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RESEARCH ARTICLE

Genetic Parameters, Diversity and Population Structure in Tomato based on Quantitative Traits and Microsatellite Markers

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Fifty accessions of tomato, including related species were evaluated to study genetics for yield and quality attributes and diversity based on quantitative traits and molecular markers. The results have shown high heritability and genetic advance for the number of fruits per plant (96.14 and 193.5), fruit weight (87.61 and 96.70), yield per plant (95.45 and 78.90) and lycopene content (98.31and 43.44) which indicated that these traits based selection is adequate. Among the accessions, extensive genetic diversity was observed for both quantitative traits and molecular analysis. From cluster means, accession Khasi Local was superior for fruit weight (80.30 g), LE-1-2 for lycopene (12.23 mg/100g) and *S. peruvianum* (1) for TSS (6.17) and vitamin-C (46.63mg/100g) content. In molecular analysis (35 microsatellite markers), a total of 118 alleles were observed with an average of 3.47 alleles per marker, and polymorphism information content ranged from 0.25 (SLM6-38) to 0.79 (LEat-015). From group based genetic diversity analyses, maximum diversity was observed in common cultivated tomato (100%) followed by cherry tomato (82.35%) and local landraces (Khasi Local and Tura-1). The molecular markers associated to QTLs for tolerance to bacterial wilt (SLM6-17, SLM6-14), fruit firmness (LEga7, LEaat7, and LEaat1), shelf life (LEat16) and yield (LEaat1 and LEaat18) have also shown wider diversity. Moreover, marker SLM6-17(150bp) tightly linked to major QTL associated with bacterial wilt was unique to accession LE-1-2, DMT-1 and BWT-3 and SLM-6-14 (240bp) for Sel-3, BWT-3, and RCT-3. These markers could be utilized in the selection of genotype having multiple desirable traits through MAS approaches.

Key Words: Genetic diversity, Quality, Molecular markers, Tomato, Yield

Introduction

Tomato (*Solanum lycopersicum* L.) is a worldwide grown Solanaceous vegetable crop. It is an important source of antioxidants such as lycopene, β-carotene, and ascorbic acid (Hanson *et al*., 2004) and used for cooking, table purpose, and in preparation of different processed products.

To develop varieties having a higher yield and superior quality, the information on the genetics of yield and quality attributes is essential. Deployment of a particular breeding strategy needs an insight into the components of genetic variability. The phenotypic expression of the plant is mainly controlled by the genetic makeup and the environment, in which it is growing. Therefore, it is necessary to partition the observed phenotypic variability into its heritable and non-heritable components with suitable parameters such as phenotypic and genotypic coefficient of variation, heritability, and genetic advance. Further, the genetic advance can be used to predict the efficiency of selection. It is a known fact that more the variability, higher the chance of getting desirable genotype. However, it is only the genetic variation which is heritable and hence significant. Solanaceae crops viz., potato, tomato, and pepper have greatly benefited from the use of wild relatives in breeding programs. Virtually all of the disease resistance in modern tomato varieties originated in related wild species (Rick and Chetelat, 1995). Among the crop species, genetics and genomics of tomato are well studied (Foolad, 2007). Genetic diversity can be evaluated using morphological traits or molecular markers. Morphological traits are the simplest way to investigate genetic diversity but they are often influenced by the environment. Molecular markers help to understand genetic variation at the DNA level without any influence of the environment. Further, breeding efficiency in tomato can be improved by using DNA markers to tag and transfer useful alleles from germplasm to elite cultivars (Foolad, 2007). There are several molecular marker systems which have been applied in tomato to the study of genetic diversity,

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fine mapping and marker-assisted selection of QTLs associated with yield and quality (Yogendra *et al*., 2013), drought (Foolad *et al*., 2003), heat (Xu *et al*., 2017), low temperature (Liu *et al*., 2016) bacterial wilt and late blight (Thoquet *et al*., 1996, Moreau *et al*., 1998).

The North-eastern region of India is known for diverse genetic resources in different crop species. The region falls under high rainfall zone, and due to mild warm temp (20-30 ºC) and humid (>75%) weather during crop season which is conducive for diseases, the incidence of bacterial wilt and late blight are severe (up to 100%). Besides yield loss, the quality of the product is also affected. These problems are more severe to the farmers adopting organic production package. The primary research work at country level in India has been focused on breeding of varieties against moisture stress and tomato leaf curl viruses, and limited work has been done for resistance to bacterial wilt and blight, but they are the primary production constraints of the region. For the development of tomato varieties well suited to the region, the present research work was initiated with following objectives: To study the genetics of yield and quality traits of tomato including wild relatives, and diversity among the accessions at the quantitative and molecular level.

Materials and Methods

Plant materials

This experiment was conducted using 50 accessions of tomato including commercial cultivar and relative species collected from different of institutes of Indian Council of Agricultural Research (ICAR) and state agricultural universities (Table 1). Among the accessions, the incidence of bacterial wilt ranged from 7.5-27.0 %. Advance breeding lines MCTR- 4B, RCMT-8, MT-11, MT-3, MT-2, MCTR-3, MCTR-4, Sel-2 and resistant check BT-317 were found resistant to bacterial wilt with < 10% incidence of bacterial wilt.

Genetic parameters and diversity based on quantitative traits

All the accessions were evaluated under open field condition for three consecutive years (January to April 2013-2016). The experimental site was Horticulture Farm, ICAR Research Complex for NEH Region, Umiam, Meghalaya (latitude 25º41'N and 92º55'E longitude) located at 960 m above mean sea level. Yearly, rainfall ranged from 2,200-2,551 mm and the average maximum and minimum temperatures during crop period were 28.3°C and 18.0°C, respectively. This location has inceptisol soils of sandy texture and acidic in reaction (pH: 5.4). The pre-treated seeds with Captan were sown in the nursery each year during the first week of January. One-month-old seedlings were transplanted on raised beds (3.5 m × 2.0 m size) at 45 cm × 30 cm spacing between the line to line and plant to plant, respectively. The accessions were evaluated in Augmented Block Design. Recommended doses of FYM (10 t/ha) and chemical fertilizers NPK applied at 120:80:60 kg ha^{-1} from urea, single super phosphate (SSP) and muriate of potash (MOP), respectively. Full dose of P and K with one-third of N was applied at the time of land preparation. The remaining dose of

Table 1. Accessions of tomatoes and their sources

S. No.	Name of the accessions	Source
1.	DVRT-2, H-86, BT-317, DMT-5, LE-1-2, BWT-3, LE-626, DMT-1, BT-1, Var-801, KSS-227, Cherry Tomato-1, Cherry Tomato-2, Cherry Tomato-3, Cherry Tomato-4 & Cherry Tomato-5	ICAR- AICRP on Vegetable Crops
2.	*S. peruvianum* (1), *S. peruvianum* (2) & *S. pimpinelifollium*	ICAR-IIVR, Varanasi
3.	TMC-1, TMC-2, RCT-3, RCMT-8, MT-5, MT-6, MT-11, MCTR-3, MCTR-4, MCTR-4A, MCTR-4B, MCTR-5, MCTR-7B, Sel-1, Sel-2, Sel-3, Sel-8, Sel-9A & Sel-11	ICAR Research Complex for NEH Region, Manipur Centre, Manipur
4.	MT-1-1, MT-2, MT-3 (Big), MT-10, Tura-1 & Khasi Local	ICAR Research Complex for NEH Region, Umiam, Meghalaya
5.	Pusa Rohini	ICAR-IARI, New Delhi
6.	Junagadh Tomato -1	Junagadh Agricultural University, Junagarh, Gujarat
7.	Arka Vikash	ICAR-IIHR, Bengaluru, Karnataka
8.	Pant T-10	GBPUAT, Pantnagar, Uttarakhand
9.	Solan Lalima	Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himanchal Pradesh
10.	V L Tamatar - 4	ICAR-Vivekananda Parvatiya Krishi Anushandhan Sansthan, Almora, Uttarakhand

N was applied in two split dosages at 30 and 60 days after planting. To control leaf miner and fruit borer insecticide, Imidacloprid (0.5 ml/L water) was sprayed prophylactically at 30 and 60 days after transplanting. Manual weeding and hoeing were done during 25-30 and 60-75 days after transplanting. Observations for growth and yield attributes were taken on six plants in each replication. Quality parameters such as TSS (^{o}B), acidity, total sugar (%), ascorbic acid (mg/100g) and lycopene content (mg/100g) were estimated by following the procedure as suggested by Ranganna (1985). The mean values of each replication for all the 13 quantitative traits were used to assess genetic parameters and dissimilarity between accessions.

Quantitative data analyses

The analysis of variance for the design of the experiment was carried out according to the procedure outlined by Panse and Sukhatme (1967). Phenotypic and genotypic coefficient of variability were calculated according to the method suggested by Burton and de Vane (1953). For estimation of genetic parameters such as heritability (broad sense), genetic advance and correlation were calculated according to Johnson *et al*. (1955). Genetic diversity was estimated following Mahalanobis's (1936) generalized distance (D^2) extended by Rao. Tocher's method (Rao, 1952) was followed for determining the group constellations.

Molecular characterization

Young actively growing leaves from one-month-old plants (pooled 5 plants in each accessions) were collected and used for DNA extraction. Total genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Saghai–Maroof *et al*., 1984). Nanodrop™ 1000 Spectrophotometer (Thermo Scientific, USA) used for DNA quantification. Total 40 SSR markers, of which 22 SSR (LE series associated with QTL for yield and quality attributes) developed by He *et al*. (2003) using Gene Bank Database and 18 SSR (SLM series associated with QTL for resistance to bacterial wilt) developed by Geethanjali *et al*., 2010 using genomic sequences of anchored BAC clones on chromosome 6 were surveyed to identify primers that were reproducible and generated the most polymorphic patterns. Veriti PCR machine (Applied Biosystem, USA) was used to carry out PCR reaction. The amplification products were electrophoresed on 3% agarose gel at 60 volts. Gels were stained with ethidium bromide and documented using a Chemidoc gel documentation unit (BioRad, California, USA).

Molecular data analyses

Molecular weights of bands were estimated by using 50 bp DNA ladder, and the homology of bands was based on the distance of migration in the gel. SSR amplicons obtained from each entry were resolved as a band on the gel system, and the data sets were used to calculate major allele frequency and the polymorphism information content (PIC) for each locus using Power Marker software. GenAlEx v.6.1 software was used to calculate pair-wise Nei's genetic distance and private alleles. Neighbor-joining dendrogram (cluster analysis) based on Nei's genetic distance for genic-SSR was generated using MEGA 7 software.

Population structure analysis was carried out using STRUCTURE 2.3.4 software (Pritchard *et al*., 2000). The optimum value of K was determined in Structure Harvester online software (Earl and vonHoldt 2012; http://taylor0.biology.ucla.edu/structureHarvester/) by loading STRUCTURE 2.3.4 software results. Principal coordinate analysis (PCoA) based on allele frequencies was done by using XLSTAT software.

Results and Discussion

Nowadays, the focus of crop improvement in tomato is not only limited to yield but also quality parameters with tolerance to biotic and abiotic stresses. To breed the varieties with multiple desirable traits, diverse genetic materials for different traits are essential. The genotypic coefficient of variation (GCV) is considered as the real indicator of the extent of genetic variability in the population. In present investigation, the coefficient of variability for quantitative traits, indicated the higher value for phenotypic coefficient of variation (PCV) than the GCV for all the traits (Table 2). However, the differences between PCV and GCV were small and indicated the influence of higher degree of the genotype over environment on phenotypic expression of these characters. It also suggests that selection based on all these traits would be useful for future crossing program. Among the traits number of fruits/plant (95.64% and 97.54%), fruit weight (50.06% and 53.48%) and yield per plant (48.0 and 56.0%) exhibited higher GCV and PCV values, respectively, indicating the higher variability for these traits in the accessions. These traits has also contributed maximum toward genetic diversity. Other traits like plant height, number of primary branches, fruit

Table 2. Genetic parameters for different quantitative traits in 50 tomato accessions

Traits	Mean	Range	Vg	Vp	GCV	PCV	h^2 (Broad Sense)	Genetic Advance	Genetic advance as percentage of mean	Contribution to genetic diversity (%)
Plant height (cm)	80.32	42.0-136.3	341.11	368.78	22.99	23.91	92.50	36.64	45.60	6.12
No of primary branches	7.47	4.0-14.0	2.45	2.70	20.95	22.00	90.74	3.08	41.20	0.08
No of flowers/cluster	6.10	4.1-9.0	0.91	1.18	15.63	17.78	77.23	1.73	28.31	0.00
Fruit length (cm)	3.93	0.8-5.9	0.78	0.81	22.47	22.90	96.30	1.79	45.50	0.33
Fruit diameter (cm)	4.15	0.8-6.6	1.28	1.30	27.26	27.47	98.46	2.32	55.80	0.90
Fruit weight (g)	38.08	1.5-88.0	363.34	414.72	50.06	53.48	87.61	36.81	96.70	21.55
No of fruits/plant	30.07	9.6-130.0	827.08	860.33	95.64	97.54	96.14	58.17	193.5	16.73
Yield/plant (kg)	1.17	0.2-2.3	0.21	0.22	48.0	56.0	95.45	0.92	78.90	23.59
TSS (^{0}B)	4.75	3.5-6.5	0.19	0.20	9.09	9.27	95.88	0.87	18.36	0.65
Acidity (%)	0.71	0.4-0.9	0.01	0.01	16.27	16.73	92.86	0.23	32.59	0.33
Total Sugar (%)	1.56	1.2-2.2	0.04	0.04	12.90	13.13	97.62	0.41	26.13	4.08
Vitamin-c (mg/100g)	23.72	14.0-48.0	23.09	33.37	24.24	24.34	69.21	8.25	34.78	13.80
Lycopene (mg/100g)	9.74	5.7-13.6	4.30	4.37	21.27	21.46	98.31	4.24	43.44	11.84

length and diameter, vitamin – C, and lycopene content exhibited moderate PCV, GCV values indicating that a moderate level of genetic variability (Table 2). Similar findings in tomato were also reported by Kouam *et al*. (2018) for these traits.

Further, perusal of data presented in Table 2 has also shown high heritability coupled with high genetic advance, as a percent of the mean for the observed characters like number of fruits per plant, fruit weight, yield per plant, fruit diameter, fruit length, plant height, and lycopene content. It indicates a strong influence of an additive gene on these traits are under action and hence, simple selection based on the phenotypic performance of these traits would be effective and efficient. Johnson *et al*. (1955) have suggested that traits with high heritability coupled with high genetic advance would respond to selection better than those with high heritability and low genetic advance. High heritability and moderate genetic advance as a percentage of mean (GAM) values were observed for total sugar and acidity, indicating expression of these traits are influenced by non-additive gene action and environment. These traits could be exploited through the manifestation of dominance and epistatic components. Similar findings was also observed by Dar and Sharma *et al*. (2011) while, Yogendra *et al*. (2013) observed low heritability and genetic advance for lycopene in the F_2 population.

For effective use of parental lines in crossing programs, it is considered imperative to have analyses of genetic divergence based on morphological along with molecular analysis. Selection of parents based on the extent of genetic divergence has been successfully utilized in different crop species. The concept of genetic distance for this has been of essential utility in differentiating distinct populations. Several measures of distance have been proposed to suit various objectives, of which Mahalanobis generalized distance (Mahalanobis, 1936 and Rao, 1952) has occupied a unique place in plant breeding. The results of cluster analysis based on quantitative data (D^2 analysis), 50 accessions were grouped into seven clusters. Cluster I and III comprised maximum 33 and 7 accessions, respectively. However, clusters IV, V, VI, and VII were mono-genotypic with accession LE-1-2, BT-1, Khasi Local, and *S. peruvianum*-1, respectively and found most diverse from other accessions. Between the clusters, maximum genetic distance (3064.0) was observed between cluster VI (Khasi Local) and cluster VII (*S. peruvianum*-1) followed by cluster II and cluster VII. Likewise, maximum intra-cluster distance (394.44) was observed in cluster III comprising accessions of *S. cerasiformae, S. pimpinellifolium* and *S. peruvianum*-2. The intra-cluster divergence indicated the existence of genetic diversity within the cluster III. Average inter and intra-cluster distances revealed that, in general, inter-cluster distances were much higher than those of intra-cluster distances, suggesting homogeneous and heterogeneous nature of the germplasm lines within and between the clusters, respectively. Similar findings were also observed in tomato by Thapa *et al*. (2014).

From cluster mean, accession Khasi Local of cluster VI was found superior for fruit weight (80.30g), and

fruit diameter (5.30 cm) hence could be utilized for higher yield. Moreover, wild species *S. peruvianum* (1) of cluster VII was found to be superior and could be utilized in a breeding program for traits like number of fruits (124.2) per plant, TSS (6.17) and vitamin-C (46.63 mg/100g) content. Similarly, accession LE-1-2 of cluster IV was superior for lycopene (12.23 mg/100g) content. The results of genetic analysis have also shown high heritability and genetic advance for yield and quality traits (number of fruits per plant, fruit weight, yield per plant, and lycopene content). Therefore, identified superior line with unique traits from the diverse group could be utilized for hybridization to get novel recombinants.

Among the traits, positive and significant association of fruit yield per plant was observed with plant height, number of primary branches, fruit length, diameter, and weight (Table 3). This may be explained by the higher photosynthetic products available for partitioning to fruit production. The direct selection of these traits will be very useful for identifying genotype having a higher yield. Similar findings were also reported by Kouam *et al*. (2018). Moreover, among the quality parameters, TSS and acidity were negatively correlated while lycopene content was positively correlated with total sugar, TSS. Thus, these traits can be selected together for identifying lines having superior quality. The results of the present study are also in agreement with the result reported Singh *et al*. (2018).

Principal component analysis (PCA) is a technique which identifies plant traits which contributed most to the observed variation within a group of genotypes and has a practical application in the selection of parental lines for the breeding purpose In present investigation, the cumulative variance of 72.17% by the first four principal components with eigenvalues of more than 1.0 indicated that the identified traits within this axes exhibited significant influence on the phenotype of the cultivars and could effectively be used for selection among them. Similar findings were also observed by Chernet *et al*. (2014). The first three PC was contributed by traits such as fruit weight, fruit length, fruit diameter, yield per plant, vitamin-C and lycopene content and are essential for improving yield and quality traits. Therefore, these variables might be taken into consideration for a valid selection of parents for hybridization programs to broaden the genetic base in the population as well as to develop elite lines or F_1 hybrids.

The power of molecular markers for analyzing genetic diversity has been well established in a range of vegetable crops, including tomato. In present investigation, polymorphism survey was carried out with a total of 40 SSR primers out of which 35 had shown amplification, and 34 (19 EST and 15 genomic SSR) markers were polymorphic (Table 4). Further, molecular analysis has also shown wide allelic diversity and frequency among the different accessions. The

Table 3. Phenotypic correlation coefficients between 13 quantitative traits in 50 tomato accessions

Traits	Plant height (cm)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	No of fruits/ plant	No of primary branches	No of flowers/ cluster	TSS (0B)	Acidity	Total sugar (%)	Vitamin-C (mg/100g)	Lycopene (mg/100g)
Plant height (cm)												
Fruit length (cm)	0.050											
Fruit diameter (cm)	0.066	0.856**										
Fruit weight (g)	-0.019*	0.829**	0.930**									
No of fruits/plant	0.193	-0.716**	-0.723**	-0.77**								
No of primary branches	0.338**	0.061	0.217**	0.144	-0.076							
No of flowers/ cluster	-0.009	0.035	0.036	-0.043	0.167*	0.128						
TSS (0B)	0.118	-0.638**	-0.446**	-0.437**	0.714**	0.192*	0.162*					
Acidity	0.556**	0.140	0.237**	0.137	-0.033	0.432**	0.014	-0.057				
Total sugar (%)	0.210**	-0.341**	-0.279**	-0.309**	0.335**	-0.029	-0.054	0.284**	0.120			
Vitamin-C (mg/100g)	0.065	-0.203*	-0.326**	-0.345**	0.509**	-0.297**	0.004	0.327**	-0.168*	0.158		
Lycopene content (mg/100g)	0.028	-0.138	-0.134	-0.177*	0.221**	0.192*	0.118	0.241**	-0.028	0.246**	0.023	
Yield/plant(kg)	0.246**	0.594**	0.647**	0.680**	-0.216**	0.248**	0.130	-0.059	0.270**	-0.070	-0.069	0.006

*,** Significant at 1 and 5% level of significance, respectively

Table 4. Details of SSR primers used for genetic diversity analysis of tomato 50 accessions

Locus	Na	Ne	I	Ho	He	u He	Allele Frequency	PIC	Size of alleles (bp)
LEaat-006	4	1.94	0.91	0.10	0.48	0.49	0.66	0.50	160-190
LEat-016	4	2.07	0.94	0.10	0.52	0.52	0.64	0.45	160-195
LEcag-003	4	2.09	0.95	0.02	0.52	0.53	0.64	0.49	140-180
LEcaa-001	3	1.71	0.67	-	0.41	0.42	0.72	0.34	120-160
LEaat-001	5	2.65	1.17	0.10	0.62	0.63	0.50	0.63	160-240
LEaat-002	3	1.90	0.81	-	0.47	0.48	0.68	0.41	110-130
LEaat-007	4	3.56	1.32	0.02	0.72	0.73	0.34	0.68	95-125
LEat-015	4	3.83	1.37	0.91	0.74	0.75	0.26	0.79	380-410
LEat-017	3	2.40	0.95	1.00	0.58	0.59	0.78	0.32	160-210
LEat-018	4	3.55	1.33	0.88	0.72	0.73	0.34	0.75	400-450
LEct-003	5	3.68	1.40	0.07	0.73	0.74	0.32	0.75	220-245
LEctat-001	5	1.62	0.80	0.02	0.38	0.39	0.64	0.52	260-305
LEga-003	3	2.80	1.07	0.96	0.64	0.65	0.42	0.60	220-245
LEga-007	2	1.84	0.65	0.04	0.46	0.46	0.60	0.46	195-205
LEta-003	3	1.18	0.33	0.08	0.15	0.15	0.90	0.18	100-150
LEta-0019	2	1.17	0.27	0.02	0.14	0.15	0.82	0.29	240-245
LEta-0020	2	1.74	0.62	0.19	0.43	0.43	0.56	0.56	200-225
LEta-0014	2	2.00	0.69	-	0.50	0.50	0.42	0.61	150-170
LEta-0024	2	1.53	0.53	0.00	0.35	0.35	0.62	0.41	260-280
SLM-6-3	2	1.84	0.65	0.00	0.46	0.46	0.40	0.72	125-130
SLM-6-4	4	2.69	1.14	0.12	0.63	0.64	0.44	0.71	120-180
SLM-6-5	5	2.77	1.23	0.09	0.64	0.65	0.76	0.39	130-210
SLM-6-7	3	1.37	0.52	0.09	0.27	0.27	0.42	0.72	230-290
SLM-6-14	4	2.95	1.23	0.07	0.66	0.67	0.52	0.63	240-300
SLM-6-15	3	2.07	0.88	0.15	0.52	0.52	0.38	0.66	230-250
SLM-6-17	4	2.84	1.14	0.12	0.65	0.66	0.50	0.48	150-220
SLM-6-18	4	2.27	0.92	0.02	0.56	0.57	0.54	0.60	130-160
SLM-6-12	5	2.08	1.06	0.32	0.52	0.52	0.56	0.55	210-280
SLM-6-25	3	2.16	0.90	-	0.54	0.54	0.56	0.56	180-225
SLM-6-13	3	2.20	0.92	-	0.54	0.55	0.76	0.37	160-190
SLM-6-36	3	1.44	0.54	0.04	0.30	0.31	0.66	0.52	145-170
SLM-6-38	6	2.78	1.20	0.90	0.64	0.65	0.84	0.25	200-280
SLM-6-56	2	1.32	0.41	-	0.25	0.25	0.48	0.61	145-150
SLM-6-57	3	2.47	0.99	-	0.60	0.60	0.57	0.53	90-105
Mean	3.47	2.25	0.90	0.19	0.51	0.52	0.57	0.53	-

* Where: Na = No. of different alleles, Ne = No. of effective alleles, I = Shannon's Information Index, Ho = Observed heterozygosity, He = Expected heterozygosity, uHe = Unbiased expected heterozygosity, PIC = polymorphic information content (mean)

total numbers of observed alleles were 118, with an average of 3.47 numbers of alleles per marker. The number of alleles varied from 2 to 6 with allele size ranging from 90-105 bp (SLM-6-57) to 400-450 bp (LEat-018). The maximum number of alleles per marker was 6 in genomic marker SLM6-38. However, the most informative markers were EST marker LEat-015 with PIC (0.79) followed by LEat-018 and LEct-003 (0.75 each). The markers have also shown heterozygosity in the accessions, and the observed heterozygosity (Ho) was lower than the expected heterozygosity (He) and this may be due to an intensive selection of the accessions (Table 4). A large variation in polymorphism information content (PIC) of markers was observed for all the SSR loci and ranged from 0.25 (SLM6-38) to 0.79 (LEat-015) with the mean polymorphism of 0.53 per markers. The wide variation in PIC of SSR loci was also observed by Geethanjali *et al*. (2010), Zhou *et al*. (2015), and Gharsallah *et al*. (2016) in different accessions of tomato. According to high, medium and low locus polymorphism is defined as PIC > 0.5; PIC 0.25 - 0.5 and PIC < 0.25, respectively. Accordingly, PIC in our investigation indicated a wider genetic base by high locus polymorphism. This could be due to the involvement of cultivated as well as wild species of the tomato. Kwon *et al*. (2009), also reported higher PIC

value (0.628) ranging from 0.210 to 0.880 using 33 SSR markers while screening 63 tomato varieties.

The cluster analysis has shown broader diversity in the accessions of the tomato. Neighbor-joining dendrogram presented in Figure 1 shows the genetic relationships among the cultivated and relative species of tomato based on Nei's genetic distance using 34 cross transferable SSRs. All the accessions were grouped into six major clusters. Except *S. peruvianum* other species were distributed mixed with common cultivated tomato. *S. pimpinellifolium* was found closer to tomato cultivar BWT-3 while landrace Khasi Local was found closer to Cherry Tomato-5. Further, molecular analysis has also shown maximum variation within the population (86%) followed by between the populations (14%). From group based diversity analysis the maximum polymorphism was observed in common cultivated tomato followed by cherry tomato and local landraces (Khasi Local and Tura-1) while it was least in *S. pimpinellifolium* and indicated the broad genetic base of the cultivated species over the other species. Maximum genetic diversity was found in common and cherry tomato, and it could be due to the broad genetic base by hybridization and selection of the desired recombinants. Among the populations based on pairwise Nei genetic distance, the maximum genetic distance (1.34) was observed between *S. pimpinelifollium* and *S. peruvianum* followed by local landrace of cultivated tomato and accessions of *S. peruvianum.* This could be due to incompatibility between of *S. lycopersicum, S. pimpinelifollium* to *S. peruvianum* (Alvarez *et al*., 2001). Higher levels of genetic diversity in the self-incompatible species (*S. peruvianum*, *S. hirsutum*, *S. pennellii*, and *S. chilense*) than the self-compatible species (*S. esculentum*, *S. pimpinellifolium*, *S. cheesmanii*, *S. parviflorum*, and *S. chmielewskii*) have been reported (Miller and Tanksley, 1990). Further, principal coordinate analysis (PCoA)

Fig. 1. Neighbour-joining dendogram showing genetic relationships among the cultivated and wild species of tomato based on Nei's genetic distance using 34 cross transferable SSRs

proved the wider diversity in the common and cherry tomato. First three coordinates explained 38.0% of the total variation, with 18.61% defined by the first coordinate and 11.56% by the second coordinate. The accessions of *S. lycopersicum* and cherry tomato were distributed in both the coordinates. However, accessions of *S. peruvianum* and *S. pimpinelifllium* was separated by second coordinate.

Under structure analysis, the optimum number of the cluster was determined as per the procedure described by Evanno *et al*. (2005) using software STRUCTURE. The analysis detected the maximal ΔK at K=2, indicating that the entire population could be grouped into two sub-populations. The ΔK value decreases with an increase in K (Figure 2). Clusters differentiate ideally between and within the crop species. The genetic differentiation between clusters was low to moderate (F*st* = 0.10 – 0.59). Further, the structure result also agreed with the clustering and principal coordinate analysis. Among the accessions, the proportions of accessions with admixture were few, and it may be due to the different geographical origin. Moreover, the admixture was common in cultivated *S. lycopersicum* and cherry tomato (*S. lycopersicum* var. *cerasiforme*) which may be due to closeness, free natural gene flow between them, hybridization, and selection.

The markers used in the present studies were also associated with desirable QTLs and have differentiated the accessions. The more extensive allelic variation was observed for the markers associated with QTL for tolerance to bacterial wilt as well as fruit quality and yield. Among the tomato accessions, bacterial wilt tolerant lines BT-317 and MT-1-1 were identified by four

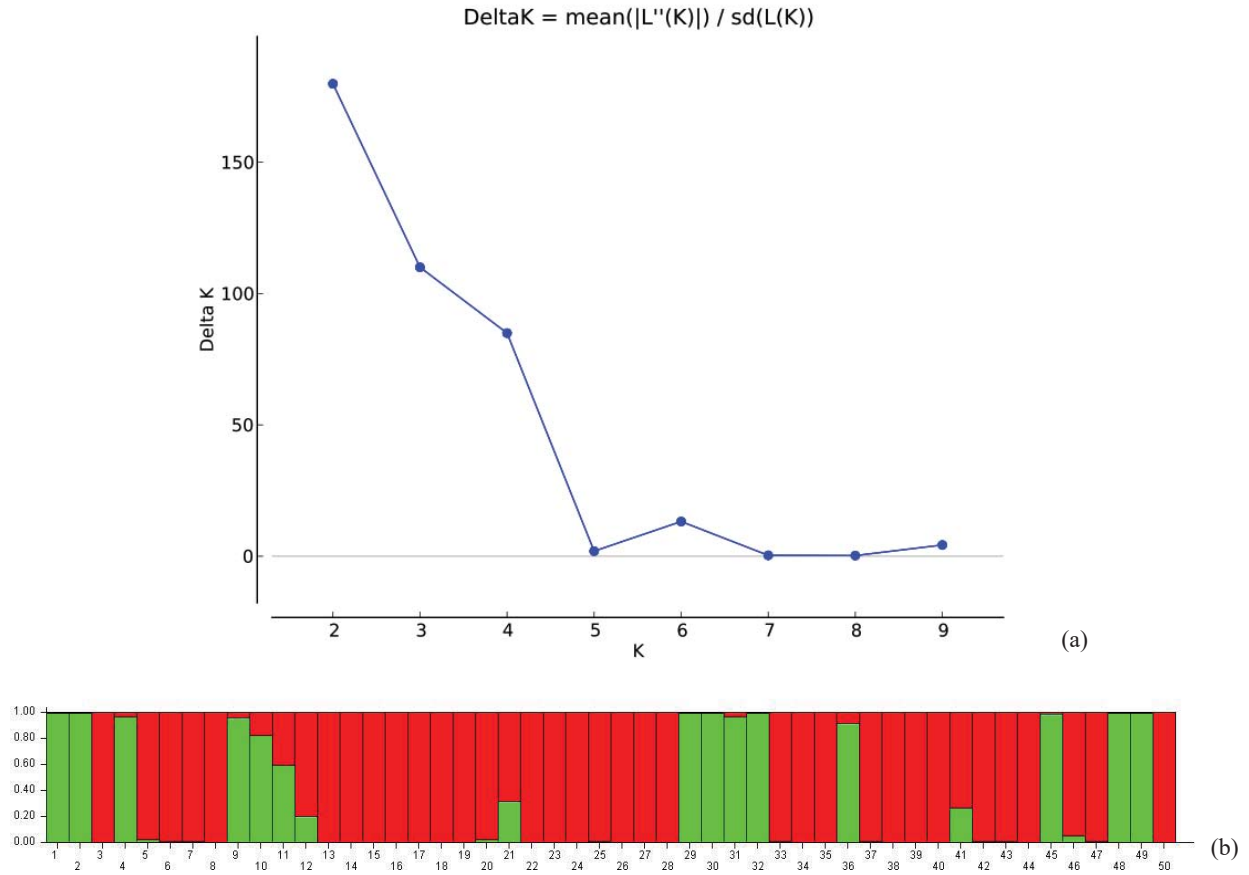

Fig. 2. Population structure of the accessions of cultivated and wild tomato accessions based on SSR markers. (a) ΔK graph, (b) population structure at ΔK =2.

Accessions Name: 1. DVRT-2; 2. VL Tamatar-4; 3. TMC-1; 4. TMC-2; 5. Pusa Rohini; 6. Junagadh Tomato-1; 7. Solan Lalima; 8. H-86; 9. RCT-3; 10. RCMT-8; 11. BT-317; 12. DMT-5; 13. LE-1-2; 14. BWT-3; 15. Arka Vikash; 16. LE-626; 17. Pant T-10; 18. DMT-1; 19. BT-1; 20. Var-801; 21. KSS-227; 22. MT-1-1; 23. MT-2; 24. MT-3 (Big); 25. MT-5; 26. MT-6; 27. MT-10; 28. MT-11; 29. MCTR-3; 30. MCTR-4; 31. MCTR-4A; 32. MCTR-4B; 33. MCTR-5; 34. MCTR-7B; 35. Sel-1; 36. Sel-2; 37. Sel-3; 38. Sel-8; 39. Sel-9A; 40. Sel-11; 41. Tura-1; 42. Khasi Local; 43. Cherry Tomato-1;44. Cherry Tomato-2; 45. Cherry Tomato-3; 46. Cherry Tomato-4; 47. Cherry Tomato-5; 48. *S. peruvianum* (1); 49. *S. peruvianum* (2); 50. *S. pimpinelifollium*

Which is correct: LEga-7 or **LEat-16 or LEaat-1 or LEaat-18?????**

markers SLM6-4 (120bp), SLM6-5 (130bp), SLM6-7 (230bp) and SLM6-12 (280bp) of chromosome-6. In earlier studies, Geethanjali *et al*. (2010) also identified these markers associated with QTL resistance to bacterial wilt strain Pss4. Further, marker SLM6-17 (150bp) tightly linked to major QTL associated with bacterial wilt was unique to LE-1-2, DMT-1, and BWT-3. Marker SLM6-14 (240bp) was also unique to bacterial wilt tolerant line Sel-3, BWT-3, and RCT-3. These identified lines and markers could be utilized in the mapping the gene linked to bacterial wilt and future improvement programs. Like quantitative traits, the molecular markers have also shown wider genetic diversity for fruit characteristics. Markers LEga-7, LEaat-7, and LEaat-1 from linkage group 3, 4 and eight associated with QTL for fruit firmness (Yogendra *et al*., 2013) have shown wider variability with PIC of 0.46, 0.68 and 0.63, respectively. Similarly, LEat-16 associated with QTL for shelf life was also found informative with PIC of 0.45 while marker LEaat-1 and LEaat-18 shown PIC value 0.63 and 0.75, respectively were associated with QTL for yield. These markers could be utilized in the selection of genotypes having multiple desirable traits through MAS approaches.

Conclusions

The overall analysis of the present investigation has shown the existence of wide genetic variability for different quantitative traits due to the significant contribution of the genotypic coefficient of variation. The traits for yield (number of fruits, size, and weight) and quality (lycopene) have shown high heritability, and genetic advance indicates for the higher role of additive gene action, and hence selection for these traits will be useful. Further, results of genetic diversity based on quantitative traits as well as molecular analysis revealed wider genetic diversity among the accessions for yield and quality attributes. The lines with unique traits from the diverse group could be utilized for developing new recombinants with many desirable traits. Moreover, molecular markers associated with unique traits (QTLs) could be used for MAS breeding. Further, there is also a need to introduce new lines having resistance to diseases, especially for early and late blight.

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