

Microsatellite polymorphism analysis: SSR primers

Table 2. Details of SSR primers used for the molecular characterization of twenty five tomato germplasm.

S. No.	Primer code	Sequence	Tm (°C)	Amplified product	Alleles	Monomorphic bands	Polymorphic bands	Percent Polymorphism	PIC
1	SSR111	F 5'-TTCCTCCCTCCATCAGTCT-3' R 5'-TTTGTGCTGCTATACGTGACA-3'	53.80	100- 200bp	2	0	2	100	0.48
2	SSR20	F 5'-GAGGACGACAAACAACGA-3' R 5'-GACATGCCACTTAGATCCACAA-3'	55.05	100- 200bp	2	1	1	50	0.49
3	SSR43	F 5'-CTCCAAATGGCAATAACA-3' R 5'-TTAGGAAGTTGCATTAGGCCA-3'	52.55	100- 200bp	2	1	1	50	0.54
4	ST1032	F 5'-TGGGAAGAATCCTGAAATGG-3' R 5'-TGTCTTACCAATTAACGGGCA-3'	63.40	120bp	1	0	1	100	0.0
5	ST10003	F 5'-ACCAATCCACCATGCAATGC-3' R 5'-CTCATGGATGGTGTCAITGG-3'	64.90	180bp	2	0	2	100	0.54
6	ST10023	F 5'-GCCAATGACAGGACAAGAGG-3' R 5'-TGCCACTGCTACCATAACCA-3'	64.45	100- 200bp	2	0	2	100	0.40
7	STM1045	F 5'-GTACATATATATACAAATTAATTAACCG-3' R 5'-TTCCTATGTTAGGCTAGAGTG-3'	51.80	200bp	1	0	1	100	0.0
8	STM1050	F 5'-TTCGATAAATGTAGAAATCATATGATCG-3' R 5'-AAGGCTGTGATACCTTAGCCTG-3'	58.55	120bp	1	0	1	100	0.0
9	ST10012	F 5'-GAAAGGACTTCCAAATCAGA-3' R 5'-AAAAGGAGGAATAGAAAACCAAAA-3'	62.90	100- 300bp	2	0	2	100	0.49
10	STGBSS	F 5'-AAATCGGTGATAAATGTGAATGC-3' R 5'-ATGCTTGCCATGTGATGT-3'	64.30	120bp	1	0	1	100	0.0
11	STM36	F 5'-TGATCTCTGCCTACTGTAAATCG-3' R 5'-CAAAGTGGTGTGAAAGCTGTGA-3'	63.50	120bp	1	0	1	100	0.0
12	STG0016	F 5'-AGCTGCTCAGCATCAAGAGA-3' R 5'-ACCACCTCAGGCATTCATC-3'	63.65	120bp	1	0	1	100	0.0
13	STM1106	F 5'-TCCAGCTGATGGTAAAGTTG-3' R 5'-ATGCGAATCTACTCGTCAATGG-3'	65.50	100- 300bp	2	0	2	100	0.32
14	ST124	F 5'-CGCCATCTCAGATCAGTCA-3' R 5'-GCTGCAGCAGTTGTTGTTG-3'	63.50	100- 200bp	2	1	1	50	0.50
15	SSR136	F 5'-GAAAACCGCTCTTTCACCTTG-3' R 5'-GCAATCTCAACTGCAATCG-3'	51.9	180bp	1	1	0	0	0
16	ATT-7	F 5'-GCATTTGATGAACATTCATTCGTCC-3' R 5'-ATTTTGTCCACCAACTAACCCG-3'	55.1	180bp	1	1	0	0	0
17	SSR565	F 5'-GAGGATGATGAACTCGCC-3' R 5'-TCAGAGGCTTCGGGTCAAGT-3'	56.5	180bp	1	1	0	0	0
18	SSR606	F 5'-GTCCTTGACAAAAGCGTC-3' R 5'-ATGTCAAAACCTACAGATGCA-3'	55.0	180bp	1	1	0	0	0
19	SSR46	F 5'-CCGAGGCGAATCTGAATAC-3' R 5'-GCACCATCTTGTGCCTCT-3'	55.5	220bp	1	1	0	0	0
20	SSR-4	F 5'-TTCCTCGGAGACGAAGGTA-3' R 5'-CTTCAATCCTCCAGATCCA-3'	54.45	180bp	1	1	0	0	0
Total					22 ^a	3 ^a	19 ^a	89.28 ^a	0.27 ^a
Average					1.57 ^a	0.21 ^a	1.36 ^a	89.28 ^a	0.27 ^a

^a primer that did not show polymorphism were not included for estimating total and average value

Table 3. Summary of SSR amplified fragments.

Total number of primer tested	20
Number of polymorphic primers	14
Total number of monomorphic primer	06
Total number of unique bands	02
Number of primer that gave unique bands	02
Total number of polymorphic bands	244
Total number of monomorphic bands	125
Total number of bands	369
Size range of amplified products (bp)	100-300bp
Percent polymorphism	50-100%

produced different numbers of DNA fragments, depending upon their simple sequence repeat motifs. The DNA was good in quality and quantity, free from proteins and RNA contamination of all 25 tomato genotypes used in this study. Out of twenty SSRs, six SSR markers *viz.*, SSR136, ATT-7, SSR565, SSR606, SSR46 and SSR-4 amplified monomorphic banding patterns, hence were not considered for further analysis. Fourteen markers were thus used for final analysis based on scoreable amplified fragments. The number of SSR alleles scored, polymorphism detected, polymorphic information content observed for each 14 primers in 25 genotypes. DNA amplification profile for marker SSR111, STM1106, STI0012 and STI003 are presented in Fig.1. Fourteen SSR markers exhibited 369 bands in the twenty five tomato germplasm of which polymorphic and monomorphic bands were 244 and 125, respectively. The range of amplified products was 100-300bp approximately. Unique band were also observed by the primer namely STI0012 and STI0003 of approximate size of 210 bp and 170 bp, respectively. The primer STI0012 gave a unique band in CO-3 and primer STI0003 gave unique in germplasm DARL-69. A summary of SSR amplified fragments is presented in Table 2.

Similarity coefficient and cluster analysis: Data scored on twenty five tomato germplasm with 14 microsatellite (SSR) markers were used to generate Jaccard's similarity coefficient presented in Table 3. In tomato germplasm, Jaccard's similarity coefficient varied from 0.65 between germplasm EC519821 and CO-3 to a maximum of 1.0 between varieties EC519769 and DARL-66, with an average value of 0.83. UPGMA cluster analysis of genetic similarity matrix resulted in the dendrogram. All twenty five germplasm were demarcated at approximately 75 per cent similarity and further divided into two major clusters A and B (Fig.2) at 75 per cent similarity. The germplasm EC519821 was most diverse among all genotypes.

Molecular diversity was done to access the degree of genetic diversity or relatedness and to establish distinctiveness among the varieties. The PCR based molecu-

lar marker system which SSRs were used for the study. The results are reported and discussed under the following heads. Fourteen SSR markers exhibited 369 bands in the twenty five tomato germplasm.

Benor *et al.* (2008) studied the genetic diversity of 39 determinate and indeterminate tomato lines and also reported the range of amplified products of 100-400 bp. Two unique bands were reported with two markers STI0012 and STI0003 in genotype CO-3 and DARL-69. These unique bands could have a number of potential applications including the determination of cultivar purity, efficient use and management of genetic resources collection and the establishment of property rights. In tomato germplasm, total numbers of 22 SSR alleles were generated by 14 SSR markers, out of which 19 were polymorphic and 3 were monomorphic, with an average of 1.57 alleles per locus. Al-Qadumii *et al.* (2012) reported 27 SSR alleles in land races of tomato grown by farmers with seventeen SSR markers. A range of polymorphism was observed from 50% (SSR20, SSR43, and STI24) to 100% (SSR111, STI032, STI0003, STI0023, STM1045, STM1050, STI0012, STGBSS, STM36, STG0016, and STM1106) with an average of 89.28%. El-Awady *et al.* (2012), observed 38 amplified alleles in their study on Egyptian tomato cultivars, with 20 SSR markers out of which 23 alleles were polymorphic. The PIC value ranged from 0 to 0.54 with an average of 0.27. The highest PIC (0.54) was observed with markers STI0003 and SSR 43. In a study on 39 tomato line the range of PIC from 0.05 to 0.60 were reported by Benore *et al.* (2008).

Similarity coefficient and cluster analysis: In tomato germplasm, Jaccard's similarity coefficient varied from 0.65 between germplasm EC519821 and CO-3 to a maximum of 1.0 between varieties EC519769 and DARL-66, with an average value of 0.83. Similar results were reported by Sanghani and mandivia (2013) in tomato. The germplasm DARL-66 and EC519769 was found 100% similar to each other. The low range of similarity coefficient between any two genotypes indicated the presence of narrow genetic variability among the genotype studied.

The phylogenetic tree was constructed through NTSYSpc cluster analysis software using UPGMA (Un-weighted pair group method with arithmetic mean). UPGMA cluster analysis of genetic similarity matrix resulted in the dendrogram. All twenty five germplasm were demarcated at approximately 75 per cent similarity and further divided into two major clusters A and B (Fig.2) at 75 per cent similarity.

Cluster A comprised most diverse germplasm (EC519821). Cluster B was again bifurcated into sub clusters namely cluster b₁ and b₂ at 78% simi-

Table 4. Jaccard similarity coefficient matrix of SSR for Tomato genotypes.

G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	G ₇	G ₈	G ₉	G ₁₀	G ₁₁	G ₁₂	G ₁₃	G ₁₄	G ₁₅	G ₁₆	G ₁₇	G ₁₈	G ₁₉	G ₂₀	G ₂₁	G ₂₂	G ₂₃	G ₂₄	G ₂₅	
G ₁	1																								
G ₂	0.86	1																							
G ₃	0.86	0.86	1																						
G ₄	0.89	0.89	0.96	1																					
G ₅	0.86	0.86	0.93	0.96	1																				
G ₆	0.89	0.83	0.89	0.93	0.96	1																			
G ₇	0.86	0.86	0.93	0.96	1.0	0.96	1																		
G ₈	0.82	0.89	0.82	0.86	0.89	0.86	0.89	1																	
G ₉	0.79	0.79	0.86	0.89	0.93	0.89	0.93	0.89	1																
G ₁₀	0.89	0.82	0.89	0.93	0.96	0.93	0.96	0.86	0.89	1															
G ₁₁	0.79	0.79	0.86	0.89	0.93	0.89	0.93	0.82	0.86	0.89	1														
G ₁₂	0.79	0.79	0.86	0.89	0.93	0.89	0.93	0.89	0.93	0.89	0.93	1													
G ₁₃	0.86	0.79	0.86	0.89	0.93	0.89	0.93	0.82	0.93	0.96	0.86	0.86	1												
G ₁₄	0.89	0.89	0.89	0.93	0.96	0.93	0.96	0.93	0.89	0.93	0.89	0.89	0.89	1											
G ₁₅	0.86	0.79	0.93	0.89	0.86	0.82	0.86	0.75	0.79	0.89	0.79	0.86	0.82	1											
G ₁₆	0.82	0.82	0.89	0.93	0.96	0.93	0.96	0.93	0.96	0.93	0.89	0.96	0.93	0.82	1										
G ₁₇	0.79	0.79	0.86	0.89	0.93	0.89	0.93	0.82	0.86	0.89	0.86	0.86	0.93	0.79	0.89	1									
G ₁₈	0.79	0.79	0.86	0.89	0.93	0.89	0.93	0.82	0.93	0.89	0.86	0.93	0.89	0.79	0.89	0.93	1								
G ₁₉	0.89	0.82	0.82	0.86	0.89	0.86	0.89	0.86	0.82	0.93	0.89	0.89	0.93	0.82	0.86	0.82	0.82	1							
G ₂₀	0.86	0.86	0.86	0.89	0.93	0.89	0.93	0.89	0.86	0.89	0.86	0.86	0.96	0.86	0.89	0.86	0.86	0.89	1						
G ₂₁	0.93	0.86	0.86	0.89	0.93	0.89	0.93	0.89	0.86	0.96	0.86	0.93	0.96	0.86	0.89	0.86	0.86	0.96	0.93	1					
G ₂₂	0.89	0.75	0.75	0.79	0.82	0.86	0.82	0.86	0.89	0.86	0.75	0.82	0.89	0.86	0.75	0.86	0.75	0.82	0.86	0.82	0.89	1			
G ₂₃	0.89	0.82	0.82	0.86	0.89	0.86	0.89	0.86	0.82	0.93	0.82	0.89	0.93	0.82	0.86	0.82	0.82	0.93	0.89	0.96	0.86	1			
G ₂₄	0.72	0.72	0.79	0.75	0.79	0.75	0.79	0.75	0.72	0.75	0.79	0.72	0.72	0.72	0.75	0.72	0.72	0.75	0.79	0.79	0.68	0.82	1		
G ₂₅	0.79	0.72	0.72	0.75	0.79	0.75	0.79	0.75	0.79	0.82	0.72	0.72	0.86	0.82	0.79	0.75	0.72	0.79	0.82	0.86	0.82	0.82	0.82	0.6	1

where G₁= DARI-67, G₂= CLN-2413, G₃= Cherry Tomato, G₄= Cherry-2, G₅= DARI-66, G₆= Cherry Sutton, G₇= EC-519769, G₈= EC-519818, G₉= EC-519812, G₁₀= EC-519812, G₁₁= DARI-68, G₁₂= PT-19, G₁₃= ARTH-3, G₁₄= AC-576, G₁₅= Sel-816-06, G₁₆= DARI-62, G₁₇= DARI-69, G₁₈= Arka Vikas, G₁₉= Pant T-3, G₂₀= Shalimar, G₂₁= PT-8, G₂₂= EC519724, G₂₃= PT-09-06, G₂₄= EC519821, G₂₅= CO-3

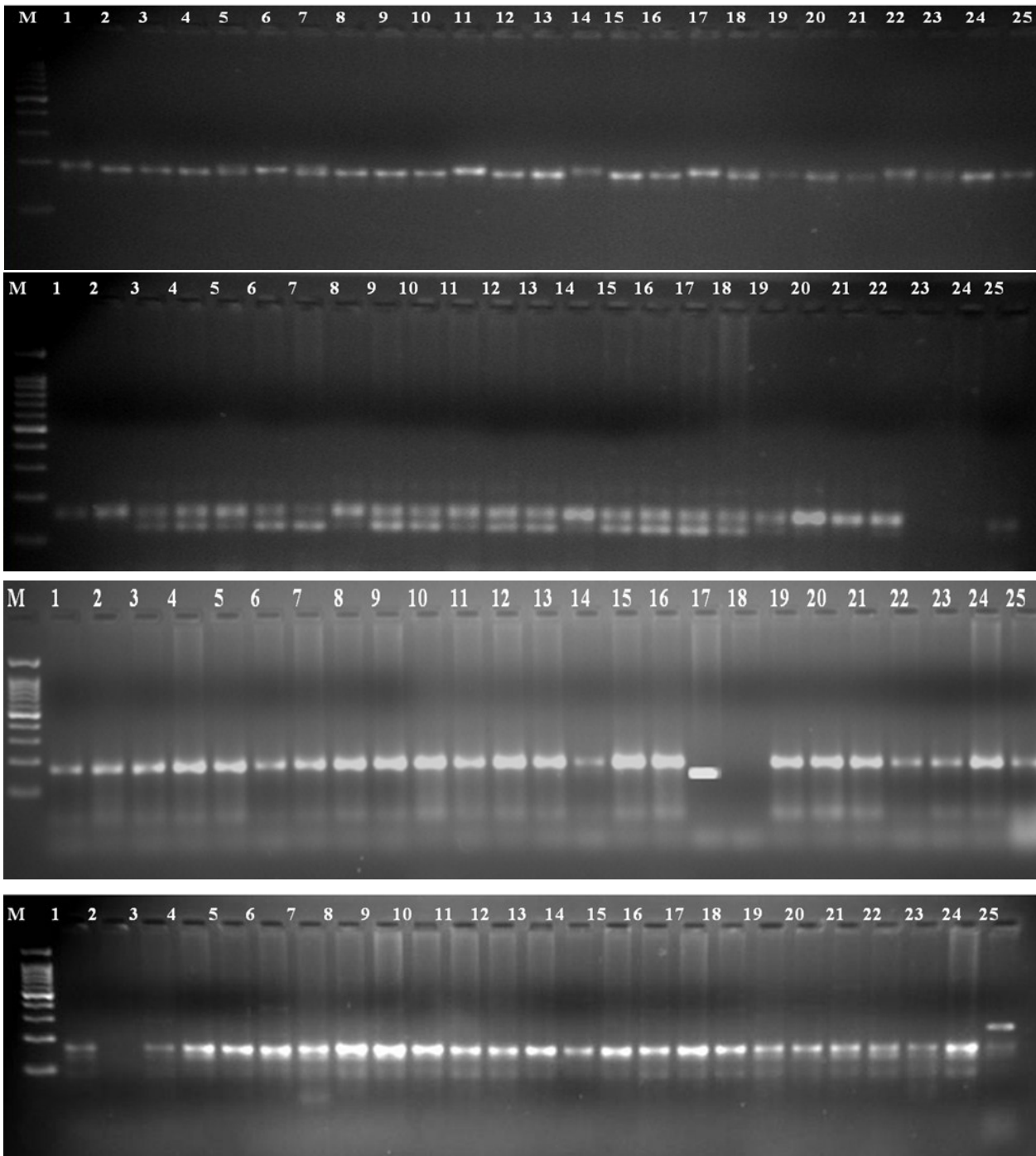


Fig. 1. Amplification profile of 25 genotypes of tomato with SSR111, STM1106, STI0012 and STI003. (M=Marker 100 bp ladder, line1-25(1= DABL-67, 2= CLN-2413, 3= Cherry Tomato, 4= Cheryy-2', 5= DABL-66, 6= Cherry Sutton, 7= EC519769, 8= EC-519977, 9= EC-519818, 10= EC519812, 11= DABL-68, 12= PT-19, 13= ARTH-3, 14= AC-576, 15= Sel-816-06, 16= DABL-62, 17= DABL-69, 18= ArkaVikas, 19= Pant T-3, 20= Shalimar, 21= PT-8, 22= EC519724, 23=PT-09-06, 24= EC519821, 25= CO-3)

larity. Cluster B1 consisted of 23 germplasm namely, DABL-67, EC519812, ARTH-3, Pant T-3, PT-8, PT-09-06, EC-519724, Cherry Tomato, Cheryy-2, DABL-66, EC519769, Cherry Sutton, AC-576, Shalimar, EC519818, DABL-62, PT-19, DABL-68, DABL-69, ArkaVikas, CLN-2413, EC519977 and

Sel-816-06, while cluster B₂ containing the single germplasm CO-3. Germplasm which are same by fruit size namely Cherry Tomato and Cheryy-2 were trends in similar cluster similar with approximately 96% similarity. CO-3 which is mutant variety showed deviation from existing cluster was also

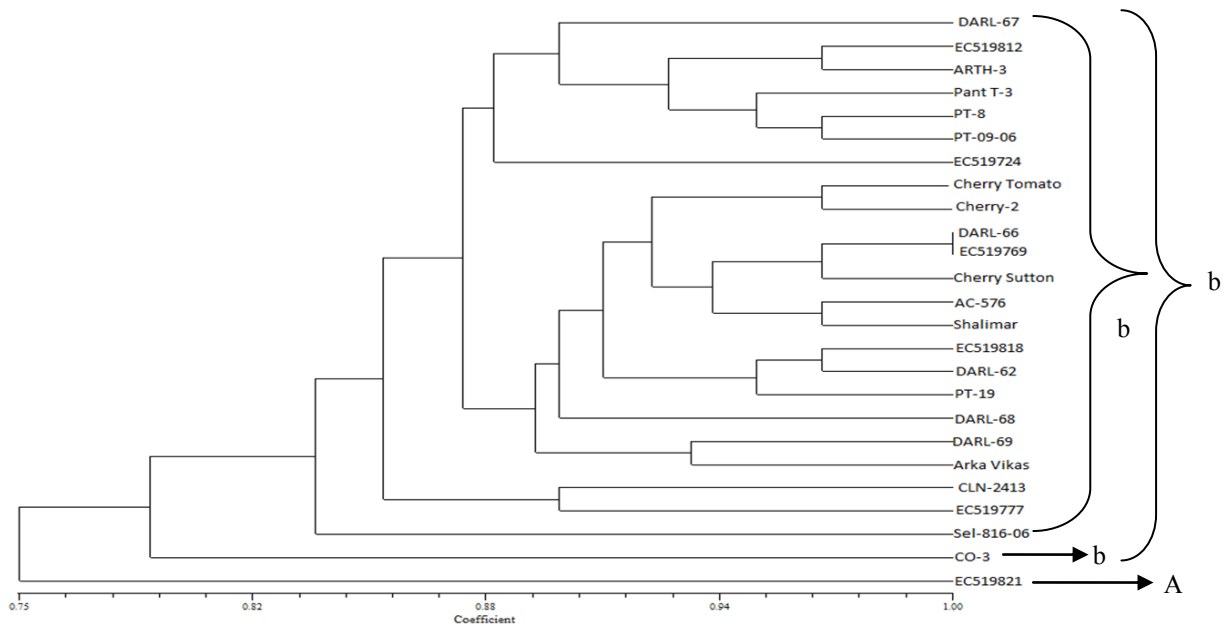


Fig.2. Dendrogram depicting the classification of the twenty five germplasm of tomato constructed using UPGMA method and based on SSR. The scale at the bottom is Jaccard's coefficient of similarity.

diverse with respect to their genetic makeup and morphological traits. The highest similarity Dendrograms showed a good fit between the graphical representation of distances and their original matrices. The germplasm which showed similar morphological and genetic trends were grouped more or less together in both these cases were a few. The very small fruited germplasm falls in most diverse cluster was EC519821 in cluster A, the present finding are consistent with the earlier report of Dhaliwal *et al* (2011). Cluster analysis revealed that the genotypes were not grouped according to the geographical distribution indicating that geographical distribution may not be criteria of genetic diversity in tomato. More or less they are grouped on the basis of horticultural and genetically make up of genotypes.

Conclusion

It can be concluded that the SSR proved helpful for estimating the magnitude of genetic diversity in tomato at molecular level and also established genetic relatedness among genotypes studied. Cluster analysis revealed that the mutant variety CO-3 is diverse with other genotypes studied among *Solanum lycopersicum* L. genotypes. The genotype EC915821, a very small fruited genotype of *Solanum pimpinellifolium* L. falls in most diverse cluster A. Hence SSR could differentiated diversity between *S. lycopersicum* L. and *S. pimpinellifolium* L. at species level. On the basis of banding pattern, SSR was effectively used for molecular characterization of tomato genotypes in the present study.

Therefore, there is need to support morphological diversity. It opens new perspectives towards conservation of tomato genetic resources and their further use in breeding programme.

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