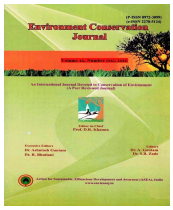
Environment Conservation Journal 23 (3):113-121, 2022

Journal homepage: https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)



Morphological, biochemical and SSR marker based genetic diversity and identification of trait-specific accessions in exotic germplasm collection of tomato (*Solanum lycopersicum* L.)

Amarnath, M.✉
Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, India.
Shyamalamma, S.
Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, India.
Anilkumar, C.
ICAR-National Rice Research Institute, Cuttack, India.
Brijesh Patil M.P
Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, India.

ARTICLE INFO

Received : 08 February 2022
Revised : 28 April 2022
Accepted : 11 May 2022

Available online: 18 September 2022

Key Words:
Exotic collections
K-means
PIC value
Trait-specific accessions
UPGMA

ABSTRACT

Characterization and evaluation of genetic base of exotic collections of germplasm hastens the process of crop breeding. Exotic collections of 25 tomato germplasm accessions along with a local check 'Vaibhav' were characterized at morphological, biochemical and DNA marker level in the University of Agricultural Sciences, Bangalore. Both morphometric and biochemical trait data divided the accessions into five clusters by model-based K-means cluster analysis. Accessions EC-620481 and EC-620554 were found highly diverse and promising to broaden the genetic base of breeding stocks in tomato. SSR marker based genetic parameter estimates inferred lower genetic differences at marker loci. However, UPGMA classification displayed similar kind of diversity as exhibited at morphometric level. Traits specific accessions identified have potential to accelerate trait specific breeding for economically important traits. This investigation resulted in the identification of such potential accessions for their use in commercial tomato breeding.

Introduction

Among the members of nightshade family Solanaceae, tomato is the major vegetable crop in the world (Rothan*et al.*, 2019). The advanced plant breeding activities narrowed down the genetic base of available tomato breeding lines due to repeated selections (Miller and Tanksley, 1990). Introducing new variation into available breeding lines in tomato is need of the hour to broaden the genetic base. Inclusion of exotic variation into tomato breeding programs is necessary to introduce new gene combinations (Bergougnoux, 2014). Assessment of genetic diversity of such exotic germplasm accessions provides an insight about its value (Rick and Chetelat, 1995). Characterization based on morphological trait expression is most commonly employed for assessing genetic differences among individuals in a population (Anilkumar *et al*., 2017). In complement to it, the biochemical products produced during different stages of plant development also serve the purpose. Nevertheless, expression of these morphological and biochemical characters are highly influenced by environment and hinders the estimates of genetic diversity (Brunlop and Finckh, 2010). However, DNA markers are crop non-stage specific and environmentally neutral complement to the morphological characters in diversity study (Milevska *et al.,* 2011). Supplementing morphological character based diversity with biochemical and DNA marker data assure breeders to strategically select appropriate genotype for breeding programs (Herraiz *et al.,* 2015). The

Corresponding author E-mail: amarhortbci@gmail.com
Doi: https://doi.org/10.36953/ECJ.10862263

objective of present investigation is to examine genetic diversity among exotic collection of tomato germplasm accessions at morphological, biochemical and SSR marker loci.

Material and Methods

The genetic material for the investigation consisted of 25 exotic germplasm collections obtained from National Bureau of Plant Genetic Resources (NBPGR), Regional station, Rajendranagar, Hyderabad, India and a local check variety 'Vaibhav' released by University of Agricultural Sciences, Bangalore (Table-1). The genotypes were evaluated at experimental plots of Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore. The experiment was laid out in randomized complete block design with two replications. Four weeks old seedlings from nursery trays were transplanted to field maintaining a spacing of 75cm between rows and 45cm between plants. To ensure a healthy crop, the recommended tomato management practices like spacing and regular watering were followed.

Table1: List of exotic collection of tomato accessions used in the study.

Sl. No	Accessions	Sl. No	Accessions
1	EC-620437	**14**	EC-620553
2	EC-620438	**15**	EC-620554
3	EC-620456	**16**	EC-620557
4	EC-620460	**17**	EC-620560
5	EC-620472	**18**	EC-620563
6	EC-620474	**19**	EC-620567
7	EC-620481	**20**	EC-620568
8	EC-620521	**21**	EC-614997
9	EC-620543	**22**	EC-614998
10	EC-620544	**23**	EC-620343
11	EC-620545	**24**	EC-620394
12	EC-620546	**25**	EC-632946
13	EC-620550	**26**	Vaibhav (Check)

Ten randomly tagged plants were considered for recording observations on 11 morphometric traits. The traits recorded were days to flowering, days to flowering to fruit set, plant height at 65 days, number of branches, fruits per cluster, fruits per plant, fruit length, fruit width, fruit weight, number of locules and plant yield. Apart from these, qualitative trait data were also recorded on flower color, fruit color and growth habit of experimental material (Table 2 and Figure 1). The data on biochemical parameters *viz*., lycopene content (Ranganna, 1976); total soluble solids (TSS) and ascorbic acid content (Johnson, 1948) were recorded. TSS was recorded from five randomly selected fruitsfrom all accessions by squeezing the juice on Erma hand refractometer (0-32° Brix) atroom temperature and mean was worked out.

The genomic DNA of 26 genotypes was extracted from young and healthy leaves using CTAB method (Doyle and Doyle, 1987). A total of 42 SSR markers (Table 3)were used to differentiate 26 genotypes at SSR marker loci. The size variations of amplicons produced at SSR priming regions in the genomic DNA were scored as different alleles at each SSR loci and subjected to analysis.

Table 2: Qualitative characters in different tomato accessions.

SN	Accessions	Growth type	Flower color	Fruit color
1	EC-620437	Indeterminate	Yellow	Orange red
2	EC-620438	Indeterminate	Yellow	Deep red
3	EC-620456	Indeterminate	Yellow	Light yellow
4	EC-620460	Indeterminate	Yellow	Orange red
5	EC-620472	Indeterminate	Yellow	Deep red
6	EC-620474	Determinate	Yellow	Orange red
7	EC-620481	Indeterminate	Yellow	Deep red
8	EC-620521	Indeterminate	Yellow	Light yellow
9	EC-620543	Determinate	Yellow	Orange red
10	EC-620544	Indeterminate	Yellow	Deep red
11	EC-620545	Indeterminate	Yellow	Light yellow
12	EC-620546	Indeterminate	Yellow	Orange red
13	EC-620550	Indeterminate	Yellow	Light yellow
14	EC-620553	Determinate	Yellow	Light yellow
15	EC-620554	Determinate	Yellow	Deep red
16	EC-620557	Determinate	Yellow	Orange red
17	EC-620560	Indeterminate	Yellow	Light yellow
18	EC-620563	Indeterminate	Yellow	Orange red
19	EC-620567	Determinate	Yellow	Orange red
20	EC-620568	Indeterminate	Yellow	Light yellow
21	EC-614997	Determinate	Yellow	Light yellow
22	EC-614998	Indeterminate	Yellow	Light yellow
23	EC-620343	Indeterminate	Yellow	Orange red
24	EC-620394	Determinate	Yellow	Orange red
25	EC-632946	Indeterminate	Yellow	Light yellow
26	Vaibhav	Indeterminate	Yellow	Deep red

Statistical analysis

The mean data on 11 morphological characters recorded over two years was subjected to Levene's

test (Levene, 1960) of variance using SPSS V.16 software. The test results assured pooling of data from two years as their variances did not differ significantly. The pooled data was subjected to K-means cluster analysis using RStudio version 1.2.133 (RStudio team, 2019). Trait specific accessions were identified for each morphological traits based on comparative performance of accessions with check. Among the 42 SSR markers used, 23 were found polymorphic across the germplasm collection. Powermarker V3.25 (Liu and Muse, 2005) was used for estimation of various population genetic parameters such as polymorphic information content (PIC), major allele frequency and gene diversity. The same data was also subjected to UPGMA classification using DARwin 6 software to classify the accessions.

Results and Discussion

The highest plant height was recorded in EC-620563 and EC-620546 (126.25 and 124.25 cm). The number of branches per plant were higher in EC-614998 (63.50) followed by EC-620546 (62.00). Among twenty five tomato accessions studied, seventeen were indeterminate type and eight accessions were determinate type. There was no significant difference in the number of days taken for flowering and it ranged from 31.00 to 36.00. There was significant variation among the accessions for the individual fruit weight. The higher fruit weight was recorded in EC-620998 (104g) and the lower fruit weight was recorded in EC-620554 (33g). Three accessions recorded higher number of fruits per plant *viz* EC-620546, EC-620568, and EC-614997 (70.00 to 72.25 fruits/plant) whereas the check variety Vaibhav recorded around 46.75 fruits/plant which was on par with the average mean. The number of fruits per cluster varied from 4.50 to 12.50, the higher number was recorded in EC-620557 and EC-620568 followed by EC-620553 as compared to Vaibhav with 5.50 fruits per cluster. The total fruit yield per plant varied from 1.43kg to 6.30kg/plant. The higheryields were obtained in EC-614997 (6.30kg) followed by EC-620560 (6.14kg). However the average mean yield per plant was 3.59kg. Similar type of variability with respect to yield parameters was reported by Pradeep Kumar *et al*. (2001); Kaushik *et al*. (2011) and Reddy *et al*. (2013). The growth type of determinate (17) and indeterminate (8) were observed in the accessions. Flower color in all the accessions was yellow. Fruit color varied from deep red to light yellow. Study conducted by Hussain *et al.* (2021) also indicated that the PCV and GCV were found to be higher for number of fruits plant-1, average fruit weight (g), fruit yield hectare-1 (q), and pericarp thickness (mm), indicating greater phenotypic and genotypic variability among the accessions. Significantly higher lycopene content was observed in two accessions viz., EC620521 (5.47mg/100g) and EC-620550 (5.21mg/100g). Higher TSS was observed in EC-632946 (6.95^0Brix) followed by EC-620557 and EC-620550 (6.15^0Brix and 6.05^0Brix). The ascorbic acid levels were very high in some of the accessions. It varied from 8.76mg/100g to 55mg/100g. Similar reports on the variations in the lycopene content, TSS and ascorbic acid content are reported by Bose *et al.* (2002); Collins and Veazie (2006) and Panthee *et al.* (2012). Hussain *et al.* (2021) reported that the PCV and GCV for total Soluble Solids (^{0}Brix) ranged from 6.86 to 48.78 for reducing sugar (%).

The pooled mean values of 11 morphological characters subjected to K-means clustering divided 26 accessions into five different clusters. Among five clusters, cluster four was included highest number of accessions and cluster two was found solitary with only accession EC-620481 inferring its distinctness from other accessions (Fig. 2). The heat map of Euclidian genetic distance among 26 accessions clearly depicted the differences among themselves (Fig.3). The estimate of genetic distance for accession EC-620554 (15) and EC-620481 (7) were higher from any other accessions under the study inferring the genetic worth of these accessions. Genotypes with higher genetic distances can be best utilized for developing heterotic hybrids in practical plant breeding schemes (Anilkumar and Lohithaswa, 2018).

Based on three biochemical traits, genotypes were grouped into five clusters (Fig. 4), while the composition of these clusters differed with those based on morphometric traits. However, the results from biochemical data paved the path for selection of genotypes in breeding programs targeted to improve quality. Observed gene diversity and Polymorphic information content (PIC) value

Table 3: List of SSR markers used in the study.

Marker	Sequence	Expected product size (bp)
SSR111	F: TTCTTCCCTTCCATCAGTTCT	190
	R: TTTGCTGCTATACTGCTGACA	
SSR134	F: CCCTCTTGCCTAAACATCCA	175
	R: CGTTGCGAATTCAGATTAGTTG	
SSR146	F: TATGGCCATGGCTGAACC	220
	R: CGAACGCCACCACTATACCT	
SSR248	F: GCATTCGCTGTAGCTCGTTT	270
	R: GGGAGCTTCATCATAGTAACG	
SSR310	F: GCGATGAGGATGACATTGAG	175
	R: TTTACAGGCTGTCGCTTCCT	
SSR318	F: GCAGAGGATATTGCATTCGC	180
	R: CAAACCGAACTCATCAAGGG	
TOM49	F: AAGAAACTTTTTGAATGTTGC	190
	R: ATTACAATTTAGAGAGTCAAGG	
TOM144	F: CTGTTTACTTCAAGAAGGCTG	180
	R: ACTTTAACTTTATTATTGCGACG	
TOM152	F: ATTCAAGGAACTTTTAGCTCC	190
	R: TGCATTAAGGTTCATAAATGA	
TOM184	F: CAACCCCTCTCCTATTCT	180
	R: CTGCTTTGTCGAGTTTGAA	
TOM210	F: CGTTGGATTACTGAGAGGTTTA	205
	R: ACAAAAATTCACCCACATCG	
TOM236	F: GTTTTTTCAACATCAAAGAGCT	200
	R: GGATAGGTTTCGTTAGTGAACT	
TGS0385	F: ATGCCAAAAAGTGATCAGGG	163
	R: GGGACAAACGTGTAACACACA	
TGS2259	F: ACGCAAGCTGAAGCCATAAT	205
	R: GTCTCCCTGCTGCTTACTGC	
Eaat001	F: GATGGACACCCTTCAATTTATGGT	136
	R: TCCAAGTATCAGGCACACCAGC	
LEaat002	F: GCGAAGAAGATGAGTCTAGAGCATAG	106
	R: CTCTCTCCCATGAGTTCTCCTCTTC	
LEaat003	F: CTTGAGGTGGAAATATGAACAC	189
	R: AAGCAGGTGATGTTGATGAG	
LEaat006	F: GCCACGTAGTCATGATATACATAG	174
	R: GCCTCGGACAATGAATTG	
LEaat007	F: CAACAGCATAGTGGAGGAGG	100
	R: TACATTTCTCTCTCTCCCATGAG	
LEaat008	F: GAGTCAACAGCATAGTGGAGGAGG	178
	R: CGTCGCAATTCTCAGGCATG	
LEat006	F: CATAATCACAAGCTTCTTTCGCCA	166
	R: CATATCCGCTCGTTTCGTTATGTAAT	
LEat012	F: CGGCAAAGGGACTCGAATTG	110
	R: GTGGCGGAGTAGAAACCTTAGGA	
LEat013	F: ATCACAAGCTTCTTTCGCCACA	163
	R: ACCCATATCCGCTCGTTTCG	
LEat018	F: CGGCGTATTCAAACTCTTGG	120
	R: GCGGACCTTTGTTTTGGTAA	
LEat020	F: ACTGCCTCTCTTCAAAGATAAAGC	212
	R: ACGGAAAGTTCTCTCAAAGGAGTTG	
LEga003	F: TTCGGTTTATTCTGCCAACC	241
	R: GCCTGTAGGATTTTCGCCTA	
LEga005	F: TTGGCCTAATCCTTTGTCAT	314
	R: AACAATGTGACGTCTTATAAGGG	
LEga006	F: CCGTCCAGAAGACGATGTAA	248
	R: CAAAGTCTTGCCAACAATCC	

Marker	Sequence	Expected product size (bp)
LEga007	F: CCTTGCAGTTGAGGTGAATT	193
	R: TCAAGCACCTACAATCAATCA	
LEgata002	F: TTGGTAATTTATGTTCGGGA	344
	R: TTGAGCCAATTGATTAATAAGTT	
LEta002	F: GCCTCCCACAACAATCATCTATACA	190
	R: TCCTCCGTACTTTGATCATCTTGTT	
LEta003	F: GCTCTGTCCTTACAAATGATACCTCC	111
	R: CAATGCTGGGACAGAAGATTTAATG	
LEta006	F: CCCTCTTGCCTAAACATCC	167
	R: TCTACTCGTTGCGAATTCAG	
LEta007	F: GCCGTTCTTGGTGGATTAG	291
	R: CCTCCTTTCGTGTCTTTGTC	
LEta015	F: ATATGCATGGACAAATCTTGAGGG	107
	R: CTCGCGCATCAAATTAATGTATCAG	
LEta016	F: AGGTTGATGAAAGCTAAATCTGGC	174
	R: CAACCACCAATGTTCATTACAAGAC	
LEta020	F: AACGGTGGAAACTATTGAAAGG	275
	R: CACCACCAAACCCATCGTC	
LEtaa002	F: TGAGAGAGATCAACCAACTCC	133
	R: ACTACTCCTGCCTCTCTATATCC	
LEtca001	F: TGCATGGCAACATTAAAGTC	176
	R: CGTGGATGCAACTTCATTG	
SSR45	F: TGTATCCTGGTGGACCAATG	260
	R: TCCAAGTATCAGGCACACCA	
SSR96	F: GGGTTATCAATGATGCAATGG	210
	R: CCTTTATGTCAGCCGGTGTT	
SSR104	F: TTCCATTTGAATTCCAACCC	220
	R: CCCACTGCACATCAACTGAC	

Figure 1: Variation in number of locules as high as 10 (EC-620481) to as low as 2 (EC-620456).

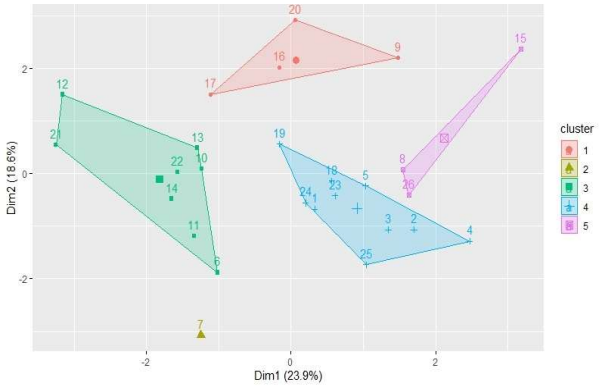


Figure 2: Grouping of genotypes using K-means cluster analysis based on 11 morphological characters.

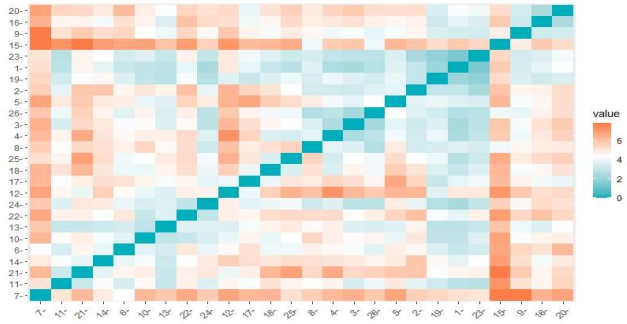


Figure 3: Heat map representing Euclidian genetic distance between germplasm accessions based on 11 morphological characters.

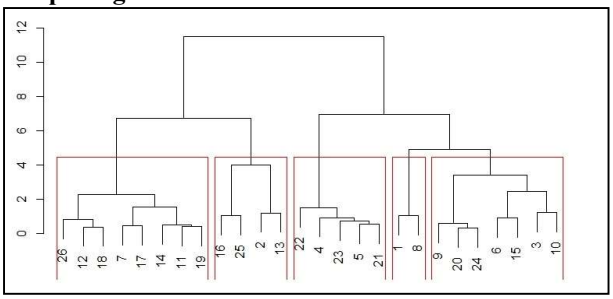


Figure 4: Clustering of genotypes based on data recorded on three important biochemical parameters.

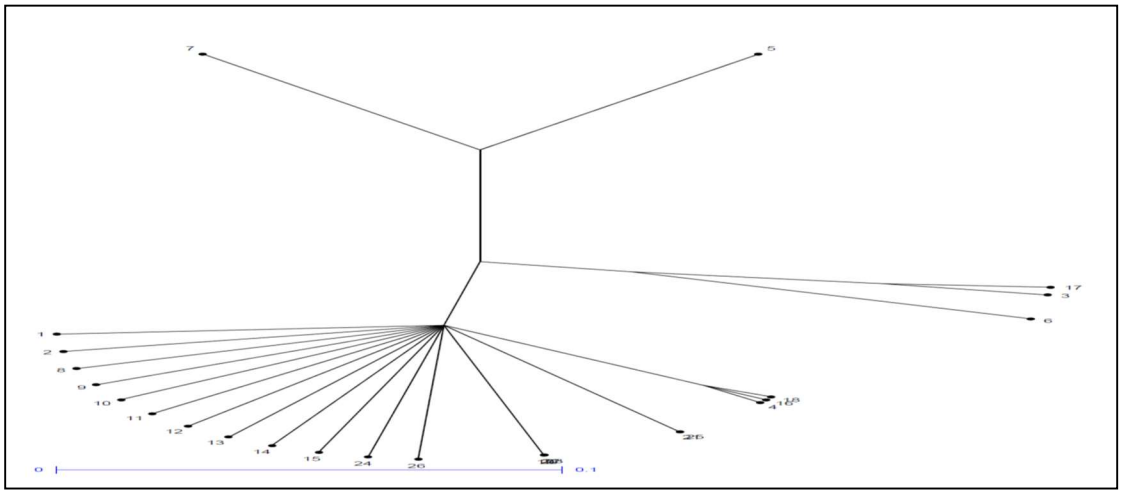


Figure 5: Tree diagram depicting SSR marker based UPGMA classification of germplasm accessions.

Table 4: List of 23 polymorphic markers and their major allele frequency, gene diversity, polymorphic information and content (PIC).

Marker name	Major Allele Frequency	Allele No.	Gene Diversity	PIC
SSR111	0.81	2.00	0.30	0.26
SSR134	0.94	1.50	0.10	0.09
SSR146	1.00	1.00	0.00	0.00
SSR318	1.00	1.00	0.00	0.00
TOM49	0.96	1.50	0.07	0.07
TOM144	0.92	1.50	0.13	0.11
TOM152	1.00	1.00	0.00	0.00
TOM184	0.81	2.00	0.28	0.23
TGS0385	0.85	1.50	0.21	0.17
Eaat001	0.98	1.50	0.04	0.04
LEaat002	1.00	1.00	0.00	0.00
LEat013	0.96	1.50	0.07	0.07
LEat020	1.00	1.00	0.00	0.00
LEga003	0.85	1.50	0.21	0.17
LEga005	0.94	1.50	0.10	0.09
LEta006	0.75	2.00	0.34	0.27
LEta015	0.75	1.50	0.25	0.19
LEta016	1.00	1.00	0.00	0.00
LEta020	0.87	1.50	0.20	0.16
SSR45	0.85	1.50	0.21	0.17
SSR96	0.65	2.00	0.45	0.35
SSR104	0.75	2.00	0.36	0.29
LEaat007	0.81	2.00	0.31	0.26

Table 5: Promising trait specific accessions in exotic collection of tomato germplasm.

Traits	Selection criteria	Range	Vaibhav (Check)	Accessions
Days to flowering	Earliness	62-73	69	EC-620474, EC-620546, EC-614997, EC-620472, EC-620544, EC-620550, EC-620553, EC-620481, EC-620521, EC-620545, EC-620567, EC-614998, EC-620438 and EC-620394
Flowering to fruit set	Earliness	6-12	10	All the accessions except EC-614998 and EC-620554
Plant height at 65 days	High	124-252 cm	185 cm	EC-620563, EC-620546, EC-620544, EC-620474, EC-632946, EC-620560, EC-620543, EC-620550, EC-620557, EC-620437, EC-620567, EC-614998, EC-620553 and EC-620343
Number of branches (primary, secondary and tertiary)	Medium	42-125	46	EC-620456, EC-620460 and EC-620472
Fruits per cluster	High	9-12.5	11	EC-614998, EC-620438, EC-620474, EC-620521, EC-620437, EC-620550, EC-620554, EC-620560, EC-620567, EC-614997, EC-620343, EC-632946, EC-620460, EC-620544, EC-620481, EC-620472, EC-620543, EC-620563, EC-620553, EC-620557 and EC-620568
Fruits per plant	High	32-170	94	EC-620554, EC-620546, EC-620568, EC-614997, EC-620543, EC-620521, EC-620560, EC-620557, EC-620550, EC-620567, EC-620343 and EC-620545
Fruit length	High	8.24 – 13.82 cm	10.48 cm	EC-620543, EC-614997, EC-620472, EC-614998, EC-620544, EC-620545, EC-620553, EC-620550, EC-620568, EC-620557, EC-620456, EC-620437, EC-620546, EC-620343, EC-620567, EC-620460, EC-620438, EC-620394, EC-632946, EC-620474 and EC-620560
Fruit width	High	8.52-12.90 cm	9.82 cm	EC-620481, EC-620550, EC-614997, EC-614998, EC-620546, EC-620553, EC-620545, EC-620544, EC-620472, EC-620474, EC-620568, EC-620343, EC-620456, EC-620437, EC-620567, EC-620560, EC-620557, EC-632946, EC-620394 and EC-620438
Fruit weight	High	66-208 g	144.20 g	EC-614998, EC-614997, EC-620553, EC-620563, EC-620394, EC-620560, EC-620545, EC-620481, EC-620474, EC-620460, EC-620456, EC-620437, EC-620544 and EC-620521
Number of locules	Fewer	2-10	7	EC-620456, EC-620472, EC-620543, EC-620460, EC-620394, EC-620554, EC-620567, EC-620438, EC-620544, EC-620560, EC-620563, EC-620568, EC-614998, EC-620521, EC-620550 and EC-620557
Plant yield	High	2.88-12.61 Kg	6.58 kg	EC-614997, EC-620560, EC-620546, EC-620557, EC-620553, EC-620568, EC-620550, EC-620545, EC-620521, EC-614998, EC-620567, EC-620554 and EC-620481

inferred the lower differences between accessions at SSR marker loci (Table 4). However, tree constructed based on SSR amplicon data using UPGMA approach provided the visibility of diversity among accessions (Fig. 5). The accessionsEC-620481 (7) and EC-620472 (5) formed solitary branches away from other accessions, proving its distinctness from other accessions. However, accession EC-620472 (5) was in cluster four under morphometric cluster analysis. This might be due to differences at genomic regions that amplified at SSR loci. These results indicated the significant differences at genetic level which can be exploited by strategic breeding programs. Brake *et al.* (2021) in their study, among the genotypes screened for variability using ISSR markers observed the lowest genetic similarity value (0.46) was found between landraces Jo964 and Jo955, while the highest (0.94) was obtained between landraces Jo983 and 29. The accessions were compared with check variety to identify some accessions which are superior to check variety with respect to 11 morphological traits (Table 5). Such accessions which surpassed local check variety for given trait were considered as trait-specific accessions. The germplasm accessions identified for specific traits can be potentially used for trait specific breeding activity without further evaluation, saving breeder's time and resources.

Conclusion

It is concluded that this investigation resulted in the identification of specific traits which are economically important, and are having potential to be utilised in commercial tomato breeding.

Acknowledgement

Senior authors are thankful to Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore for supporting for this experimentation and to NBPGR, Regional station Hyderabad for sharing genetic material.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Anilkumar, C., & Lohithaswa, H. C. (2018). Heterotic hybrid frequency in relation to combining ability and parental genetic divergence in maize. *Electronic journal of Plant Breeding,* 9(4): 1322-1334.

Anilkumar, C., Lohithaswa, H. C., & Pavan, R. (2017). Assessment of genetic diversity in newly developed inbred lines of maize (*Zea mays* L.). *Electronic journal of Plant Breeding,* 8(1): 193-200.

Bergougnoux, V. (2014). The history of tomato: from domestication to biopharming. *Biotechnology advances*, 32:170-189.

Bose, T. K., Kabir, J., Maity, T. K., Parthasarathy, V. A. & Som, M, G. (2002). *Vegetable Crops*.1. NayaProkash, Calcutta.

Brake, M. H., Al-Gharaibeh, M. A., Hamasha H. R., Al-Sakarneh, N. S., Alshomali, I. A., Migdadi, H. M., Qaryouti, M. M., & Haddad, N. J. (2021). Assessment of genetic variability among Jordanian tomato landrace using inter-simple sequence repeats markers. *Jordan Journal of Biological Sciences,* 14(1): 91-95.

Brunlop, S., & Finckh, M. R. (2010). Application and potentials of markers assisted selection (MAS) in plant breeding. Germany: *Federal Agency for Nature Conservation.*

Collins, J. K., & Veazie, P. P. (2006). Lycopene from plants to humans. *Horticulture Science*, 41(5): 1135-1144.

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus,*12:13–15.

Herraiz, F. J., Vilanova, S., Andujar, I., Torrent, D., Plazas, M., & Gramazio, P. (2015). Morphological and molecular characterization of local varieties, modern cultivars and wild relatives of an emerging vegetable crop, the pepino (*Solanum muricatum*), provides insight into its diversity, relationships and breeding history. *Euphytica,* 206(2): 301-318.

Hussain, K., Lone, S., Malik, A., Masoodi, K. Z., Dar, Z. A., Nazir, N., Ali, G., & Farwah, S. (2021). Genetic variability studies in cherry tomato for growth, yield, and quality traits in open field conditions. *International Journal of Agricultural and Applied Sciences,* 2(2):60-64.

Johnson, B. C. (1948). Methods of Vitamin Determination. *Burgess Publishing Co. Minneapolis, Minnesota,* Pp- 98.

Kaushik, S. K., Tomar, D. S., & Dixit, A. K.(2011). Genetics of fruit yield and it's contributing characters in tomato *(Solanum lycopersicum). Journal of Agricultural Biotechnology,* 3(10): 209-213.

Levene, H. (1960). Robust tests for equality of variances. In Contributions to probability and statistics: Essays in honour of Harold Hotelling, ed. Olkin, *Stanford University Press, Stanford, Connecticut.* 278–92.

Liu, K., Muse, S. V. (2005). Power Marker: An integrated analysis environment for genetic marker analysis. *Bioinformatics,* 21: 2128-2129.

Milevska, E. M., Popovski, Z. T., Dimitrievska, B. R., & Bandzo, K. (2011). Determination of genetic diversity among different tomato varieties using SSR markers. *Acta Agriculturae Serbica*, 16(31): 9–17.

Miller,J. C, & Tanksley, S. D. (1990). RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theoretical and Applied Genetics,* 80:437-448.

Panthee, D. R., Cao, C., Debenport, S. J., Rodrguez, G. R., Labate, J. A., Robertson, L. D., Andrew, P. B., Vander K. E., Brian, B., & Gardener. (2012). Magnitude of genotype x environment interactions affecting tomato fruit quality. *Horticulture Science,* 47(6): 721–726.

Pradeepkumar, T., Bastian, D., Joy, M., Radhakrishnan, N. V., Aipe, K. C. (2001). Genetic variability in tomato for yield and resistance to bacterial wilt. *Journal of Tropical Agriculture,* 39: 157-158.

Ranganna, S. (1976). In: manual of analysis of fruits and vegetable products, *McGraw hill,* New Delhi*., Pp*-77.

Reddy, B. R., Reddy, M. P., Begum, H., & Sunil, N. (2013). Genetic diversity studies in tomato (*Solanum lycopersicum* L.). *IOSR Journal of Agriculture and Veterinary Science,* 4(2): 53- 55.

Rick, C. M., Chetelat, R. T., (1995). Utilization of related wild species for tomato improvement. *Acta Horticulturae,* 412: 21–38.

Rothan, C., Diouf, I., Causse, M. (2019). Trait Discovery and Editing in Tomato. *Plant Journal,* 97:73-90.

RStudio Team (2019). RStudio: integrated development for R. *RStudio, Inc.,* Boston. http://www.rstudio.com/