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RESEARCH ARTICLE

Genetic diversity, population structure and validation of SSR markers linked to *Sw-5* and *I-2* genes in tomato germplasm

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Abstract Tomato is the world's second largest cultivated vegetable crop. Tomato spotted wilt virus (TSWV) and fusarium wilt (FW) are the two major biotic stresses in India limiting tomato production. Identification and utilization of resistant lines to realize the full genetic potential of varieties for yield gain is an eco-friendly approach. The present research work involved genetic diversity study of 48 genotypes, augmented from different exotic, and indigenous sources belonging to three species using SSR markers. A total of 195 alleles were generated by employing 84 polymorphic markers. The PIC value was ranged from 0.12 to 0.93. Two sub-populations (K = 2) were revealed by model based structure analysis. The cluster analysis using the UPGMA method classified the genotypes into 6 clusters. Pusa Ruby, EC-310310 and EC-620452 were found to be highly diverse. Molecular characterization of 48 genotypes with SSR markers divulged seven genotypes with *Sw-5* gene and nine genotypes with *I-2* gene showing resistance to TSWV and FW, respectively and further, on artificial screening, they were found to be phenotypically resistant. Out of 195 alleles generated from 84 polymorphic SSR markers, 43 alleles from 26 SSR markers were identified with positive average allele effect distributed across nine chromosomes and positive average allele effect was identified for the average weight of the fruit, the number of fruits formed per plant, and fusarium wilt PDI score. Fruit weight and fruit yield per plant registered a significant and positive correlations. The identified genotypes with varied backgrounds and performances will be very useful as diversified sources in resistant breeding programs of tomato.

Keywords Fusarium wilt · Genetic diversity · *I-2* · *Sw-5* · SSR markers · Tomato spotted wilt virus · Population structure · Correlation

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Introduction

Solanum lycopersicum L. (Tomato), with diploid chromosome number of 24, is originated in South America. It occupies the second position in terms of the cultivated area of vegetable crops. It has a wide range of growth habitats in different climate conditions. It is considered as the richest source of dietary fiber, vitamins A, C, minerals, lycopene (Frusciante et al. 2007) and having anticancer properties (Bhuvaneswari and Nagini 2005).

The production of tomato is constrained by abiotic stresses like heat, drought, salinity (Parankusam et al. 2017) and biotic stresses like viral, bacterial, and fungal diseases. The major viral pathogens like Tomato Spotted Wilt Virus, Yellow Leaf Curl Virus, Tomato Mosaic Virus, Tobacco Mosaic Virus and Cucumber Mosaic Virus; bacterial pathogens and fungal pathogens affect tomato production and productivity. Out of these, TSW is a major viral disease caused by Tomato Spotted Wilt Virus belonging to *tospovirus* with a wide host range. Thrips play a vital role in disease transmission and symptoms include ring spots on fruits and reduced fruit yield (Saidi and Warade 2008). Insecticidal sprays result in almost no effect on the spread of this virus (Agrios 2005). A number of resistant genes were reported against TSWV disease *i.e., Sw-1, Sw-2, Sw-3, Sw-4, Sw-5, Sw-6* and *Sw-7* (Price et al. 2007; Saidi and Warade 2008). When compared among the reported genes, *Sw-5* and *Sw-6* as well demonstrated partial resistance to thrips incidence with a definite range of resistance to various isolates of the virus (Rosello et al. 2001), whereas three recessive genes, *Sw-2*, *Sw-3*, and *Sw-4* and two dominant genes *Sw-1a* and *Sw-1b* were observed to be able to quickly overcome resistance, therefore were not under use in commercial tomato production (Price et al. 2007; Saidi and Warade 2008).

Among the fungal diseases, fusarium wilt is one of the major diseases in tomato, causing significant yield loss. Fusarium wilt is caused by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) (FOL). Fusarium wilt is a vascular disease. As a soil borne disease, the pathogen can enter through damaged roots and thereby infect the crop during all its growth stages. FW induced wilting of leaves, stunting of plants, browning of the vascular system and cessation of fruit bearing also occur. FOL causes disease exclusively on species belonging to *Lycopersicon* genus (Currently under *Solanum*) and thereby causing yield loss to a great extent, resulting in limitation of tomato production worldwide. The deployment of resistant cultivars will be very effective in the control of this disease (Wong 2003) and the *I-2* gene, which is dominant in nature, brings in resistance against FOL race 2 in tomato. The gene is introgressed from *S. pimpinellifolium* (Stall and Walter 1965) and Laterrot 1976 reported that the gene is genetically identified on chromosome 11.

Solanum lycopersicum is highly diversified, consisting of 16 wild species harboring genes with potential application in breeding for incorporation of genes/ QTLs resistant to biotic and abiotic stresses into popular cultivars. Investigation of genetic variation in tomato genotypes using morphological, biochemical and molecular markers enables the selection of useful parental lines. Among these, morphological markers are often used in genetic diversity analyses which are often misrepresented by abiotic conditions (Cooke et al 2003). Various biochemical markers are also used to analyze the genetic diversity, which have provided tremendous information compared to morphological markers. Molecular markers are very informative in unraveling diversity and are useful in Marker-Assisted Breeding (MAB), which involves trait specific selection using foreground and background selection methods. These also can speed up the genome recovery in molecular breeding techniques (Narshimulu et al. 2011). At present, the genetic diversity in tomato is performed with DNA based markers like Amplified Fragment Length Polymorphic markers (AFLP) (Park et al. 2004), Restriction Fragment Length Polymorphic markers (RFLP) (Miller et al. 1990), Randomly Amplified Polymorphic DNA (RAPDs) (Tabassum et al. 2013), Inter Simple Sequence Repeats (ISSRs) (Henareh et al. 2016), Single Nucleotide Polymorphic markers (SNPs) (Wang et al. 2016) and Simple Sequence Repeats (SSRs)/ Microsatellites (Zhou et al. 2015; Aguirre et al. 2017). SSRs are the most widely used among the molecular markers due to high reproducibility and their co-dominant nature. Utility of SSRs in genetic diversity studies in tomato was taken up by different researchers (Alvarez et al. 2001; Bredemeijer et al. 2002; He et al. 2003; Frary et al. 2005; Yang et al. 2005; Garcia-Martinez et al. 2005; Mazzucat et al. 2008; Benor et al. 2008; Kwon et al. 2006, 2009: Pritesh et al. 2010; Zhou et al. 2015; Kaushal et al. 2017 and Jaafar et al. 2018).

In the present investigation, 48 genotypes were evaluated for their genetic diversity using 130 SSRs along with two gene specific primers to confirm the resistance of genotypes against TSWV and FW diseases, in addition to phenotypic screening of genotypes for resistance to both the diseases.

Materials and methods

Tomato germplasm augmented

A total of 48 tomato genotypes (Table 1), which include 32 genotypes collected from ICAR-NBPGR, India, 14 genotypes imported from Tomato Genetics Resource Center, University of California, Davis, CA, USA and two were superior released varieties. This experimental material was maintained at the farm of SKLTSHU during *Kharif,* 2018 under Genetics and Plant Breeding department to take up genetic diversity analysis.

DNA isolation and SSR analysis

Fresh leaf samples were collected from all 48 genotypes from 21 days old seedlings and isolation of genomic DNA

Table 1 Tomato genotypes and their sources used for diversity analysis

S. No.	Accession Number	Collection Source	Taxon	S. No.	Accession Number	Collection Source	Taxon
1	EC-251717	NBPGR, New Delhi, India	*Solanum lycopersicum*	25	EC-615047	NBPGR, New Delhi, India	*Solanum lycopersicum*
2	EC-273966	NBPGR, New Delhi, India	*Solanum lycopersicum*	26	EC-514013	NBPGR, New Delhi, India	*Solanum lycopersicum*
3	EC-310310	NBPGR, New Delhi, India	*Solanum lycopersicum*	27	EC-620428	NBPGR, New Delhi, India	*Solanum lycopersicum*
4	EC-620503	NBPGR, New Delhi, India	*Solanum lycopersicum*	28	EC-620452	NBPGR, New Delhi, India	*Solanum lycopersicum*
5	EC-625644	NBPGR, New Delhi, India	*Solanum lycopersicum*	29	EC-620446	NBPGR, New Delhi, India	*Solanum lycopersicum*
6	EC-615055	NBPGR, New Delhi, India	*Solanum lycopersicum*	30	EC-636482	NBPGR, New Delhi, India	*Solanum lycopersicum*
7	EC-617059	NBPGR, New Delhi, India	*Solanum lycopersicum*	31	EC-251751	NBPGR, New Delhi, India	*Solanum lycopersicum*
8	EC-617066	NBPGR, New Delhi, India	*Solanum lycopersicum*	32	EC-320565	NBPGR, New Delhi, India	*Solanum lycopersicum*
9	EC-617068	NBPGR, New Delhi, India	*Solanum lycopersicum*	33	EC-145057	NBPGR, New Delhi, India	*Solanum lycopersicum*
10	EC-620434	NBPGR, New Delhi, India	*Solanum lycopersicum*	34	EC-251518	NBPGR, New Delhi, India	*Solanum lycopersicum*
11	EC-620463	NBPGR, New Delhi, India	*Solanum lycopersicum*	35	LA-1940	TGRC, Davis, USA	*Solanum pennelli*
12	EC-620522	NBPGR, New Delhi, India	*Solanum lycopersicum*	36	LA-3120	TGRC, Davis	*Solanum lycopersicum*
13	EC-164295	NBPGR, New Delhi, India	*Solanum lycopersicum*	37	LA-3006	TGRC, Davis	*Solanum lycopersicum*
14	EC-631356	NBPGR, New Delhi, India	*Solanum lycopersicum*	38	LA-2662	TGRC, Davis	*Solanum lycopersicum*
15	EC-251694	NBPGR, New Delhi, India	*Solanum lycopersicum*	39	LA-0490	TGRC, Davis	*Solanum lycopersicum*
16	EC-631962	NBPGR, New Delhi, India	*Solanum lycopersicum*	40	LA-4345	TGRC, Davis	*Solanum lycopersicum*
17	EC-638302	NBPGR, New Delhi, India	*Solanum lycopersicum*	41	LA-3005	TGRC, Davis	*Solanum lycopersicum*
18	EC-676742	NBPGR, New Delhi, India	*Solanum lycopersicum*	42	LA-0535	TGRC, Davis	*Solanum lycopersicum*
19	Arka Vikas	IIHR, Bangalore, India	*Solanum lycopersicum*	43	LA-3847	TGRC, Davis	*Solanum lycopersicum*
20	LA-1589	TGRC, Davis, USA	*Solanum pimpinellifolium*	44	AVTO-9802	WVC, Taiwan	*Solanum lycopersicum*
21	EC-567305	NBPGR, New Delhi, India	*Solanum lycopersicum*	45	LA-1500	TGRC, Davis	*Solanum pimpinellifolium*
22	LA-3667	TGRC, Davis	*Solanum lycopersicum*	46	LA-1015	TGRC, Davis	*Solanum cheesmani*
23	Pusa Ruby	IARI, New Delhi, India	*Solanum lycopersicum*	47	LA-1664	TGRC, Davis	*Solanum lycopersicum*
24	AVTO-1219	WVC, Taiwan	*Solanum lycopersicum*	48	EC-620570	NBPGR, New Delhi, India	*Solanum lycopersicum*

was executed through modified CTAB method (Murray and Thompson 1980). Quantification and quality of the isolated DNA was performed with Biophotometer (Eppendorf) and 0.8% Agarose Gel Electrophoresis. Finally,

DNA was diluted using nuclease free water to a concentration of 50 ng/µl to perform PCR analysis using SSRs. A total of 130 SSRs were selected from different tomato genomic resources to analyze genetic diversity among 48 genotypes along with two gene specific markers against TSWV and Fusarium wilt (Table 2). Polymerase chain reaction (PCR) was performed with the SSR markers with a slight modification of the annealing temperature (55 °C). The amplified products were separated in a 3% agarose gel along with the marker in 0.5 Tris – Boric acid–EDTA (TBE) buffer and resolved using Vilber gel documentation unit.

Data analysis

The observed polymorphic bands were scored based on the molecular weight of the amplified product. The binary matrix generated using scoring data was analyzed for allelic frequency. All polymorphic markers were assessed for polymorphism information content (PIC) and was calculated according to formula PIC = 1 − Σpi2 (Botstein et al. 1980). The allelic matrix was employed in cluster analysis and dendrogram was constructed based on UPGMA (unweighted pair-group method with arithmetic mean) using NTSYS V 2.0 software (Rohlf 2000).

Model-based clustering program STRUCTURE V2.3.4 (Pritchard et al. 2000) was employed to deduce the population structure of all the 48 accessions. Number of populations (K) was determined with a burn-in period of 5000 and Markov Chine Monte Carlo of 50,000. Three independent runs were performed for each K varying from 1 to 10 and most probable K-value was defined based on ΔK method (Evanno et al. 2005) by running the Structure Harvester software (Earl and von Holdt 2012). Analysis of molecular variance (AMOVA) was performed using GENALEX 6.5 (Peakall and Smouse 2012) to estimate the genetic structure within and among populations estimated based on the ΔK method with 999 permutations.

Evaluation of genotypes for fusarium wilt resistance

Forty eight genotypes were sown in the pro-trays filled with coconut compost and were raised by the following general cultural practices. Artificial screening of genotypes for resistance to Fusarium wilt was conducted using root dip method with 21 days old seedlings. FW affected tomato plant samples were collected from five different disease infected areas, *i.e*., Cheruvu annaram, and Marrooru village in Nalgonda district; Amdapur village, Surangal village, and Dharmanna gudem village in Rangareddy District of Telangana state, India. Conidia of all the isolates were recovered from one week old cultures. All the isolates were characterized and tested for their pathogenicity and further most pathogenic (Surangal) isolate was utilized for phenotype screening for disease resistance. Seedlings were carefully removed from the protrays, and were washed with tap water to remove adhering soil particles. The roots were submerged in the conidial suspension for 30 min, with prior root trimming using a sterile scissor. The inoculated seedlings were transplanted to poly bags (15 cm diameter), after surface sterilized with 0.1% mercuric chloride containing soil and sand 1:1 ratio. The severity of the disease was assessed from 2 weeks after inoculation up to 45 days (Nirmaladevi and Srinivas 2012). The percent incidence for *Fusarium oxysporum* was calculated using a scale 0 to 4 as given by Silme and Cagirgan (2010), which was based on infection percent, as follows: Where, 0—highly resistant (0%), 1—resistant (0.33 to 25%), 2—moderately resistant (26 to 50%), 3—moderately susceptible (51 to 66.66%), 4—susceptible and highly susceptible (66.67–100%).

Evaluation for TSWV resistance under induced disease conditions

The TSWV isolates (fruit and leaf samples) were collected from Kanakamaidi, Sriramnagar, and Urella villages of Ranga Reddy district, which were confirmed for the presence of virus through ELISA and the most virulent strain (Sriramnagar-1) was utilized for screening all the 48 genotypes. The Sriramnagar-1 (local virulent) strain maintained on cowpea leaves was used to inoculate the genotypes artificially when they were at 2–4 leaf stage (Paterson et al. 1989). The plants were inoculated three times a week and continued till fruiting and scored for disease symptoms. Scoring of genotypes for the disease symptoms was taken up for TOSPO (TSWV) virus and were categorized (Juliatti et al. 1994) into 5 viz. whole

Table 2 Markers associated with *Sw-5* and *I-2* genes

S.No.	Marker	Forward primer	Reverse primer	Chromosome	Reference
1	Sw-5-F3	CGGAACCTGTAACTTGACTG	GAGCTCTCATCCATTTTCCG	9	Shi et al. (2011)
2	FWZ1063	ATTTGAAAGCGTGGTATTGC	CTTAAACTCACCATTAAATC	11	Arens et al. (2010)

plant diseased (scored as 1); plants diseased with few green stem and leaves (scored as 2); the 50% plant diseased and may have one or two fruits (scored as 3); except for the top leaves, stem and other plant parts are healthy (scored as 4), and healthy plants (scored as 5). The plant phenotypic symptoms were recorded at 40, 60 and 80 days after transplanting of seedlings in the field during *Kharif*, 2018.

Allelic effect of polymorphic SSRs

The average allele effect (AAE) of 84 polymorphic SSRs was estimated according to Benjamini and Hochberg (1995). The AAE calculated with the formulae used in the reference (Benjamini and Hochberg 1995) were presented in table (supplementary table 2) as positive alleles (showing positive AAE values) and negative alleles (showing negative AAE values).

Correlation studies

Correlations were carried out between yield, its attributes, fusarium wilt and TSWV scores with R language software (R Core Team 2012) and its significance was reported, where the positive association was indicated by blue colour and the negative association was by red color. The color intensity indicates the level of association from high to low range.

Results

A total of 130 SSR markers were used to analyze the genetic diversity in 48 tomato genotypes. Out of 130 SSR markers, 84 (64.6%) were observed to be polymorphic, 36 (27.7%) were monomorphic and the remaining 10 (7.7%) markers did not amplify. These 84 polymorphic markers (Supplementary Table 1) only were used in the analysis of genetic diversity, which yielded allelic data. Clear allelic variation was only considered to prepare the binary matrix (Fig. 1). The number of alleles/ locus varied from 2 to 4, with an average of 2.32/ marker with TES-478 and TGS 3032 yielding the highest number (4) of alleles.

The polymorphic markers covered all the 12 chromosomes with the maximum number of 13 primers on chromosome 1 followed by 10 primers each on chromosome number 2 and 11, whereas chromosome 8 was with only 2 primers. Polymorphism information content (PIC) value among SSRs varied widely from 0.17 to 0.74 with an average of 0.45, indicating good genetic diversity among the tomato genotypes.

Allelic effect of polymorphic SSRs

AAE for all the markers was presented in supplementary table 2. Among the total of 195 alleles from the 84 polymorphic SSR markers, 43 alleles were identified with positive average allele effect from 26 SSR markers distributed across nine chromosomes (Table 3). Chromosome wise distribution of positive alleles was presented in Fig. 2. Positive average allele effect was identified for three traits viz., average fruit weight, and fruits per plant and Fw PDI score.

STRUCTURE and AMOVA analysis

The population structure of 48 tomato accessions was deduced with Structure V2.3.4 from 84 SSR markers. The most likely number of clusters was evaluated using ΔK

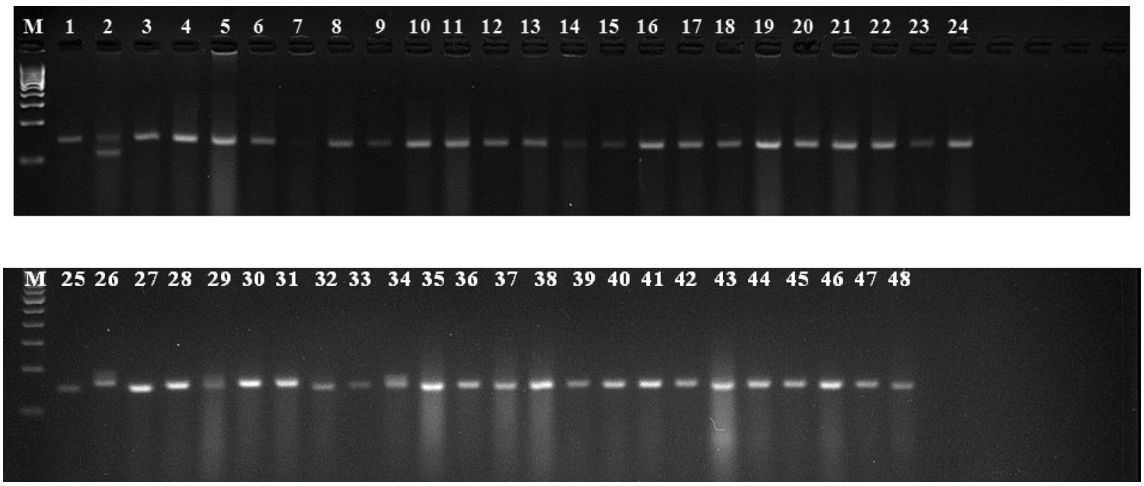

Fig. 1 Genetic diversity of tomato as revealed by TES-478 marker

Table 3 Alleles with positive effect from 84 polymorphic SSR markers for various traits in tomato

S.No.	Marker	Chromosome	Trait	AAE	Allele size
1	TES939	1	Average fruit weight	8.715	244
2	TES939	1	Average fruit weight	2.215	250
3	TES939	1	Average fruit weight	1.924	255
4	TGS1030	1	Average fruit weight	11.314	255
5	TES609	1	Number of fruits per plant	0.521	210
6	SSR135	1	Average fruit weight	0.911	220
7	SSR135	1	Average fruit weight	4.504	235
8	TGS2126	1	Average fruit weight	11.314	261
9	TES1683	1	Average fruit weight	2.248	245
10	TES1683	1	Average fruit weight	1.397	252
11	TEI0866	1	Number of fruits per plant	0.225	149
12	TES1673	2	Number of fruits per plant	0.050	291
13	TES1132	2	Number of fruits per plant	0.646	210
14	TES373	2	Average fruit weight	1.792	185
15	TES373	2	Average fruit weight	1.916	192
16	TGS3418	2	Number of fruits per plant	0.922	262
17	TES0498	3	Number of fruits per plant	2.186	176
18	SSRB50753	3	Number of fruits per plant	0.487	244
19	TGS2288	3	Number of fruits per plant	0.200	275
20	SSR3.171.1	3	Number of fruits per plant	2.930	263
21	TES0077	3	Average fruit weight	3.227	147
22	TES0077	3	Average fruit weight	1.169	155
23	SSR.111	3	Average fruit weight	8.752	150
24	SSR.111	3	Average fruit weight	8.868	115
25	TEI0139	4	Number of fruits per plant	0.097	140
26	TGS1360	5	Number of fruits per plant	0.922	225
27	TGS266	6	Number of fruits per plant	0.286	195
28	TGS1145	6	Average fruit weight	1.380	210
29	TGS1145	6	Average fruit weight	3.227	220
30	TGS1145	6	Average fruit weight	10.284	225
31	TES422	6	Fusarium wilt PDI score	4.471	200
32	TES422	6	Fusarium wilt PDI score	7.813	219
33	TES422	6	Fusarium wilt PDI score	2.720	225
34	TES537	8	Fusarium wilt PDI score	4.977	143
35	TES537	8	Fusarium wilt PDI score	16.145	155
36	TES537	8	Fusarium wilt PDI score	2.184	160
37	TEI0795	11	Fusarium wilt PDI score	2.533	225
38	TEI0795	11	Fusarium wilt PDI score	8.854	235
39	TEI0795	11	Fusarium wilt PDI score	4.717	240
40	TES1970	11	Number of fruits per plant	1.832	215
41	TES1970	11	Number of fruits per plant	2.686	220
42	TES1502	12	Number of fruits per plant	0.560	220
43	TGS3032	12	Fusarium wilt PDI score	4.336	260

method. Based on the highest peak of ΔK for the tested accessions (K = 2) (Fig. 3A, 3B), the entire germplasm was grouped into two sub-populations (pop1, pop2) with the alpha mean value of 0.28. The overall proportion of membership of the samples in each of the two clusters at K = 2 was 0.43 and 0.57 for pop1 and pop2, respectively. Among these populations, 0.18 allele frequency divergences were observed using point estimation and the average distance between individuals in the same cluster was 0.12 and 0.34, respectively. Most interestingly mean

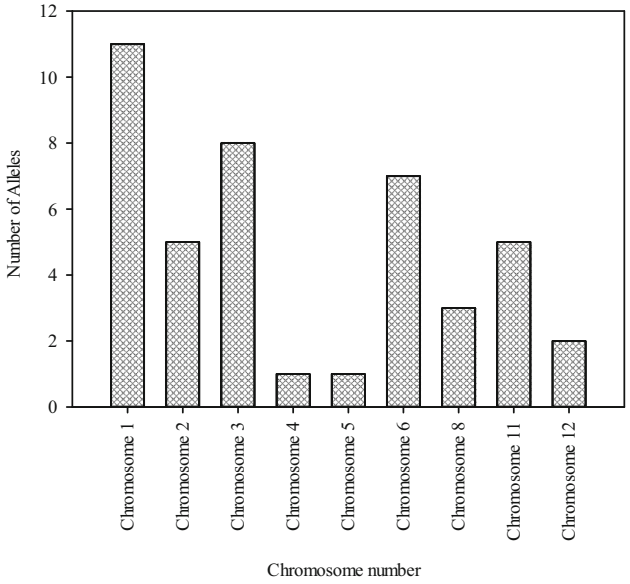

Fig. 2 Chromosome wise distribution of AAE for alleles with positive effect in tomato

value of 0.75 for Fst1 and 0.09 for Fst2 were observed for each population.

In addition to this, using these subpopulations data from structure analysis, the distribution of molecular variance was estimated using AMOVA. The results revealed that 12% of the variation was observed among the populations, while 88% of the variations were observed within the populations (Fig. 3C and Table 4) along with 0.15 φPT value.

Cluster analysis

The similarity coefficient of tomato genotypes varied from 0.20 to 0.96 reflecting a huge range, which further explains the diverse relationship of genotypes. UPGMA method based cluster analysis of the 48 tomato genotypes using 84 polymorphic markers resulted in (Fig. 4) grouping the genotypes into six clusters based on genetic distances (Table 5). While only two genotypes EC-251717 and EC-625644 were present in Cluster I, Cluster II was considered as a major cluster with 38 genotypes, which further truncated into two sub clusters. Genotypes EC-273966, EC-620503, EC-617059, EC-617066, EC-615055, EC-615047, EC-514013, EC-620428, EC-620446, EC-251751, EC-636482 and EC-320565 were grouped together in sub cluster I and Arka Vikas, LA-1589, EC-567305, AVTO-1219, LA-3667, EC-620434, EC-320463, EC-620522, EC-631356, EC-638302, EC-145057, EC-251518, LA-3120, LA-3006, LA-2662, LA-4345, LA-3005, LA-0490, LA-0535, LA-3847, AVTO-9802, LA-1500, LA-1015, LA-1664, EC-620570 and EC-164295 in sub cluster—II with EC-164295 showing the highest deviation. Two genotypes viz., LA-3847 and AVTO-9802, which showed the highest similarity coefficient of 0.96 were, placed in sub cluster II only. Additionally, all the UC Davis lines were also grouped in to this sub cluster, except LA 1940, which was in separate cluster (cluster III) with EC-251694. With regard to other clusters, cluster IV contained three genotypes viz., EC-617068, EC-631962 and EC-676742, Cluster V consisted of Pusa Ruby alone, indicating it's unique and distinctiveness from other genotypes under study and Cluster VI consisted of two genotypes viz., EC-310310 and EC-620452.

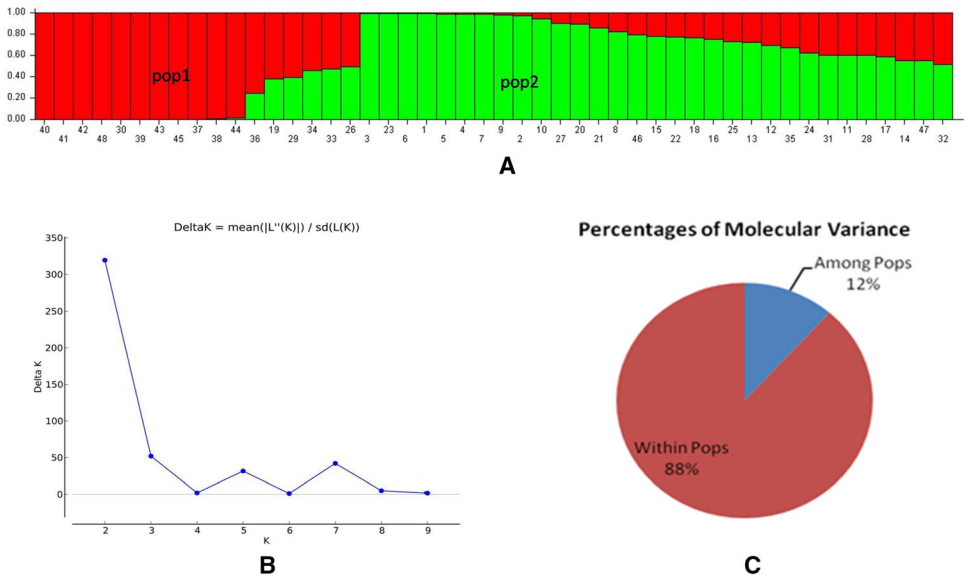

Fig. 3 **A** Population structure inferred by the STRUCTURE software based on SSRs at K = 2 in tomato. **B** Estimated ΔK of the 48 tomato accessions over 3 runs of each K value. **C** AMOVA analysis of 48 tomato genotypes

Table 4 Summary of AMOVA in tomato genotypes

Source	Degrees of freedom	Sum of squares	Mean sum of squares	Est. variation	%	Stat	Value	*P* value
Among populations	1	116.99	116.99	3.95	12			
Within population	46	1395.09	30.33	30.33	88			
Total	47	1512.08		34.28	100	φPT	0.15	0.001

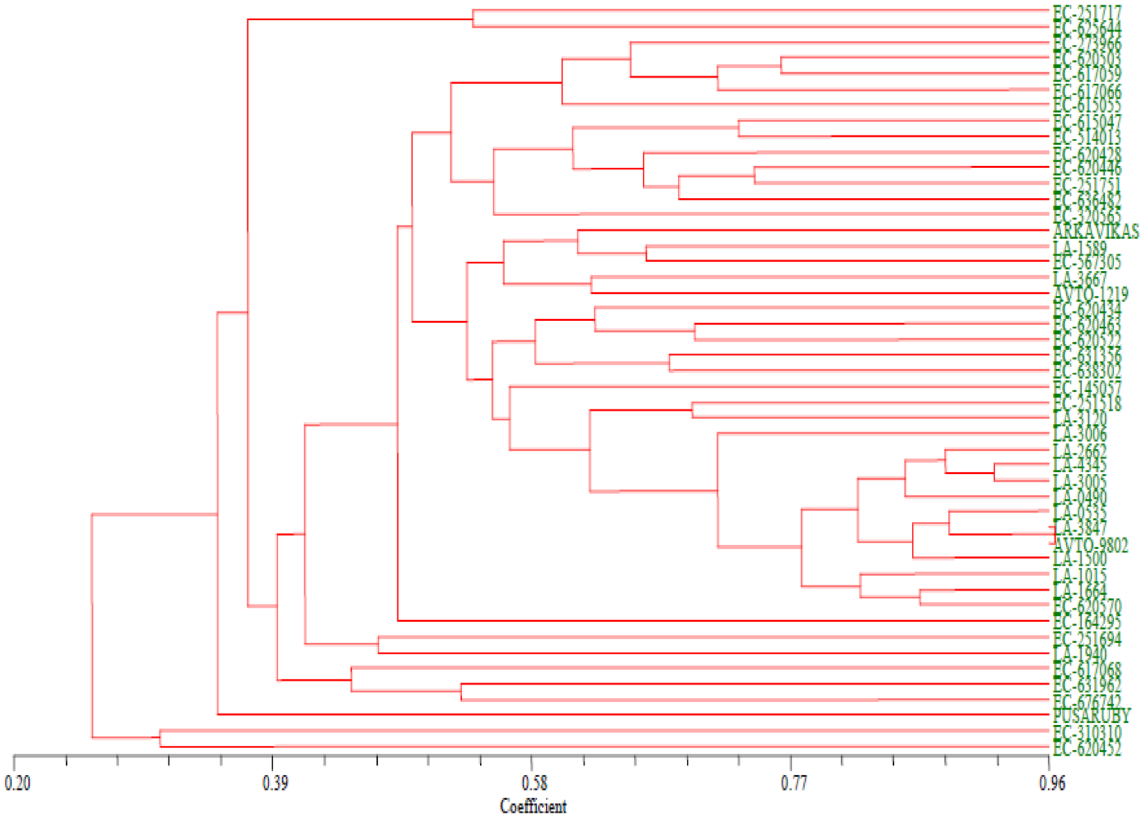

Fig. 4 Dendrogram obtained from SSR analysis in tomato using UPGMA with NTSYSpc2.0 software

Validation of gene specific markers (*Sw-5*, *I-2*) in 48 genotypes of tomato

All the 48 tomato genotypes were tested with two gene specific markers of *Sw-5* (Shi et al. 2011) and *I-2* (Arens et al. 2010) genes, which were previously reported for their use in the selection of resistance genotypes against TSWV and Fusarium wilt. Out of 48 genotypes, seven genotypes, EC-251717, EC-273966, EC-625644, EC-251694, LA-1589, LA-1940 and LA-3667 amplified the desired band above 540 bp with *Sw-5* gene specific marker, Sw-5-F3 (Fig. 5).

Nine genotypes *i.e.* EC-617066, EC-620463, EC-631356, LA-3667, AVTO-1219, EC-620428, LA-3847, AVTO-9802 and EC-620570 produced the desired product at 940 bp with Fusarium wilt gene specific marker, FWZ1063 (Fig. 6).

Evaluation of genotypes for fusarium wilt resistance

Out of 48 genotypes studied for fusarium wilt resistance based on morphological symptoms using the scale (0–4), nine genotypes viz. EC-617066, EC-620463, EC-631356, LA-3667, AVTO-1219, EC-620428, LA-3847, AVTO-9802 and EC-620570 were scored "0", no genotypes were scored in the range of 0–1, four genotypes *i.e.* EC-251717, EC-620434, EC-676742 and LA-0535 with 1–2 score, four genotypes viz. EC-273966, EC-617068, EC-638302 and EC-567305 with 2–3 score and the remaining 31 genotypes *i.e.* EC-620522, EC-251694, Arka Vikas, LA-1589, EC-620503, EC-625644, EC-617059, EC-514013, EC-620446,

Table 5 Clustering pattern of tomato genotypes obtained by genetic diversity analysis

Cluster number	Number of genotypes	Name of the genotype/s
I	2	EC-251717 and EC-625644
II	38	Sub cluster-I
		EC-273966, EC-620503, EC-617059, EC-617066, EC-615055, EC-615047, EC-514013, EC-620428, EC-620446, EC-251751, EC-636482 and EC-320565
		Sub cluster-II
		Arka Vikas, LA-1589, EC-567305, AVTO-1219, LA-3667, EC-620434, EC-320463, EC-620522, EC-631356, EC-638302, EC-145057, EC-251518, LA-3120, LA-3006, LA-2662, LA-4345, LA-3005, LA-0490, LA-0535, LA-3847, AVTO-9802, LA-1500, LA-1015, LA-1664, EC-620570 and EC-164295
III	2	EC-251694 and LA-1940
IV	3	EC-617068, EC-631962 and EC-676742
V	1	Pusa Ruby
VI	2	EC-310310 and EC-620452

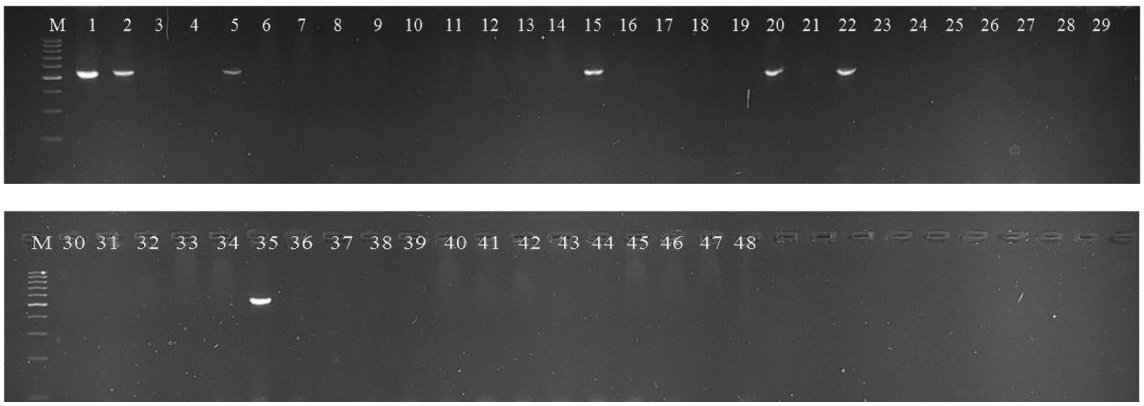

Fig. 5 Screening of 48 tomato genotypes with *Sw-5* gene specific marker, Sw-5-F3

EC-320565, LA-1940, LA-0490, LA-1015, LA-3006, EC-310310, EC-615055, EC-164295, EC-631962, Pusa Ruby, EC-615047, EC-620452, EC-636482, EC-251751, EC-145057, EC-251518, LA-3120, LA-2662, LA-4345, LA-3005, LA-1500 and LA-1664 were recorded with the score 3–4. Simultaneously, the percent of incidence was also calculated and it was in the range of zero to hundred. Different levels of percent incidence were observed in genotypes, where zero per cent disease incidence was observed in EC-617066, EC-620463, EC-631356, LA-3667, AVTO-1219, EC-620428, LA-3847, AVTO-9802 and EC-620570, while 100% incidence was observed in EC-620522, EC-251694, Arka Vikas and LA-1589 (Tables 6, 7).

Evaluation for TSWV resistance under induced disease conditions

Out of the 48 genotypes screened for TSWV resistance based on morphological symptoms using 1–5 score, as many as 26 genotypes, EC-310310, EC-620503, EC-617066, EC-617068, EC-620434, EC-620463, EC-620522, EC-164295, EC-631356, EC-631962, EC-638302, EC-676742, Arka Vikas, EC-567305, EC-620452, EC-620446, EC-636482, EC-251751, EC-320565, LA-3006, LA-2662, LA-0490, LA-4345, LA-3005, LA-1015 and LA-1664 exhibited symptoms of whole plant diseased (Table 7) and scored as 1; 12 genotypes *i.e*. Pusa Ruby, EC-615047, EC-514013, EC-251518, LA-3120, LA-0535, AVTO-9802, EC-620570, EC-615055, EC-617059, EC-145057 and LA-3847 were diseased with few green stems and leaves, scored as 2; two genotypes, AVTO-1219 and EC-620428 were the plants with 50% diseased and had one to two fruits (scored as 3); the stem and other plant parts were healthy in case of LA-1500 and scored as 4, and healthy plants with score of 5 were EC-251717, EC-273966, EC-625644, EC-251694, LA-1589, LA-3667 and LA-1940.

Correlation study

A significant positive correlation was observed between fruit weight and fruit yield per plant (Fig. 7) and negative

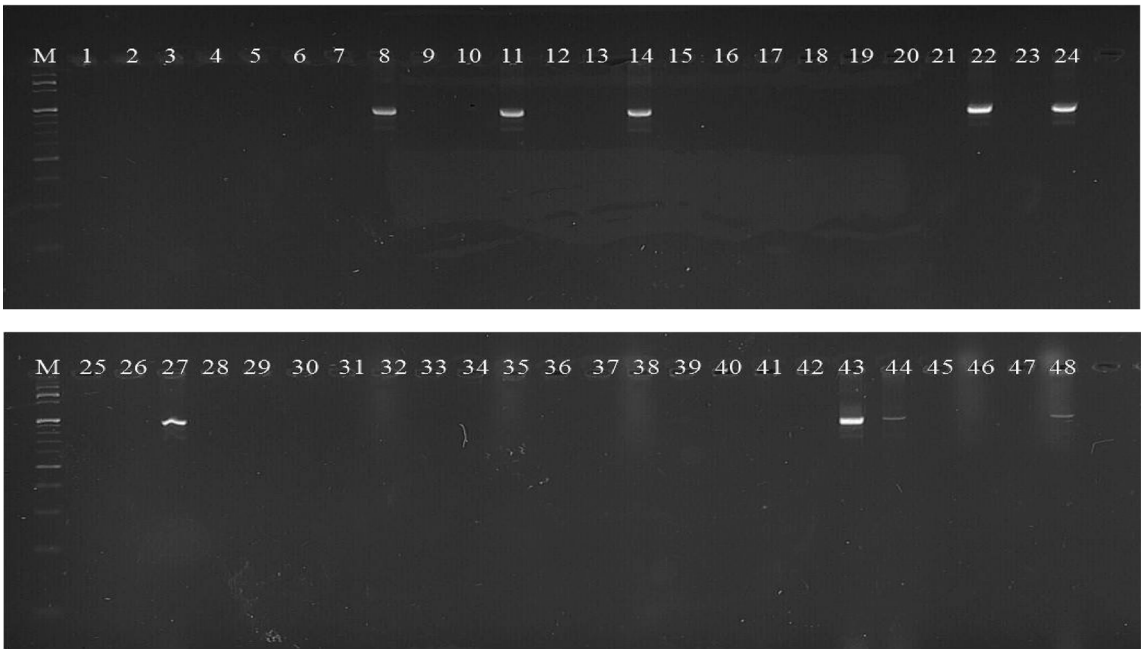

Fig. 6 Screening of 48 tomato genotypes with *I-2* gene specific marker, FWZ1063

Table 6 Reaction of genotypes against fusarium wilt disease in tomato

S. No.	Reaction	Score	Genotypes
1	Highly resistant (HR)	0	EC-617066, EC-620463, EC-631356, LA-3667, AVTO-1219, EC-620428, LA-3847, AVTO-9802, EC-620570
2	Resistant (R)	1 (0–1)	Nil
3	Moderately resistant (MR)	2 (1–2)	EC-251717, EC-620434, EC-676742, LA-0535
4	Moderately susceptible (MS)	3 (2–3)	EC-273966, EC-617068, EC-638302, EC-567305
5	Susceptible (S) and highly Susceptible (HS)	4 (3–4)	EC-620522, EC-251694, Arka Vikas, LA-1589, EC-620503, EC-625644, EC-617059, EC-514013, EC-620446, EC-320565, LA-1940, LA-0490, LA-1015, LA-3006, EC-310310, EC-615055, EC-164295, EC-631962, Pusa Ruby, EC-615047, EC-620452, EC-636482, EC-251751, EC-145057, EC-251518, LA-3120, LA-2662, LA-4345, LA-3005, LA-1500, LA-1664

correlation was observed for fruit number per plant with fruit weight and fruit yield per plant. The remaining correlations were neutral and non significant.

Discussion

Genetic improvement of yield and other agronomical traits in a crop will be easier, if the assessment of diversity analysis is proper. The discovery and use of molecular markers has advantages and among these, SSRs are playing a vital role in molecular diversity studies and in identifying better parents for heterosis breeding. The present investigation found the existence of a high level of diversity among 48 tomato genotypes using 130 SSRs. Out of these, 84 polymorphic primers yielded 195 alleles with 64.6% polymorphism. Polymorphism was observed in different studies varying from 27.75% (Velpula et al. 2017) to 100% (Chen et al. 2009; San et al. 2008). Earlier, in the study of genetic diversity and DNA finger printing of 10 Egyptian tomato varieties, Mohamed et al. (2012), reported almost similar level of polymorphism (60.5%) using 20 SSRs, a range of polymorphism by Pidigam et al. 2019 using RAPDs in yardlong bean and Saidaiah et al. 2021 in *Lablab purpureus* with RAPD markers. The level of polymorphism may be due to the amount variability and

Table 7 Scores and per cent incidence of fusarium wilt in 48 tomato genotypes

S. No.	Genotype	Per cent disease incidence of Fusarium wilt	Disease score of TSWV	S. No.	Genotype	Per cent disease incidence of Fusarium wilt	Disease score of TSWV
1	EC-251717	50.00	5.00	25	EC-615047	83.33	1.67
2	EC-273966	66.66	5.00	26	EC-514013	91.66	1.67
3	EC-310310	83.33	1.00	27	EC-620428	0.00	3.00
4	EC-620503	91.66	1.00	28	EC-620452	83.33	1.00
5	EC-625644	91.66	5.00	29	EC-620446	91.66	1.00
6	EC-615055	83.33	1.60	30	EC-636482	83.33	1.00
7	EC-617059	91.66	1.60	31	EC-251751	83.33	1.00
8	EC-617066	0.00	1.00	32	EC-320565	91.66	1.00
9	EC-617068	66.66	1.00	33	EC-145057	83.33	1.33
10	EC-620434	50.00	1.00	34	EC-251518	83.33	1.67
11	EC-620463	0.00	1.00	35	LA-1940	91.66	5.00
12	EC-620522	100.00	1.00	36	LA-3120	83.33	1.67
13	EC-164295	83.33	1.00	37	LA-3006	86.33	1.00
14	EC-631356	0.00	1.00	38	LA-2662	83.33	1.00
15	EC-251694	100.00	5.00	39	LA-0490	91.66	1.00
16	EC-631962	83.33	1.00	40	LA-4345	83.33	1.00
17	EC-638302	66.66	1.00	41	LA-3005	83.33	1.00
18	EC-676742	50.00	1.00	42	LA-0535	50.00	1.67
19	Arka Vikas	100.00	1.00	43	LA-3847	0.00	1.33
20	LA-1589	100.00	5.00	44	AVTO-9802	0.00	1.67
21	EC-567305	66.66	1.00	45	LA-1500	83.33	3.70
22	LA-3667	0.00	5.00	46	LA-1015	91.66	1.00
23	Pusa Ruby	83.33	1.67	47	LA-1664	83.33	1.00
24	AVTO-1219	0.00	3.00	48	EC-620570	0.00	1.67

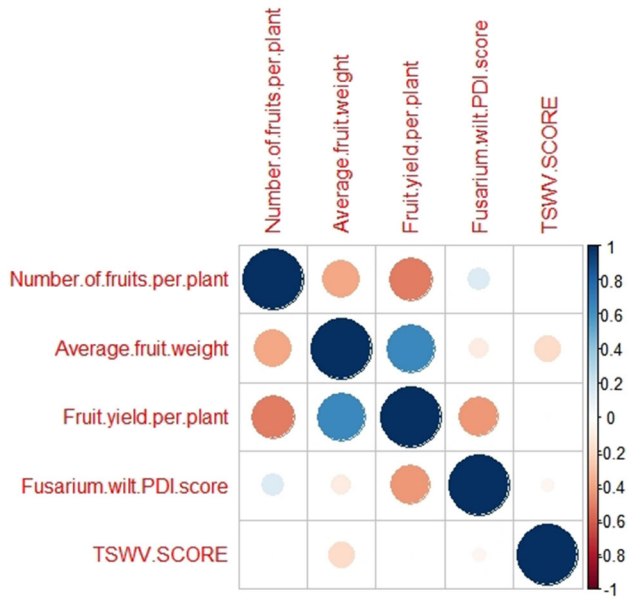

Fig. 7 Correlations analysis among yield, yield attributes, TSWV score and fusarium wilt PDI score in tomato

levels association of selected SSRs with reference to genotypes. Average of 2.32 alleles/ marker was recorded in the present study and 29.7% primers showed alleles above the mean level, which is comparable to the other studies (Chen et al. 2009; Velpula et al. 2017). Of these, TES478 and TGS3032 showed highest level of polymorphism with four alleles each. The PIC values range reported was between 0.12 and 0.93 with an average of 0.69, showing higher values than the earlier reported PIC values of 0.27 (Saravanan et al. 2014), 0.31 (Benor et al. 2008), 0.37 (He et al. 2003), 0.39 (Frary et al. 2005), 0.40 (Bredemeijer et al. 2002), 0.45 (Glogovac et al. 2013) and 0.58 (Sardaro et al. 2013) in different diversity studies in tomato. Whereas, Known et al. (2006 and 2009) observed very nearer PIC values similar to the present study. In this study, 56% of markers showed the highest PIC value (> 0.5). TGS2446 recorded the highest PIC with 0.93. TGS1360, TGS2288, SSR-111, TGS-522, TES20 and TES1028 followed with more than 0.9 values indicating that these primers would be further useful for selectivity and to

determine the genetic variability in tomato germplasm. In these, SSR111 also proved its importance in identifying genetic diversity in tomato with 100% polymorphism (Kaushal et al. 2017) and 3 alleles (Frary et al. 2005; Chen et al. 2009) and recommended for further studies having the highest PIC values (Glogovac et al. 2013). Zhao et al. (2016) reported a significant association of sucrose with TES1028 loci on chromosome 9. About 43 alleles were identified having a positive average allele effect from 26 SSR markers (out of 84 polymorphic SSR markers) distributed across nine chromosomes. The positive average allele effect was associated with fruit weight, fruits formed per plant and fusarium wilt PDI score. Besides supporting the results of present investigation, all the above research studies also unravel the importance of genetic diversity in tomato.

Structure analysis can estimate the sub-populations number, degree of admixture along with the genetic relatedness among germplasm. In the present study, structure clustering revealed that the 48 accessions of tomato germplasm were divided into 2 distinct sub-populations (K = 2) along with different levels of admixture. This admixture level was higher in sub-population 2 than sub-population1, indicating existence of diverse genetic bases in tomato germplasm. The low value of K obtained in this study may be due to high amount of gene flow. Similar level of K value was also obtained in different studies on tomato using structure analysis (Sim et al. 2012; Henareh et al. 2016; Pailles et al. 2017). Interestingly, the majority of the LA lines clustered together in sub-population 1 may be genetically similar. Moreover, the mean Fst values of pop1 and pop2 were observed with 0.75 and 0.09, respectively suggesting strong episode of genetic drift in these populations, which was more in sub-population 1 than sub-population 2 (Henareh et al. 2016; Pailles et al. 2017). AMOVA analysis revealed a higher percentage of molecular variation with in a population (88%), when compared with population variation (12%) at p values of 0.001, suggesting that, the molecular variance was significant. This was also comparable with the previous results in the diversity analysis of tomato using SSRs (Aquirre et al. 2017; Raveendar et al. 2016). UPGMA method based phylogenetic study was constructed using binary matrix data retrieved from polymorphic alleles of 84 SSRs truncated tomato genotypes into six clusters. The highest similarity (96%) was observed between AVTO-9802 and LA-3847 (Cluster II). The lowest similarity of 20% was observed between four different pairs *i.e*. Pusa Ruby with EC-310310, EC-625644, and AVTO-9802 with EC-620452 and LA-1015 with EC-310310. Besides lowest similarity, Pusa Ruby also formed into separate cluster (Cluster V) by showing its diversified and superior nature with other genotypes (Yogendra and Gowda 2013). The two AVTO genotypes (AVTO-1219, AVTO-9802) involved in the present study showed 55% similarity due to the similarity in origin of taxon (*S. lycopersicum*) and differ in level of heat tolerance, disease resistance and fruit shape. Both the AVTO genotypes paired with LA-3667 and LA-3847 genotypes, a 96% similarity was observed between AVTO-9802 and LA-3847, which may be due to their tolerance to high temperatures (AVRDC database; Xu et al. 2017) along with other disease resistant loci. Cluster I contained two genotypes, EC-251717 and EC-625644 with 0.54 similarity coefficients. Cluster II had 38 genotypes, considered as a major group, again bifurcated into two sub-clusters. Interestingly, all the LA lines were placed in sub-cluster II B with above 58% similarity due to their similar origin and cultivar type. Whereas, LA-1940 was the only one under *S. pennellii* with wild type characters, separated from other genotypes into a different cluster (Cluster III) along with EC-251694. Even though LA1500 and LA1589 belong to *S. pimpinellifolium*, they got placed in the same cluster with other *S. lycopersicum* lines as reported by Aflitos et al. (2014) that, the grouping of *S. lycopersicum* genotypes with *S. pimpinellifolium* and *S. pennellii* as sister groups to *S. habrochaites*. The separation of *S. pennellii* form *S. lycopersicum* may be due to its superior agronomic performance (Rick and Tanksley 1981), divergent phenotypic variation in relation to fruit development, maturation, metabolism (Schauer et al. 2008; Steinhauser et al. 2010; Kochevenko et al. 2011) and stress tolerance (Frary et al. 2010; Bolger et al. 2014). Overall, the genotypes in the present study were clearly discriminated based on binary data yielded from polymorphic loci of SSRs. Thus, this investigation of genetic dissimilarities among tomato genotypes will be further useful in tomato improvement programs.

In addition to polymorphic primers, two gene specific primers were used to screen 48 genotypes for their resistance against TSWV (*Sw-5*) and fusarium wilt (*I-2*). Among all the genotypes, seven genotypes (EC-251717, EC-273966, EC-625644, EC-251694, LA-1589, LA-1940 and LA-3667) produced the desired resistant allele of *Sw-5* resistant gene. Similar results are also reported by Shi et al. (2011) in tomato breeding approach with marker assisted selection (MAS) for *Sw-5* gene using same primer with a product size of 541 bp. Both the studies represented LA-3667 as a resistant genotype, confirming the true resistance of other six genotypes with resistance loci in the present study (Shi et al. 2011). On other hand, genotypes, EC-617066, EC-620463, EC-631356, EC- EC-567305, EC-620570, EC-620428, AVTO-1219, AVTO-9802 and LA-3847 showed the amplification of fusarium wilt resistant gene (*I-2*) with FWZ1063 specific primer at 940 bp similar to Arens et al. (2010). Tomato genotype LA-3667 appears to be most promising with the presence of both *Sw-5* and *I-*

2 genes governing the resistance to TSWV and Fusarium wilt, respectively. The presence of *Sw-5* and *I-2* genes against TSWV (Stevens et al. 1991; Robbins et al. 2010; Parrella et al. 2002) and fusarium wilt (Chaerani et al. 2007; Arens et al. 2010) diseases proves an opportunity for selection and identification of suitable donors for introgression of desired genes into elite cultivars through MAS.

All the 48 genotypes of tomato were screened for fusarium wilt resistance after proper confirmation of the presence of the most pathogenic (Surangal) isolate of *Fusarium oxysporium.* Different levels of resistance were observed among the genotypes, which indicates the occurrence of high genetic diversity among the genotypes. Nine genotypes, EC-617066, EC-620463, EC-631356, LA-3667, AVTO-1219, EC-620428, LA-3847, AVTO-9802 and EC-620570 were with 0% disease incidence and scored with 1 were showing their high resistant nature against Fusarium Wilt. The resistant nature of AVTO-1219 was also explained by Loganathan (2012–13) of World Vegetable Centre. Genotypes EC-620522, EC-251694, Arka Vikas and LA-1589 resulted as susceptible genotypes with 100 percent disease index with 3–4 score. The plant showing 0% incidence or 0 severity level symptoms were healthier and plants with the maximum disease severity level (incidence ranged from 80% -100%) were diseased. Mahmoud et al. (2006), Ahmadvand et al. (2010), Bahattin et al. (2010) and Antonio et al. (2017) also presented similar findings. Based on morphological scoring, EC-251717, EC-273966, EC-625644, EC-251694, LA-1589, LA-3667 and LA-1940 were identified as healthy plants, free of TSWV with a score of 5 followed by the genotype, LA-1500, exhibited healthy stem and other plant parts with a score of 4 for TSWV reaction.

Correlation

The correlation between fruit weight and fruit yield obtained per plant was significant and positive, while it was negative for fruit number per plant with fruit yield per plant. The correlation of either fusarium wilt PDI or TSWV disease score with the yield and its components was non-significant. To improve the tomato yield, even when fusarium wilt or TSWV incidence is there, increased fruit number per plant will enhance the per se plant fruit yield and similar results are reported earlier by several investigators (Raut et al. 2005; Singh et al. 2002; Ullah et al. 2015; Gupta et al. 2019). When plants were subjected to stress due to disease, the increase in the numbers of fruits per plant is not contributing towards yield enhancement.

Conclusion

In the present investigation, a multidisciplinary approach was used for assessing the genetic diversity within a collection of indigenous and exotic tomato germplasm. The results revealed differences among all the 48 genotypes at genotypic level and clustering revealed the degree of related and distinctiveness among the germplasm. Furthermore, a significant level of molecular variance was observed within and among the populations derived by structural analysis. In addition to distinct and related tomato accessions, the new resistant tomato lines were also validated using gene specific markers for resistance to TSWV and Fusarium wilt along with phenotypic screening. Around 43 alleles were identified with a positive average allele effect from 26 SSR markers for average fruit weight, number of fruits per plant and fusarium wilt PDI score. The identified accessions could be further exploited in different breeding programs for the development of tomato varieties with resistance to TSWV and Fusarium wilt for enhancing the farmer's opportunities and earnings.

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Availability of data and materials The data that support the findings of this study are available from the corresponding author upon request.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval Not applicable

Human participants and animals This article does not contain any studies with human participants or animals performed by any of the authors.

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