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Molecular Diversity Analysis in Elite Tomato Genotypes (*Solanum lycopersicum* L.) for Fruit Quality

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ABSTRACT: In the present investigation, twenty exotic and indigenous accessions of tomato along with the checks were screened for morphological, agronomic, biochemical, and molecular variability. The accessions with most favourable morphological characters identified were *viz*., EC-620545 (higher fruit weight-162.00 g/fruit and high fruit yield-8.24 kg/plant), EC-16786 (higher fruits/cluster-8), EC-159053 (high lycopene content-10.12 mg/100g), EC-168283 (high ascorbic acid content-18.07 mg/100g) and EC-151568 (high TSS- 8.08 °Brix). High genetic coefficient of variation with high heritability coupled with genetic advance over mean recorded for fruit weight, yield per plant, number of clusters per plant, number of fruits per cluster, fruit width, fruit length, TSS ^{0}Brix, total solids, fruit firmness, and plant height. The molecular diversity was assessed and fifteen fruit quality-linked markers were polymorphic among accessions. EC-165690, EC-168283, EC-249514, EC- EC-249515, EC-320574-1, EC-362944, and EC-151568 belonged to the same clade and were rich sources for Ascorbic acid, TSS, and lycopene content. Among them EC-249515 (5.3 Kg/plant), EC-620545 (8.24 Kg/plant) and EC-620521 (7.64 Kg/plant) were significantly superior to Vaibhav. The accessions with better superior fruit qualities can utilized in the breeding program for creating variability and also for introgression of novel alleles into superior varieties.

Keywords: Genetic diversity, variability, correlation and molecular markers.

INTRODUCTION

Tomato (*Solanum lycopersicum* L. 2n = 24) is one of the world's most important warm-season vegetable crops, grown in tropical, subtropical, and temperate regions. It is a member of the Solanaceae nightshade family and is originated in South America and diversified first in Peru and Mexico. It has a global area of 4.84 million hectares, a global output of 182.30 million tonnes per year, and an average productivity of 37.6 metric tonnes per hectare (FAO 2017). The major producers are China followed by India, Pakistan, Turkey, and the United States. In India, it is grown on 8.14 lakh hectares, with a production of 20.51 million metric tonnes and an average yield of 25.63 metric tonnes per hectare (NHB, 2019). Because of their palatability, nutritional and health benefits, tomatoes and other products are becoming increasingly popular among consumers.

Tomatoes are popular among consumers because of their many uses and nutritional value. It is a nutrient-dense vegetable that can be consumed raw or cooked. Tomatoes are also used to make sauces, juices, ketchup, pastes, purees, and other items. Fruit quality has been a major focus of most tomato breeding programs during the past century. Fruit qualities also determine the fresh market and processing industries. It includes fruit size, shape, total solids, color, firmness, ripening, nutritional quality, and flavor. Total solids are the important factor that regulates the yield /market price and are comprised of soluble solids (SS) and insoluble solids (ISS). Regular tomato consumption has been linked to a lower risk of chronic degenerative diseases like cancer (Giovannucci, 1999) and cardiovascular disease (Pandey, 1995). According to epidemiological evidence, the observed health benefits are due to the presence of various antioxidant molecules such as carotenoids (lycopene), ascorbic acid, vitamin E, and phenol compounds (flavonoids) (Frusciante *et al.,* 2007).

Apart from the health benefits Lycopene is the red pigment and major part of the carotenoid in tomatoes. The red color is the most visible and important quality attribute of the mature tomato fruit for both fresh consumption and processing. In processing tomatoes, fruit color influences the grades and standards of the processed commodity. In fresh market tomato, fruit color has a significant effect on its marketability. Acidity influences the storability of processed tomatoes. Lower pH reduces the risk of pathogen growth in tomato products by contributing to heat inactivation of thermophilic organisms. With this background information and importance of fruit quality characteristics the present study was carried out to screen the tomato accessions for fruit quality by phenotypically and genotypically for the better accessions for further crop improvement.

MATERIALS AND METHODS

Twenty tomato accessions obtained from NBPGR, Regional Station, Rajendranagar, Hyderabad, were evaluated in randomized block design with three replications in the field at the Department of Plant Biotechnology, GKVK, University of Agricultural Sciences, Bangalore. All the accessions were evaluated for plant growth, fruit, and yield characters along with checks Arka Rakshak and Vaibhav. Biochemical parameters like lycopene (Ranganna, 1976) and ascorbic acid (Johnson, 1948) content were estimated. Statistical analysis was carried "Indostat" to support the study. DNA was isolated from twenty tomato accessions along with two checks from the CTAB method (Doyle and Doyle 1987) and subjected to PCR with fruit quality linked SSR markers (Yogendra and Gowda 2013). The amplified gel pictures obtained from primers were scored using binary codes. The presence of a band was scored as 1 and absence was scored as 0. The binary data generated for all the accessions for the polymorphic markers was entered in the NT edit program of NTSYSpc version 2.02 software. The similarity matrix was used to generate a dendrogram using the SHAN module for cluster analysis.

RESULTS AND DISCUSSION

The analysis of variance indicated a significant difference among the genotypes for all the characters studied. The phenotypic coefficient of variation (PCV) is always higher than the genotypic coefficient of variation (GCV) and, were varies from low to high. The high PCV and GCV were observed for fruit weight (69.30% and 68.95%) followed by yield per plant, number of clusters per plant, number of fruits per cluster, fruit width, fruit length, TSS ^{0}Brix, total solids, fruit firmness and plant height (Table 1). Also, these observed traits were high for heritability and genetic advance over mean (GAM). The variability study indicated the accessions were used in this study have a good potential of genes/alleles and selection could be based on traits that could be effective due to additive gene interaction. Similar results were found by Mehta and Asati (2010); Kaushik *et al.* (2011); Manna *et al.* (2012). Fruit length and weight were positively significantly associated with yield, while fruit lycopene content was negative significantly associated with yield (Table 2). This indicates that the lycopene content gradually decreases with an increase in the yield of the fruit. Almost all the traits were positively associated with yield but some traits like plant height, fruit pH, and TSS had a negative effect similar results were observed by Dudi and Kalloo (1982); Rattan *et al.* (1983); Reddy and Gulshanlal (1990).

Growth habits, fruit shape, and fruit color were observed in the accessions (Table 3). In total, fifteen accessions were indeterminate type and other five accessions were determinate type of growth habit, and checks were observed with the determinate type of growth habit. A huge variation was observed for fruit shape, among 20 accession 13 accessions flat type, 3 accessions round type, 2 accessions ellipsoid type, one accession oxheart, and one accession belongs to heart-type, Arka Rakshak belongs to oxheart type and Vaibhav belongs to flat type. Fruit color is an important parameter that determines the lycopene content and varies from orange-red to deep red. 4 accession orange-red, 5 accessions red and 10 accessions deep red, interestingly IC- 247508 fruit color was pink.

Thirty linked markers were validated and among them, fourteen markers showed polymorphism (supplemented Table 1). The EC-313479 stands as an outer group in the dendrogram and commercially released cultivars (checks) belong to the same clade may be due to domestication and selection. EC-165690, EC-168283, EC-249514, EC-249515, EC-320574-1, EC-362944, and EC-151568 belong to the same clade (Fig. 1). These accessions were rich source of biochemical parameters like Ascorbic acid, TSS ^{0}Brix, and lycopene content. Among accession present in the single clade EC-249515 (5.30 Kg/plant) was significantly superior over Vaibhav (4.50 Kg/plant) for the yield per plant and superior for fruit quality and, can be released as the commercial cultivar. The accessions with higher lycopene content *viz.,* EC-159053 (10.12 mg/100 g), EC-241140 (8.65 mg/100 g), and EC-249514 (8.25 mg/100 g) can be exploited for further crop improvement for lycopene. The Arka Rakshak was observed with the highest yield (9.53 Kg/plant) followed by two accessions EC-620545 (8.24 Kg/plant) and EC-620521 (7.64 Kg/plant). These two exotic collections directly can be released as varieties and were almost on par with Arka Rakshak. Similar types of variability concerning yield parameters were reported by Kaushik *et al*. (2011) ; Reddy *et al*. (2013).

In the present study, the exotic and indigenous collections had potential genes/alleles for both fruit quality and productivity. Many other wild species/ accessions need to be screened for quantitative and qualitative traits. The identified trait-specific accessions have the potential to accelerate trait-specific breeding for economically important traits without further evaluation, saving breeders time and resources. This investigation resulted in the identification of such potentially useful accessions for commercial tomato breeding.

Table 1: Variability study among the twenty accessions for fruit quality and yield parameters.

Sr. No.	Characters	Range	Mean	PCV (%)	GCV (%)	h^2 (%)	Genetic advance (%)	Genetic advance over mean (%)
1.	Plant height (cm)	90.00-243.50	157.8	27.55	26.05	89.42	80.10	50.76
2.	No. of days for flowering	31.00-35.33	33.09	4.70	3.26	48.18	1.54	4.66
3.	No. of days from flowering to fruit set	11.00-15.33	12.09	14.12	10.67	57.06	2.01	16.60
4.	Fruit pH	3.49-5.80	4.38	16.86	16.63	97.35	1.48	33.81
5.	Fruit firmness (lb)	2.25-8.38	4.62	40.25	38.22	90.17	3.46	74.77
6.	Total solids (g)	1.33-5.00	3.52	28.77	28.56	98.52	2.05	58.39
7.	Lycopene content (mg/100g)	2.27-10.12	10.12	19.75	19.73	99.81	4.11	40.61
8.	TSS ^{0}Brix	3.78-8.08	5.72	20.05	20.04	99.88	2.36	41.26
9.	Ascorbic acid (mg/100g)	9.85-18.07	12.90	17.80	17.19	93.33	4.41	34.22
10.	Fruit length (cm)	2.56-6.67	4.09	34.77	34.68	99.54	29.17	71.28
11.	Fruit width (cm)	2.54-7.05	4.42	29.20	29.01	98.73	26.28	59.38
12.	Fruit weight (g)	18.0-162.0	67.37	69.30	68.95	98.98	95.21	141.31
13.	No. of clusters per plant	15.0-53.0	30.53	34.91	31.68	82.36	18.08	59.22
14.	No. of fruits per cluster	2.0-8.0	4.22	29.45	27.90	89.76	2.30	54.46
15.	Yield (kg/plant)	1.41-9.53	3.74	62.01	56.59	83.28	3.98	106.38

Table 2: Correlation among the fruit quality traits and yield parameters in twenty tomato accession.

	PHt	FpH	FL	FW	FF	TSS	FLY	FA	FPC	CPP	FWt	DTF	DTFF	TS	Y
PHt	**1**	.184	-.314	-.160	-.469*	.261	.758**	-.147	.047	-.130	-.294	-.390	-.390	-.453*	-.400
FpH	.184	**1**	-.168	-.209	-.151	.064	-.024	-.102	-.099	-.296	-.111	-.057	-.057	.006	-.110
FL	-.314	-.168	**1**	.652**	.844**	-.355	-.194	.217	-.090	-.292	.814**	.397	.397	.405	.702**
FW	-.160	-.209	.652**	**1**	.482*	-.221	-.066	.075	-.272	-.207	.635**	.226	.226	.018	.309
FF	-.469*	-.151	.844**	.482*	**1**	-.383	-.353	.189	.034	-.303	.844**	.293	.293	.470*	.804**
TSS	.261	.064	-.355	-.221	-.383	**1**	.372	.046	.187	.362	-.413	-.298	-.298	-.152	-.203
FLY	.758**	-.024	-.194	-.066	-.353	.372	**1**	.052	.022	-.226	-.257	-.507*	-.507*	-.227	-.468*
FA	-.147	-.102	.217	.075	.189	.046	.052	**1**	.236	-.077	-.049	.299	.299	.044	.182
FPC	.047	-.099	-.090	-.272	.034	.187	.022	.236	**1**	.251	-.318	-.236	-.236	.180	.201
CPP	-.130	-.296	-.292	-.207	-.303	.362	-.226	-.077	.251	**1**	-.439*	.026	.026	-.214	.044
FWt	-.294	-.111	.814**	.635**	.844**	-.413	-.257	-.049	-.318	-.439*	**1**	.247	.247	.428*	.660**
DTF	-.390	-.057	.397	.226	.293	-.298	-.507*	.299	-.236	.026	.247	**1**	1.000**	-.234	.345
DTFF	-.390	-.057	.397	.226	.293	-.298	-.507*	.299	-.236	.026	.247	1.000**	**1**	-.234	.345
TS	-.453*	.006	.405	.018	.470*	-.152	-.227	.044	.180	-.214	.428*	-.234	-.234	**1**	.400
Y	-.400	-.110	.702**	.309	.804**	-.203	-.468*	.182	.201	.044	.660**	.345	.345	.400	**1**

Plant height(PHt), Fruit Ph (FpH), Fruit length (FL), Fruit width (FW), Fruit firmness (FF), Total soluble solids (TSS), Fruit lycopene (FLY), Fruit ascorbic acid (FA), Fruit per cluster (FPC), Cluster per plant (CPP), Fruit weight (FWt), Date of flowering (DTF), Date of fruit set from flowering (DTFF), Yield (Y), Total solids (TS)

Table 3: Observations recorded for fruit quality parameters and yield contributing parameters among twenty tomato accessions.

Accessions	GH	FS	FC	PHt (cm)	DTF	DTFF	FPC	CPP	FL (cm)	FW (cm)	FWt (g)	TS (g)	FpH	FF (lbs/cm^2)	FLY (mg/100 g)	TSS (°Brix)	FA (mg/100 g)	Y (kg/plant)
EC 145054	ID	Flat	Orange red	186.50 c	33.83 a	13.83 a	4 c	25	3.57 b	4.94 a	66.5	2	5.20 b	3.38	3.51	4.13	10.61	3.07
EC 159053	ID	Ellipsoid	Deep red	243.50 a	33.33 b	13.33 b	5 b	25	2.94 c	2.82 b	18	1	4.06	3.38	10.12 a	5.98	14.67 b	2.23
EC 165690	ID	Round	Deep red	175.75 d	33.83 a	13.83 a	3 c	43 b	2.68 c	2.54 b	33	3 c	3.75	2.5	6.13	6.08	11.98	1.41
EC 168283	ID	Flat	Red	151.75	33.67 a	13.67 c	5 b	24	2.83 c	3.29 b	25.3	4 b	4.17	3	5.98	5.03	18.07 a	2.36
EC-249514	ID	Flat	Deep red	201.75 b	32.00 b	12.00 b	4 c	39 b	3.94 b	5.39 a	35.5	3 c	3.81	3	8.25 c	6.03	11.6	1.92
EC 249515	ID	Flat	Orange red	91	31.67 c	11.67 c	5 b	34 c	3.00 c	4.08 a	73.5	4 b	3.59	5.88 c	4.34	5.23	13.31 c	5.34 b
EC 320574-1	ID	Flat	Red	197.25 b	33.17 b	13.17 c	5 b	26	3.19 c	4.34 a	49.8	3 c	5.80 a	3.88	5.74	6.05	12.68 c	2.47
EC 362944	ID	Flat	Red	127	33.00 b	13.00 b	4 c	22	2.69 c	3.33 b	36	4 b	5.05 b	3.88	3.67	5.23	11.62	2.82
EC 617059	D	Flat	Deep red	177.00 d	33.17 b	13.17 b	2	22	5.49 a	7.05 a	149.50 b	3 c	4.56 c	5.25	6.97	4.78	11.63	4.34
EC-151568	ID	Flat	Deep red	158.25	33.67 a	13.67 a	4 c	37 b	3.74 b	6.87 a	32.8	2	3.77	3.38	7.36	8.08 a	16.09 a	2.03
EC-165393	ID	Flat	Deep red	155.75	31.67 c	11.67 c	5 b	42 b	2.56 c	3.53 b	20.5	4 b	5.70 a	3.63	6.38	7.38 b	12.78 c	2.42
EC-165751	D	Ellipsoid	Orange red	145	33.67 a	13.67 a	3 c	32 c	4.45 b	4.46 a	50.5	2	4.13	5.5	5.26	4.6	13.04 c	2.27
EC-16786	D	Round	Deep red	201.00 b	31.00 c	11.00 c	8 a	43 b	3.33 b	3.32 b	20.5	4 b	3.49	3.63	7.53	7.00 c	10.45	3.79
EC-241140	ID	Flat	Deep red	232.00 a	31.00 c	11.00 c	3	23	2.78 c	3.27 b	38.3	4 b	5.25 b	2.25	8.65 b	6.45	10.78	2.25
EC-313479	ID	Round	Deep red	138	32.67 b	12.67 c	3	15	5.33 a	6.10 a	157.00a	5 a	3.55	6.88 b	6.39	3.78	9.85	2.91
EC-620419	ID	Oxheart	Deep red	119.25	33.17 b	13.17 b	4 c	22	6.08 a	4.65 a	75.8	5 a	4.38 c	4.88	6.59	6	14.84 b	3.16
EC-620521	D	Heart	Orange red	162.75	32.67 b	12.67 b	5 b	27	6.67 a	5.36 a	146.80 c	5 a	4.17	8.36 a	4.76	4.95	16.85 a	7.64 a
EC-620545	D	Flat	Red	152.25	34.17 a	14.17 a	4 c	18	6.43 a	5.27 a	162.00 b	4 b	4.37 c	8.38 a	5.4	7.08 c	12.83 c	8.24 a
IC 247508	ID	Flat	Pink	124	33.67 a	13.67 b	3	53 a	2.67 c	3.42 b	55	3 c	3.81	3	2.59	7.03 c	10.73	4.18
IC 549835	ID	Flat	Red	129.25	33.33 b	13.33 b	4	24	4.01 b	2.96 b	65.5	4 b	5.60 a	5	5.69	6	12.47 c	3.4
ARKA RAKSHAK	D	Oxheart	Orange red	112.5	35.33	15.33	5	45	6.49	5.22	98.3	4	4.4	7.13	3.23	4.3	14.09	9.53
VAIBHAV	D	Flat	Red	90	34.5	14.5	5	34	5.05	5.05	71	4	3.8	5.55	2.27	4.75	13.02	4.5
MEAN				157.8	33.1	12.09	4.22	30.53	4.09	4.42	67.37	3.52	4.38	4.62	5.76	5.72	12.9	3.74
S.Em				4.16	0.65	0.65	0.28	3.2	0.69	1.03	3	0.04	0.09	0.41	0.05	0.07	0.85	0.22
CV				3.73	3.38	8.55	9.42	14.7	2.37	3.29	6.238	1.75	2.75	12.58	1.51	1.81	11.38	11.95
CD (5 %)				12.23	1.84	1.84	0.83	9.3	2.02	3.02	10	0.1	0.25	1.21	0.14	0.22	2.42	0.63

Plant height (PHt), Fruit Ph (FpH), Fruit length (FL), Fruit width (FW), Fruit firmness (FF), Total soluble solids (TSS), Fruit lycopene (FLY), Fruit ascorbic acid (FA), Fruit per cluster (FPC), Cluster per plant (CPP), Fruit weight (FWt), Date of flowering (DTF), Date of fruit set from flowering (DTFF), Yield (Y), Total solids (TS), Growth habit (GH), Fruit shape (FS), Fruit color (FC)

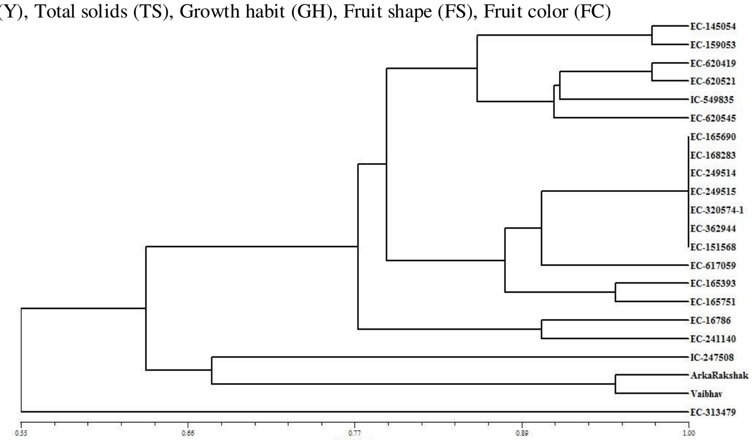

Fig. 1. Dendrogram based on the molecular data representing the relationship among twenty accessions and two check varieties.

Supplemented Table 1: Polymorphic SSR markers linked fruit quality (Yogendra and Gowda 2013).

Primer name	No/. of loci amplified	Amplified product
TOM144	2	Polymorphic
TOM210	2	Polymorphic
LEaat002	2	Polymorphic
LEaat007	2	Polymorphic
LEaat008	2	Polymorphic
LEat006	2	Polymorphic
LEga007	2	Polymorphic
LEta002	2	Polymorphic
LEta003	2	Polymorphic
LEta007	2	Polymorphic
LEta015	2	Polymorphic
LEta016	2	Polymorphic
LEta020	2	Polymorphic
SSR96	2	Polymorphic

CONCLUSIONS

In the present study, the exotic and indigenous collections had potential genes/alleles for both fruit quality and productivity. Many other wild species/ accessions need to be screened for quantitative and qualitative traits. The identified trait-specific accessions have the potential to accelerate trait-specific breeding for economically important traits without further evaluation, saving breeders time and resources. This investigation resulted in the identification of such potentially useful accessions for commercial tomato breeding.

FUTURE SCOPE

These potential avenues for future research in tomato diversity analysis highlight the interdisciplinary nature of the field, incorporating genetics, genomics, agriculture, data science, and ethical considerations. Continued exploration and innovation in these areas can contribute to the development of more robust and sustainable tomato varieties to meet the challenges of a changing world.

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Conflict of Interest. None.

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