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RESEARCH PAPER

Characterization of tomato lines for yield components, processing traits and disease resistance based on phenotype and molecular markers

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Abstract

Development of improved line is one of the major activities in hybrid tomato breeding. The aim of this study was to identify improved lines based on horticultural traits, total soluble solid (TSS) and diseases resistance particularly tomato yellow leaf curl virus (ToLCV). The genotypes consisted of improved lines, jointless tomato, cherry tomato and beta carotene rich lines. These lines were evaluated in field experiment and screened against ToLCV resistance under natural field condition for two years. Molecular markers based assays were performed for resistance genes to ToLCV (*Ty-2* and *Ty-3*), late blight (*Ph-3*) and root knot nematode (*Mi1-2*). Based on fruit yield, promising genotypes were improved lines (VRT-06, VRT-19, VRT-34 and VRT-51), jointless (EC-605037 and EC-695037), cherry tomato (VRCRT-5), and beta-carotene line (KB-20). High TSS was noticed in improved line VRT-67 (5.36 °Brix), jointless EC-695037 (5.37 °Brix) and cherry tomato VRCYT-3 (8.22 °Brix). High ToLCV resistance was recorded in improved lines (VRT-06, VRT-19, VRT-30, VRT-34, VRT-50, VRT-51, and VRT-67), jointless (EC-695037, EC-605037, EC-605094, KB-Jointless and New-Jointless), cherry tomato (VRCYT-3, VRCYT-5, VRCYT-9 and VRCYT-15), and beta carotene (KB-2, KB-3-1, KB-3-2, KB-5, KB-10, KB-14, KB-17 and KB-20). Tomato line VRT-02 having dwarf plant type was found suitable for pot culture, whereas line H-88-78-2 had very delayed and partial fruit ripening. Thus, our study identified promising lines for breeding application for economically important horticultural traits.

Keywords: Tomato, Horticultural traits, ToLCV, Molecular markers, Processing traits.

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Introduction

Tomato (*Solanum lycopersiccum* L.) is one of the most important vegetable crops used world over as fresh and processed products. It is a rich source of nutrients, vitamin A & C, antioxidants mainly lycopene and health beneficial compounds. Lycopene is a very powerful antioxidant and anti-cancerous (Tiwari et al., 2022). Hence, tomato is considered as functional and protective food, and also known as poor's man orange (Pal et al., 2023). In 2022, world tomato production was 189.13 million tonnes from an area of 5.16 million hectare with an average productivity of 36.60 t/ha (FAOSTAT 2023). China is the top producer of tomato (35.70%) followed by India (11.19%). India ranks second after China (67.53 mt) in tomato production (21.18 mt) from an area of 0.84 m ha area with 25.06 t/ha productivity (FAOSTAT 2023). Tomato crop production faces problems of several biotic stresses. A considerable advancement has been achieved in ToLCV management through resistance breeding. Wild tomato species have been the main source of resistance such as *S. hirsutum* derived ToLCV resistance through hybridization and embryo rescue methods (Vidavsky and Czosnek 1998). Currently, ToLCV resistance genes (*Ty-1* to *Ty*-6) originated from wild species, have been introgressed into cultivated tomatoes. These genes are *Ty-1/3* (chr. 6 from *S. chilense*), *Ty-2* (chr. 11 from *S. habrochaites*), *Ty-4* (chr. 3 from *S. chilense*), recessive gene *ty-5* (chr. 4, cv. Tyking or *S. peruvianum*), and *Ty-6* (chr. 10 from *S. chilense*). As a result, this has led to the development of resistant tomato varieties all over the world (El-Sappah et al., 2022; Ren et al., 2022).

Late blight caused by the oomycetes *Phytophthora infestans* (Mont.) de Bary is another problem of tomato cultivation and may cause heavy crop loss depending upon the severity of infestation. Resistance breeding has also been progressed to develop late blight resistant tomato varieties. The known late blight resistance genes are *Ph-1* (chr. 7, from *S. pimpinellifolium*), *Ph-2* (chr. 10, from *S. pimpinellifolium*), *Ph-3* (chr. 9, from *S. pimpinellifolium/ S. habrochaites*), *Ph-4* (chr. 2, from *S. habrochaites*), *Ph-5-1* (Chr. 1, from *S. pimpinellifolium*), and *Ph-5-2* (Chr. 10, from *S. pimpinellifolium*) (reviewed by Mazumdar et al. 2021). Last, root knot nematode (RKN, *Meloidogyne incognita*) is one of the major nematodes affecting tomato crop, which cause severe damage to the roots. Resistance breeding has been deployed in tomato breeding using resistance genes (*Mi-1* to *Mi-8*) originated from wild species *S. peruvianum*. The known RKN resistance genes are *Mi-1* (chr. 6), *Mi-2, Mi-3* (chr. 12), *Mi-4, Mi-5* (chr. 12), *Mi-6, Mi-7, Mi-8, Mi-9* (chr. 6 from *S. arcanum*), and *Mi-HT* (chr. 6), of which only five genes have been mapped (El-Sappah et al. 2019).

Development of improved tomato varieties is a continuous breeding process. Improvement of yield traits in combination with multiple disease-pest resistance to ToLCV, late blight and RKN are important in tomato breeding. Hence, the availability of diverse parents is the major step for developing hybrids or varieties. To achieve this, information about resistance genes and linked markers are necessary for selection at early stage and to reduce breeding cycles by MAS. With the advent of molecular markers, there has been a voluminous increase in marker-assisted selection (MAS) in tomato breeding particularly *Ty-2/Ty-3* genes for ToLCV resistance (Foolad & Panthee, 2012). Recently, we have reviewed molecular markers for various diseases and insect-pests in tomato breeding (Tiwari et al., 2022). Moreover, gene pyramiding for ToLCV, late blight and RKN resistance genes showed elite tomato lines for northern Indian farmers (Kumar et al., 2019), and *Ty-2* and *Ty-3* genes in tomato breeding lines (Prasanna et al., 2015a; 2015b). Hence, the aim of this study was to evaluate available tomato lines for horticultural traits and to screen against ToLCV in natural open field conditions over the years. Additionally, linked molecular markers were used to find host resistance genes for ToLCV, late blight and RKN.

Materials and Methods

Plant materials and field experiment

A total of 52 tomato lines comprised of improved lines, jointless, cherry tomato and beta carotene genotypes were used in this study (Table 1). The experiments were conducted in the field in RBD for two years (2021-22 and 2022-23) in main (rabi or winter) crop seasons at research farm of Indian Council of Agricultural Research – Indian Institute of Vegetable Research, Varanasi. The seeds were sown in the month of September (2nd week) and nearly 25 days old seedlings were transplanted in October (1st week) in both the years. Forty seedlings of each line were transplanted on raised bed paired-row bed at 60 x 45 cm^2 spacing in three replications following standard cultural practices.

Horticultural traits performance

Plant phenotype, yield and its components traits were recorded in all lines for each trait for two years. Data were measured from ten randomly selected plants. The measurable traits under the observations were namely earliness (based on days to first picking from date after transplanting i.e. DAT), crop duration (based don days to last picking from DAT), average per fruit weight (g, average of 10 fruits weight, fruit length (cm, i.e. polar diameter), fruit diameter (cm, i.e. equatorial diameter), locule number, pericarp thickness (cm, measured by Vernier calliper), total soluble solid (TSS, °Brix, measured by hand refractometer), yield/plant (kg) and plant growth habit (determinate, indeterminate and semi-determinate). Besides, some qualitative traits were also recorded based on physical eye/ hand appearance such as fruit shape, fruit firmness, and special traits, if any associated with any line. Pooled data of two years of horticultural traits data were statistically analysed by pooled analysis using the XLSAT software. Test for homogeneity of variance was tested and Tukeys honestly significant difference (HSD) ($p < 0.05$) was performed using the XLSTAT tool.

Field screening for tomato yellow leaf curl virus (ToLCV) resistance

All lines were evaluated for ToLCV resistance under natural field conditions for two years using control varieties like Punjab Chhuhara (susceptible control) and Kashi Aman and Kashi Chayan (resistant). Viral infection was recorded based on % disease infection on all plants at 45 and 90 days after transplanting. Disease severity scores were calculated based on a six-point scale (0-5) method (Banerjee & Kalloo, 1987), where score 0 (0-5%, HR= highly resistant), 1 (5·1–12·0%, R= resistant), 2 (12·1–25%, MR= moderately resistant), 3 (25·1–50·0% (MS= moderately susceptible), 4 (50·1–75% (S= susceptible), and 5 (75·1–100%, HS= highly susceptible).

Molecular marker assays

Fifteen improved lines were tested for multiple disease resistance such as tomato leaf curl virus (ToLCV), late blight and root knot nematode (RKN) using gene based sequence characterized amplified region (SCAR) markers. Details of the markers and primer sequences are summarised in Table 2. Leaf tissues of 15 improved lines were used for DNA isolation using the CTAB method as described by Prasanna et al. (2015). The quality of the DNA was examined on a 1% agarose gel and quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). The polymerase chain reaction (PCR) was set up in a total volume of 25 µL containing 2 µL DNA (100 ng), 1× PCR buffer consisting of 2.5 mM/L $MgCl_2$ and 200 µM/L dNTP, 0.5 µM/L each primer, and 1 U Taq polymerase (Genei Laboratories Pvt Ltd, Bangalore, India). PCR cycle was run for denaturation at 94°C for 4 min, 36 cycles (denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min), and extension at 72 °C for 10 min in a thermal cycler (BIO-RAD, CA, USA). PCR products were resolved on 1.5 % agarose gels stained with ethidium bromide and electrophoresed at 85 V for 120 min and visualized under UV light using the Alpha Innotech Gel-Doc system (Alpha Innotech, USA).

Results and Discussion

Horticultural traits performance

All 52 tomato genotypes were evaluated in the winter main crop season under field conditions for two years for 15 different horticultural traits, total soluble solid (TSS), ToLCV resistance and special traits, if any associated with the genotype (Table 1). Significant statistical differences ($p < 0.05$) were observed amongst the genotypes for all quantitative traits. Early genotype based on days to first picking were VRT-67 (70.33 DAT) followed by VRT-30 or VRT-34 (70.67 DAT). Interestingly, based on days to last picking VRT-30 was early maturing type with shortest crop duration (115 DAT). On the contrary, H-88-78-2 had very late picking date (112 DAT) and also late maturing type based on last fruit picking (148 DAT) with much delayed partial fruit ripening, which is highly undesirable trait. In jointless category, KB-Jointless line had earliest picking (71.67 DAT) combined with shortest crop duration (117.33 DAT). In cherry tomato, VRCRT-9 (60.67 DAT) had earliest picking date, whereas VRCRT-15 was early maturing type with shortest crop duration (151 DAT). Similarly, beta-carotene lines KB-9 showed earliest picking date (61.33 DAT), whereas KB-33 was early maturing type with shortest crop duration (121.00 DAT). Our study is in agreement with findings by Singh et al. (2014) performed genetic analysis to identify good combiners for yield components combined with ToLCV resistance in tomato breeding. Thus, we identified promising lines with elite traits for tomato breeding in future.

Fruit traits showed significant variations in the genotypes for various yield component traits. In improved lines, largest average per fruit weight (g) was observed in H-88-78-2 (148.00 g) followed by VRT-06 (120.26 g), VRT-19 (112.37 g) and VRT-51 (105.51 g), while smallest average per fruit weight was observed in VRT-02 (18.43 g), which had dwarf plant type and therefore identified for pot culture and kitchen garden. The largest average per fruit weight (g) was recorded in jointless line EC-605037 (100.50 g), cherry tomato VRCRT-5 (9.59 g) and beta-carotene line KB-20 (97 g). Fruit length (polar diameter) showed significant variation in genotypes studies, such as promising were improved line VRT-06 (6.18 cm), jointless EC-605037 (6.73 cm), cherry tomato VRCRT-9 (3.19 cm) and beta carotene KB-33 (4.61 cm). Fruit diameter was highest in improved line H-88-78-2 (6.60 cm), jointless EC-538441-3 (5.38 cm), cherry tomato VRCRT-14 (2.08 cm), and beta carotene tomato KB-32 (5.27 cm). Locule number was found maximum in improved line H-88-78-2 (4.60) followed by VRT-19 (4.49), jointless EC-538441-3 (3.82) and beta carotene tomato KB-1 (4.82). Pericarp thickness was observed maximum in H-88-78-2 (0.68 cm) followed by VRT-06 (0.55 cm), jointless EC-605094 (0.71 cm), cherry tomato VRCYT-3 (0.26 cm) and beta carotene KB-20 (0.65 cm). Importantly, fruit yield is the most economically important trait. Maximum fruit yield per plant was observed in ToLCV-28 (2.12 g), VRT-19 (2.09 g), VRT-34/ VRT-50 (1.94 g), VRT-51 (1.78 g) jointless EC-605037 (2.53 g), cherry tomato VRCRT-5 (1.33 g) and KB-3-1 (1.26 g). TSS is an important trait for processing tomatoes. Maximum TSS was observed in improved line VRT-67 (5.36 °Brix), jointless EC-695037 (5.37 °Brix), cherry tomato VRCYT-3 (8.22 °Brix) and beta carotene tomato KB-6 (5.45 °Brix). Our findings were supported by Singh and co-workers (2015) who investigated tomato lines for ToLCV resistance and yield related traits by genetic and molecular characterization methods. Thus, our study identified promising lines for tomato breeding.

There were many lines having resistance to ToLCV with no or very minor infection such as improved line VRT-06, VRT-19, VRT-30, VRT-34, VRT-50, VRT-51, and VRT-67, and identified promising parental lines for breeding new varieties. Jointless lines EC-695037, EC-605037, EC-605094, KB-Jointless and New-Jointless, cherry tomato VRCYT-3, VRCYT-5, VRCYT-9 and VRCYT-15, and beta carotene KB-2, KB-3-1, KB-3-2, KB-5, KB-10, KB-14, KB-17 and KB-20. Regarding fruit shape, most improved lines were round, whereas VRT-19 and H-88-78-2 are flat-round and VRT-34 is oval shape. In jointless category, VRT-69 was pear shape, whereas other lines were either oval or round type. In cherry tomato, VRCRT-15 was pear shape, whereas VRCYT-3 and VRCYT-5 were oval, and VRCRT-9 was oblong and VRCRT-14 was round. In beta carotene group, KB-1, KB-5, KB-7, KB-9, KB-11 and KB-13 were flat-round, whereas other lines were either round or oval shape. Fruit firmness is one of the important parameters of tomato for

Table 1: Mean performance of tomato improved lines for horticultural traits over two years (2021-22 and 2022-23)

S. No.	Genotype	Days to first fruit picking	Days to last fruit picking	Average per fruit wt.(g)	Fruit length (cm)	Fruit dia. (cm)	Locule no.	Pericarp thickness (cm)	TSS (°Brix)	Yield/ plant (kg)	ToLCV (%) (class)	Fruit shape	Fruit firmness	Fruit colour	Growth habit	Special traits
a) Improved lines																
1	VRT-01	85.33	155.00	81.60	5.27	4.94	4.13	0.48	3.79	1.41	23.75 (MR)	Round	Medium	Red	SD	-
2	VRT-02	70.33	140.00	23.43	3.17	2.70	2.07	0.23	4.89	1.27	30.00 (MS)	Round	Loose	Red	D	Dwarf plant and suitable for pot culture
3	VRT-06	100.00	150.00	120.26	6.18	5.93	3.37	0.55	4.27	1.40	7.50 (R)	Oval	Medium	Red	SD	Large fruit
4	VRT-16-1	85.00	160.00	54.35	4.10	4.20	2.24	0.37	4.26	1.15	30 (MS)	Round	Loose	Red	D	-
5	VRT-19	86.00	150.00	112.37	4.90	5.80	4.49	0.48	4.12	2.09	0 (HR)	Flat-Round	Loose	Red	SD	Large fruit and green shoulder
6	VRT-30	70.67	115.00	52.57	4.09	4.19	3.03	0.38	4.26	0.95	2.50 (HR)	Round	Loose	Red	D	-
7	VRT-34	70.67	140.67	65.75	4.85	4.57	2.60	0.50	4.71	1.96	2.50 (HR)	Oval	Medium	Red	SD	Moisture-stress tolerance
8	VRT-50	100.00	160.00	84.84	4.81	5.29	3.60	0.52	4.50	1.94	2.5 (HR)	Round	Medium	Red	SD	-
9	VRT-51	95.00	155.33	105.51	5.14	5.41	3.54	0.51	4.11	1.78	5 (HR)	Round	Medium	Red	SD	-
10	VRT-67	70.33	150.67	29.95	3.57	3.42	2.17	0.35	5.36	1.23	10.50 (R)	Round	Loose	Red	SD	-
11	ToLCV-16	80.33	145.33	45.42	4.73	4.57	2.73	0.43	3.62	1.15	12.50 (R)	Round	Loose	Red	SD	-
12	ToLCV-28	71.33	130.33	68.67	4.69	5.09	4.17	0.44	4.04	2.12	7.50 (R)	Flat	Loose	Red	SD	Highly serrated leaf
13	ToLCV-32	81.33	150.00	75.82	5.75	4.66	2.20	0.54	4.22	1.06	2.50 (HR)	Oval	Medium	Red	SD	-
14	H-88-78-1	88.00	136.00	39.20	3.52	3.86	2.20	0.44	4.07	1.44	0 (HR)	Round	Medium	Red	SD	-
15	H-88-78-2	112.00	148.00	188.20	5.32	6.60	4.60	0.68	5.11	1.50	0 (HR)	Flat-Round	Loose	Green yellow	SD	Delayed partial fruit ripening
b) Jointless tomato																
16	VRT-69	85.67	137.33	80.07	5.37	4.52	2.62	0.61	4.52	2.26	35 (MS)	Pear shape	Tough	Red	SD	-
17	EC-695037	89.67	135.67	97.33	5.92	4.68	2.03	0.54	5.37	2.15	0 (HR)	Oval	Medium	Red	SD	Large fruit
18	EC-605037	77.33	132.23	100.50	6.73	4.93	2	0.7	5.26	2.53	0 (HR)	Oval	Tough	Red	SD	Large fruit
19	EC-538441-2	82.52	127.67	84.60	5.86	5.20	2.33	0.63	3.73	1.75	4.50 (HR)	Oval	Tough	Red	SD	
20	EC-538441-3	87.33	138.00	78.23	5.42	5.38	3.82	0.63	3.77	1.49	30.00 (MS)	Round	Medium	Red	SD	-
21	EC-538441-4	72.33	132.33	61.27	5.45	4.56	2.34	0.54	5.02	2.75	45.50 (MS)	Oval	Medium	Red	SD	-

22	EC-538439	73.33	129.67	66.63	6.31	4.50	2.34	0.61	4.10	2.04	30.50 (MS)	Oval	Medium	Red	SD	-
23	EC-605094	95.67	140.67	85.70	6.56	4.67	2.13	0.71	5.18	2.47	0 (HR)	Oval	Medium	Red	SD	-
24	KB-Jointless	71.67	117.33	45.33	4.18	4.11	2.90	0.57	4.05	1.34	8.50 (R)	Round	Medium	Yellow	SD	-
25	New-Jointless	75.33	130.67	59.37	4.84	4.10	2.03	0.57	4.70	2.56	0 (HR)	Round	Medium	Red	SD	-
c) Cherry tomato																
26	VRCYT-3	65.00	160.67	7.89	2.95	1.80	2.13	0.26	8.22	1.13	4.50 (HR)	Oval	Medium	Yellow	I	-
27	VRCRT-5	61.33	160.67	9.59	2.61	1.96	2.03	0.20	7.78	1.33	2.50 (HR)	Oval	Medium	Red	I	-
28	VRCRT-9	60.67	160.33	6.63	3.19	1.71	2.07	0.21	7.88	0.59	5 (HR)	Oblong	Medium	Red	I	-
29	VRCRT-14	65.00	165.67	7.03	2.25	2.08	2.00	0.21	5.41	0.82	9 (R)	Round	Loose	Red	I	-
30	VRCRT-15	65.00	151.00	9.22	3.01	2.05	2.40	0.25	7.81	1.25	2.50 (HR)	Pear shape	Tough	Red-yellow	D	-
d) Beta-carotene tomato																
31	KB-1	82.00	155.00	88.32	4.45	5.09	4.82	0.37	4.78	1.19	65.50 (S)	Flat-Round	Loose	Yellow-Orange	D	Large fruit
32	KB-2	75.67	150.67	34.51	3.81	3.43	2.13	0.44	4.70	1.26	2 (HR)	Round	Medium	Yellow	D	-
33	KB-3-1	85.67	160.00	47.49	4.13	3.57	2.26	0.47	4.61	1.97	2.00 (HR)	Oval	Medium	Yellow	SD	-
34	KB-3-2	85.67	161.33	48.27	4.60	3.71	2.03	0.35	5.09	1.67	0 (HR)	Oval	Medium	Yellow	SD	-
35	KB-4	70.67	152.33	34.20	3.30	3.77	3.45	0.43	4.97	1.24	10 (R)	Round	Medium	Yellow	SD	-
36	KB-5	67.33	124.33	36.00	3.49	3.89	3.21	0.27	4.28	0.84	0 (HR)	Flat-Round	Loose	Yellow	SD	-
37	KB-6	71.33	122.33	28.67	3.93	3.15	2.31	0.35	5.45	0.69	60 (S)	Oval	Loose	Yellow	SD	-
38	KB-7	66.33	130.67	35.07	3.17	3.72	4.10	0.34	4.24	0.70	75 (HS)	Flat-Round	Loose	Yellow	SD	-
39	KB-8	75.00	125.67	26.83	3.07	2.84	2.68	0.24	4.43	0.97	70.50 (HS)	Round	Loose	Yellow	SD	-
40	KB-9	61.33	126.00	37.07	3.31	4.44	4.68	0.34	4.77	1.34	75 (HS)	Flat-Round	Loose	Yellow	SD	-
41	KB-10	73.33	122.67	18.67	2.90	3.24	3.66	0.32	5.22	0.27	0 (HR)	Round	Loose	Yellow	SD	-
42	KB-11	69.67	149.00	26.66	3.27	3.57	3.38	0.36	4.52	1.14	20.50 (MR)	Flat-Round	Loose	Yellow	SD	-
43	KB-13	66.67	131.00	46.33	3.54	5.05	5.28	0.41	4.92	1.30	65.50 (S)	Flat-Round	Loose	Yellow	SD	-
44	KB-14	64.33	137.33	36.91	3.61	3.55	2.25	0.42	4.63	1.66	10 (R)	Round	Loose	Yellow	SD	-
45	KB-17	99.67	157.33	40.94	4.15	4.26	2.12	0.40	4.51	1.16	0 (HR)	Round	Loose	Orange	SD	-
46	KB-18	72.33	131.00	34.17	3.69	3.38	2.10	0.41	4.68	0.87	90 (HS)	Round	Loose	Yellow	SD	-
47	KB-19-1	92.33	151.00	71.95	4.38	4.90	4.40	0.46	4.67	1.08	25 (MR)	Round	Medium	Orange	SD	Large fruit and green shoulder
48	KB-19-2	92.67	152.33	73.79	4.39	4.40	4.46	0.53	4.87	1.98	13.50 (MR)	Round	Medium	Orange	SD	Large fruit
49	KB-20	76.33	135.67	97.00	4.59	4.72	4.10	0.65	3.57	2.26	2.00 (HR)	Round	Medium	Orange	SD	Large fruit
50	KB-32	82.67	153.33	82.77	4.56	5.27	4.59	0.45	4.90	0.95	70.50 (S)	Round	Medium	Yellow-Orange	D	Large fruit

51	KB-33	86.00	121.00	41.33	4.61	3.96	2.32	0.54	5.18	1.47	30.50 (MS)	Oval	Loose	Yellow	SD	-
52	KB-34	75.67	125.67	35.50	3.40	3.60	3.07	0.41	3.90	0.88	85.50 (HS)	Round	Loose	Yellow	SD	-
Mean		79.04	144.34	65.19	4.45	4.41	3.21	0.47	4.71	1.86	20.94	-	-	-		
C.D. ($p < 0.05$)		12.55	10.10	5.07	7.89	7.55	0.43	2.15	0.69	1.21	5.53	-	-	-		
C.V.		8.45	8.67	7.70	9.95	7.70	8.27	6.67	9.03	8.43	9.22	-	-	-		

C.D.: Critical difference, C.V.: Coefficient of variation

Table 2: Details of molecular makers used in the study

Trait	*Gene*	*Chr.*	*Marker name (type)*	*Sequence (5′ → 3′)*	*Band size (bp)*		*Reference*
					Resistant	*Susceptible*	
Tomato yellow leaf curl virus (ToLCV)	*Ty-2*	11	AW910upF2R3 (SCAR)	F: AGAAGGTTAACGCGCTAAATTA R: AAGCCAAGAAGTTTGAAAACAC	523	821	Shen et al., 2020
	Ty-3	6	Ty3-SCAR1 (SCAR)	F: GCTCAGCATCACCTGAGACA R: TGCAGGAACAGAATGATAGAAAA	519	269	Dong et al., 2016
Late blight	*Ph-3*	9	Ph3 (SCAR)	F: CTACTCGTGCAAGAAGGTAC R: TCCACATCACCTGCCAGTTG	176	154	Zhang et al., 2014
Root knot nematode	*Mi1-2*	6	Mi23 (SCAR)	F: TGGAAAAATGTTGAATTTCTTTTG R: GCATACTATATGGCTTGTTTACCC	380	430	Garcia et al., 2007

Note: Hetero (H) indicates presence of both Resistant (R) and Susceptible (S) bands

long duration storage and distance market transport. In improved lines, VRT-01, VRT-06, VRT-34, VRT-50, VRT-51, ToLCV-32 and H-88-78-1 were medium firmness, whereas others were loose skin. Jointless type VRT-69, EC-605037 and EC-538441-2 and cherry tomato VRCRT-15 had tough fruit firmness. Plant growth habit was observed determinate in improved lines (VRT-02, VRT-16-1 and VRT-30), cherry tomato (VRCRT-15), and beta carotene (KB-1, KB-2 and KB-32). Other genotypes were semi-determinate (SD) category expect indeterminate growth habit in cherry tomato (VRCYT-3, VRCRT-5, VRCRT-9, and VRCRT-14). Some unique traits were noticed in the lines such as VRT-02 was dwarf plant type, therefore suitable for pot culture and kitchen garden/ vertical farming, VRT-06 had large fruit size, VRT-19 had also large fruit size with green shoulder, VRT-34 was moisture stress tolerant genotype, ToLCV-28 had highly serrated leaf, and most importantly H-88-78-2 had delayed partial fruit ripening and did not fully ripe until harvest stage. Jointless tomato EC-695037 and EC-605037, and beta carotene KB-1, KB-19-1, KB-19-2, KB-20 and KB-32 were large fruited type, and KB-19-1 had also green shoulder. Horticultural traits have been studied for analyzing genetic and inheritance of ToLCV resistance in tomato breeding populations (Singh et al., 2014; 2015a; 2015b)

Field screening for tomato yellow leaf curl virus (ToLCV) resistance

All 52 lines were screened for ToLCV resistance under natural open field conditions for two years. Significant differences ($p < 0.05$) were observed among the lines. Virus infection was measured based on symptoms (%) on whole plant (leaves/ stems). The improved lines were categorized into highly resistant (0-5% infection) (VRT-19, VRT-30, VRT-34, VRT-50, VRT-51, ToLCV-32, H-88-78-1 & H-88-78-2), and resistant (5·1–12·0% infection) (VRT-06, VRT-67, ToLCV-16, ToLCV-28). Besides, highly resistant lines were jointless (EC-695037, EC-605037, EC-538441-2, EC-605094 & New-Jointless), cherry tomato (VRCYT-3, VRCRT-5, VRCRT-9 & VRCRT-15), and beta carotene lines (KB-2, KB-3-1, KB-3-2, KB-5, KB-10, KB-17 & KB-20).

Molecular marker assays for ToLCV, late blight and root knot nematode resistance genes

Only 15 promising improved lines were tested for host resistance genes using molecular markers for ToLCV (*Ty-2* and *Ty-3* genes, Figure 1), late blight (*Ph-3* gene) and root knot nematode resistance (*Mi1-2* gene) (Table 3). These lines have shown potential for gene pyramiding through marker-assisted breeding in future. The improved lines such as VRT-01, VRT-06, VRT-19, VRT-34, H-88-78-1, H-88-78-2 and ToLCV-28 showed the presence of *Ty-3* gene. These lines were also found phenotypic resistance (HR/R/MR) to ToLCV under field condition except VRT-02 and VRT-16-1 lines were moderate susceptible. None of the lines amplified *Ty-2* gene. Our findings are in consistent with several studies molecular markers based gene pyramiding of *Ty-2* and *Ty-3* genes for ToLCV resistance in tomato breeding (Prasanna et al., 2015a; 2015b). Furthermore, molecular markers were applied in screening of a collection of local and foreign varieties of tomato in Kazakhstan for genetic markers of resistance

Table 3: Molecular marker-based screening of elite tomato lines for resistance genes of tomato yellow leaf curl (ToLCV), root knot nematode (RKN) and late blight diseases

Sr. No.	Genotype	*ToLCV*			*RKN (Mi-1 gene)*	*Late blight (Ph-3 gene)*
		Ty-2 gene	*Ty-3 gene*	*Field test*		
1	VRT-01	-	+	MR	-	+
2	VRT-02	-	Hetero	MS	-	-
3	VRT-06	Hetero	+	R	-	-
4	VRT-16-1	-	Hetero	MS	-	+
5	VRT-19	-	+	HR	-	-
6	VRT-30	-	+	HR	-	-
7	VRT-34	-	+	HR	-	-
8	VRT-50	-	Hetero	HR	-	-
9	VRT-51	-	+	HR	Nd	-
10	VRT-67	-	Hetero	R	-	-
11	H-88-78-1	+	-	HR	-	-
12	H-88-78-2	-	+	HR	Nd	-
13	ToLCV-16	-	Hetero	R	-	-
14	ToLCV-28	-	+	R	-	-
15	ToLCV-32	-	Hetero	HR	-	-

Presence (+)/absence (-) of resistance genes are shown and Hetero indicates presence of both bands; For phenotypic ToLCV test: HR: Highly resistant, MR: Moderately resistant, MS: Moderately susceptible; NA: Not determined

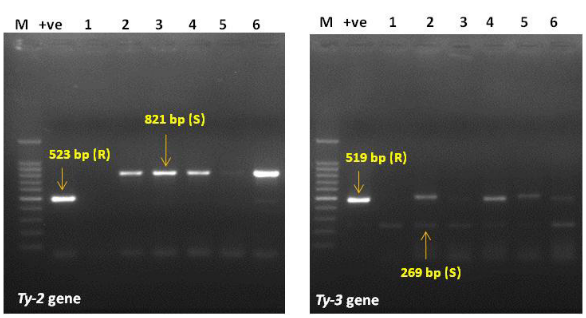

Figure 1: Screening of improved tomato lines for ToLCV resistance genes (*Ty-2* and *Ty-3*) on agarose gel (1.5%). Samples: 1) ToLCV-41, 2) ToLCV-32, 3) VRT-67, 4) VRT-50, 5) VRT-34, and 6) VRT-02; +ve control: Sankranti (*Ty-2*) and cv. Aman (*Ty-3*)

against three tomato viruses (Pozharskiy et al., 2022). Besides, all lines showed absence of RKN resistance *Mi-1* gene except VRT-51 and H-88-78-2 did not show any amplification. The presence of late blight resistance *Ph-3* gene was found in two improved lines (VRT-01 and VRT-16-1), whereas all other lines had susceptible band. Gene pyramiding in tomato with ToLCV, late blight and RKN resistance has developed elite lines with multiple disease-pest resistance (Kumar et al., 2019).

Conclusion

We identified promising lines with improved horticultural traits and ToLCV resistance based on field studies. Based on fruit yield, promising genotypes were improved lines (VRT-06, VRT-19, VRT-34 and VRT-51), jointless (EC-605037 and EC-695037), cherry tomato (VRCRT-5), and beta-carotene line (KB-20). Whereas, high TSS (>5 °Brix) was observed in improved line VRT-67, jointless EC-695037 and cherry tomato VRCYT-3. Most importantly, we identified ToLCV resistant improved lines (VRT-06, VRT-19, VRT-30, VRT-34, VRT-50, VRT-51, and VRT-67), jointless (EC-695037, EC-605037, EC-605094, KB-Jointless and New-Jointless), cherry tomato (VRCYT-3, VRCYT-5, VRCYT-9 and VRCYT-15), and beta carotene (KB-2, KB-3-1, KB-3-2, KB-5, KB-10, KB-14, KB-17 and KB-20). Notably, improved line VRT-02 was identified for dwarf plant architecture, which is suitable for pot culture, whereas H-88-78-2 had very delayed and partial fruit ripening only, which is a good line for introgression of non-ripening genes to extend shelf-life in tomato. We also identified large fruited improved lines (VRT-06 and VRT-19), jointless (EC-695037 and EC-605037), and beta carotene (KB-1, KB-19-1, KB-19-2, KB-20 and KB-32). Taken together, we identified promising lines for use as parents in breeding with diverse genetic background.

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सारांश

संकर टमाटर प्रजनन में उन्नत वंशावली का विकास प्रमुख गतिविधियों में से एक है। इस अध्ययन में बागवानी लक्षणों, कुल घुलनशील ठोस (टीएसएस) और रोग प्रतिरोधक क्षमता (टीओएलसीवी) के लिए बेहतर लाइनों की पहचान करना था। इनका मूल्यांकन खेत में दो वर्षों तक किया गया और टीओएलसीवी प्रतिरोध की भी जांच की गई। टीओएलसीवी (*Ty-2* और *Ty-3*), लेट ब्लाइट (Ph-3) और रूट नॉट नेमाटोड (*Mi1*) के प्रतिरोध जीन के लिए आणविक मार्कर का प्रयोग किया गया। फलों की उपज के आधार पर, आशाजनक जीनोटाइप में उन्नत लाइनें (वीआरटी-06, वीआरटी-19, वीआरटी-34 और वीआरटी-51), जॉइंट-लेस टमाटर (ईसी-605037 और ईसी-695037), चेरी टमाटर (वीआरसीआरटी-5), और बीटा-कैरोटीन युक्त लाइन (KB-20) का पहचान किया गया। उन्नत लाइन वीआरटी-67 (5.36 डिग्री ब्रिक्स), ज्वाइंटलेस लाइन ईसी-695037 (5.37 डिग्री ब्रिक्स) और चेरी टमाटर वीआरसीवाईटी-3 (8.22 डिग्री ब्रिक्स) में उच्च टीएसएस देखा गया। टमाटर की किस्म वीआरटी-02, जिसमें बौने पौधे हैं, पॉट कल्चर के लिए उपयुक्त पाई गई, जबकि लाइन एच-88-78-2 में बहुत देरी से और आंशिक रूप से फल पकने वाले लाइन्स थे। इस प्रकार, हमारे अध्ययन ने महत्वपूर्ण टमाटर के लाइनों की पहचान की जिनका उपयोग नए किस्मों के विकाश के लिए प्रजनन में किया जा सकता है।