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Original scientific paper

HORTICULTURAL EVALUATION OF ADVANCE BREEDING LINES POSSESSING DIFFERENT COMBINATIONS OF *Ty* AND *Ph* GENES IN TOMATO (*Solanum lycopersicum* L.)

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Agro-statistics, India is the second largest tomato producer only after China in the world. Tomato crop is grown round the year due to wider adaptability but production is adversely affected due to high incidence of tomato leaf curl virus and late blight diseases. These pathogens have enormous capacity to generate new forms and control of these pathogen are mainly achieved by chemical approaches which is not safe for environment as well as for human beings and also add an extra cost in tomato production. Therefore resistance breeding is best approach to manage these types of diseases along with improvement of yield and quality parameters. Five genes viz. *Ty-1*, *Ty-2*, *Ty-3*, *Ph-2* and *Ph-3* were tried to pyramid from different parent through hybridization to achieve resistance against tomato leaf curl virus and late blight diseases. In this study, 122 advance breeding lines of tomato were evaluated in 2016-17 and 2017-18, through phenotypic and genotypic screening to identify best lines having resistance to both the diseases with better horticultural traits. Punjab Chhuhara cultivar was used as common susceptible check to both the diseases while PVB-4 and LBR-10 were used as resistant

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checks for tomato leaf curl virus and late blight disease, respectively. Out of 122 lines evaluated, 12 lines were found to be having resistance against both the diseases with different combination of *Ty* and *Ph* genes. Among them, four lines viz. TW-4-5G-12, TW-5-1E-7, PR-DH-15-7-11 and PR-DH-28-11G-13 having resistant to both diseases and produces 3.14, 3.90, 2.74 and 3.84 yield kg plant^{-1} which is more than all the three standard checks. These resistant lines could be evaluated in multi-locations for their commercial exploitation.

Keywords: genotypic and phenotypic screening, late blight, tomato leaf curl virus

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a member of family Solanaceae is the most popular and profitable summer vegetable crop due to its versatile uses, nutritive value, unique flavour and processability. Tomato requires 25-30°C day and 15-20°C night temperature for optimum growth and fruit setting (DHALIWAL, 2012). The phylogenetic classification of the Solanaceae has been recently revised and the genus *Lycopersicon* reintegrated into the *Solanum* genus with its new nomenclature and cultivated tomato called as *Solanum lycopersicum* L. (PERALTA *et al.,* 2008). It is an important source of minerals, antioxidants, lycopene, vitamins (C and E), β-carotene, flavonoids and organic acids (DORAIS *et al.,* 2008). It has wide range of adaptability to different climatic conditions, more yield potentials and used as fresh for vegetable purpose as well as in processed form like sauce, soup, puree, chutney, ketchup, paste etc. (HE *et al.,* 2003; NWOSU *et al.,* 2014). Tomato can be grown round the year due to wider adaptability but production is adversely affected by different biotic and abiotic factors. Among biotic factors, tomato leaf curl virus and late blight diseases are major which can cause up to cent percent losses if both occurred at same time. Tomato leaf curl virus (ToLCV) is highly destructive disease during summer season crop in South India and autumn season in North India (SAIKIA and MUNIYAPPA, 1989; VIJETH *et al.,* 2018). SAIKIA and MUNIYAPPA (1989) reported that the incidence of this disease can cause 17-53% yield loss in July-November and up to 100% yield loss during February-May crop. Tomato leaf curl virus disease caused by bipartite and monopartite geminiviruses belongs to *Geminiviridae* family and *Begomovirus* genus (PRASANNA *et al.* 2015). North Indian isolates of ToLCV have been revealed to possess bipartite genome while isolates from Australia, Taiwan and South Indian isolates showed monopartite genome (PANDEY *et al.,* 2010). The white fly (*Bemisia tabaci*) acts as a vector for transmission of ToLCV, despite this white fly also damage the crop directly by sucking the cell sap. Management measures are completely based on control of the white fly population but control of vector is unlikely to be successful because of the rapid turn-over rate of the white fly population in tomato (HOLT *et al*., 1999). Under North Indian conditions, three species of *Begomoviruses* are dominant viz. "Tomato leaf curl New Delhi virus, Tomato leaf curl Palampur virus and Tomato leaf curl Karnataka virus" (TIWARI *et al.,* 2010; KANAKALA *et al.,* 2013). A best management is possible by development of resistant cultivars against ToLCV disease through resistance breeding approach (NATESHAN *et al*., 1996; PICO *et al*., 1996; LAPIDOT *et al*., 1997). The six genes viz. *Ty-1* to *Ty-6*, have been documented for resistance to leaf curl disease, which are present on different chromosomes in tomato (JI *et al.,* 2007; HUTTON *et al.,* 2012; HUTTON and SCOTT, 2014).

Late blight is a highly destructive disease of tomato caused by *Phytophthora infestans* (Mont.) de Bary, which causes significant yield losses in tomato worldwide (FRY and GOODWIN,

1997). GRUNWALD and FLIER (2005) reported that, Mexico is the centre of origin of *P. infestans*. It reproduces by both sexual and asexual means. Therefore, *P. infestans* populations found outside the Mexico were the outcome of asexual reproduction, which made them genetically identical and more stable (FRY *et al.*, 2008). Late blight is endemic in mid-altitude and highland regions of the subtropical and tropical areas during rainy season. High humidity with cool and moist climate is required for rapid development of pathogen. It causes yield losses up to 100% under favorable environment (DUBEY *et al.*, 2018). The symptoms of late blight are characterized by brownish black lesions on leaves and stem, appear water soaked lesions having chlorotic borders but expand soon and become necrotic. Infection on tomato fruit is identified by dark brown firm lesions which enlarge in later stage and destroy entire fruit. In humid and cool condition, the pathogen produces sporangia and sporangiophores on infected parts of plant (NELSON, 2008). The five genes have been identified for resistance to late blight and introgressed into cultivated tomato from wild species *S. pimpinellifolium* but only *Ph-2* and *Ph-3* genes are commercially used for advancement of resistant cultivars due to availability of molecular marker linked to *Ph-2* and *Ph-3* genes (NOWICKI *et al.*, 2012; ZHANG *et al.*, 2014). Several screening techniques have been established for the assessment of resistance to late blight and the study of parasitic fitness of *P. infestans* populations.

From long time, these diseases were mainly control by cultural practices, pesticides, biological approaches but they are not found satisfactory and also add an extra cost in tomato cultivation. In addition to this, use of chemicals also causes critical environmental pollution and human hazards due to residue in vegetable products. Resistant breeding is the appropriate and best approach to manage these types of diseases without affecting environment and living organisms. To increase productivity of tomato, it is essential to develop superior varieties having resistance to prevailing diseases at particular areas with better horticultural traits. The breeding population was developed through hybridization and pyramided five genes viz., *Ty-1, Ty-2* and *Ty-3* (ToLCV), and *Ph-2* and *Ph-3* (late blight) from different resistant lines. Disease resistance alone is insufficient to ensure farmer adoption; commercial cultivars also must have higher total yield with other important horticultural traits. Marker assisted selection (MAS) provides great opportunity for efficient selection of desirable traits; also save the time of breeder and more accurate than conventional breeding. Thus, the evaluation of developed resistance breeding lines for horticultural traits with disease resistance is essential. If they were confirmed the resistance and have acceptable horticultural traits, these lines could be release to farmers for cultivation.

MATERIALS AND METHODS

The present investigation was performed at Vegetable Research Farm, Biochemistry Laboratory and Molecular Breeding Laboratory in the Department of Vegetable Science, Punjab Agricultural University Ludhiana, India. The experimental field is situated at 30˚ 55' North latitude, 75˚54' East longitude and at an altitude of 247 m above mean sea level. The experimental material includes 122 advance breeding lines of F_5 population developed through hybridization followed by pedigree method in tomato. Punjab Chhuhara was used as a common susceptible check while PVB-4 and LBR-10 were used as resistant check for Tomato leaf curl virus and late blight, respectively. All 122 advance breeding lines were transplanted in field in the month of August, 2016 along with standard checks in Randomized Block Design (RBD) with two replications for evaluation of horticultural traits. In each replication, ten plants were transplanted in a row for each entry.

Genotypic description of all the breeding lines was mentioned in Table 1. The observations, fruit weight (g), fruit yield (kg plant^{-1}), number of locules fruit^{-1}, pericarp thickness (mm), P/E ratio, dry matter (%), TSS (°Brix), lycopene (mg.100ml^{-1}), titrable acidity (g.100ml^{-1}) were recorded for evaluation of horticultural traits. Dry matter, TSS, lycopene and Titrable acidity was estimated as suggested by Srivastava and Kumar (2006). Statistical analysis was performed using computer software 'Windostat Version 9.2' to estimate genetic parameters for all the horticultural traits.

Table 1. Genotypic description of advance breeding lines of tomato used for evaluation

Lines	**Parents involved in line development**
TH-PR-21-2-3, TH-PR-21-4G-6, TH-PR-21-5-3, TH-PR-21-6-4, TH-PR-21-10G-13, TH-PR-21-11-7, TH-PR-22-1-10, TH-PR-22-2-10, TH-PR-22-3-6, TH-PR-22-5-5, TH-PR-22-5G-1, TH-PR-22-6-13, TH-PR-22-6E-2, TH-PR-22-7-9, TH-PR-22-7E-9, TH-PR-22-12-1, TH-PR-22-14-7, TH-PR-23-10-10, TH-PR-23-11-8, TH-PR-23-13E -2, TH-PR-26-1-5, TH-PR-26-1E-1, TH-PR-26-2-17, TH-PR-26-3E-6, TH-PR-26-4-4, TH-PR-26-5-5, TH-PR-27-12-2, TH-PR-56-1E-13, TH-PR-56-2-1, TH-PR-56-4-2, TH-PR-56-4E-8, TH-PR-56-6-6, TH-PR-56-6E-8, TH-PR-56-9-5, TH-PR-56-9E-7, TH-PR-57-2E-1, TH-PR-57-4E-8, TH-PR-58-3-8, TH-PR-58-3G-10, TH-PR-58-4-3, TH-PR-58-5-8, TH-PR-58-7-12, TH-PR-58-7E-10, TH-PR-58-8-5, TH-PR-58-8E-6, TH-PR-58-9-2, TH-PR-58-9E-5, TH-PR-58-9G-8, TH-PR-58-11G-10, TH-PR-58-16-5	Punjab Ratta × CLN3022F$_2$-154-11-11-0 ↓ F_1 ⋮ F_5
TW-4-3-4, TW-4-3E-5, TW-4-5G-12, TW-4-6E-17, TW-4-7G-16, TW-5-1-17, TW-5-1E-7, TW-5-1E-18, TW-5-6-5, TW-6-7-16, TW-6-7E-2, TW-6-7G-5, TW-6-8-15, TW-8-3-5, TW-8-3G-5, TW-8-4G-12, TW-12-1-9, TW-12-1E-8, TW-12-4G-5, TW-12-5-2, TW-12-6-7, TW-12-8E-13, TW-12-10-1, TW-12-10E-2, TW-14-3E-6, TW-14-6G-2, TW-14-12G-3, TW-18-2-8, TW-18-2G-9, TW-18-3G-7, TW-18-4G-4, TW-18-5G-9, TW-18-5E-4, TW-20-1 -2, TW-20-1E-5, TW-20-2G-9, TW-20-3-6, TW-22-1-7	(8-2-1-2-5 × LBR-17) × CLN3022F$_2$-154-11-11-0 ↓ Three way F_1 ⋮ F_5
PR-DH-7-4E-15, PR-DH-14-3-5, PR-DH-14-4G-7, PR-DH-14-5E-11, PR-DH-15-2-14, PR-DH-15-3-10, PR-DH-15-3E-8, PR-DH-15-4E-8, PR-DH-15-7-11, PR-DH-15-7E-3, PR-DH-15-8G-1, PR-DH-15-16G-4, PR-DH-22-1-13, PR-DH-30-2G-10, PR-DH-30-6-1, PR-DH-28-1-7, PR-DH-28-1E-14, PR-DH-28-2-6, PR-DH-28-2G-6, PR-DH-28-3E-5, PR-DH-28-11E-17, PR-DH-28-11G-3,PR-DH-29-5G-9, PR-DH-29-11E-16, I-3-2-1-17, PR-DH-33-1E-10, PR-DH-33-4E-19, PR-DH-33-6-9, PR-DH-33-6G-4	(Punjab Ratta × CLN3024F$_2$-104-48-1-0) × (8-2-1-2-5 × LBR-11) ↓ Double hybrid F_1 ⋮ F_5
102-2-2E-1, 102-2-4E-10, 102-2-1-2, 102-2-6E-1, 102-3-12E-20	102-13-6-1 × PVB-1 ↓ F_1 ⋮ F_5

Phenotypic screening for disease resistance

The artificial screening for ToLCV was conducted from August to October during 2017 as described by SHANKARAPPA *et al.,* (2008). The susceptible check (Punjab Chhuahara) was used for maintenance of inoculums in an insect proof greenhouse. The vector (whitefly) was multiplied in disease free cotton (*Gossypium hirsutum* L.) plants within a separate greenhouse. The ten plants of each line were raised in plug trays filled with cocopeat, vermiculite and perlite in the ratio of 2:1:1 along with resistant (PVB-4) and susceptible (Punjab Chhuhara) checks inside a greenhouse. The plants at 4 leaf stage were inoculated with viruliferous whiteflies under greenhouse. The disease severity and disease incidence were recorded at 45 days after inoculation as suggested by ALEGBEJO (1997) in the scale of 0-7 (0, 1, 3, 5 and 7). The disease incidence (DI) and disease severity or per cent disease index (PDI), the coefficient of infection (CI) and decides the level of resistant reaction against ToLCV disease accordingly as mentioned by KUMAR *et al.,* 2019.

Artificial screening for late blight was conducted through the "detached leaf assay" during the month of February, 2018. In this method, detached young leaves of all the 122 lines including resistant (LBR-10) and susceptible (Punjab Chhuhara) checks were thoroughly washed with tap-water, air-dry and placed in plastic trays lined with moist blotting paper, with the adaxial surface facing upwards to make easy penetration of pathogen. The leaves were sprayed with sporangial suspension of 4.5×10^4 sporangia/ml concentrations using an atomizer (GILL *et al.,* 1999). The inoculated trays were covered with polythene bag and water was sprayed inside the bag to maintain the high relative humidity (RH) of 80-100%. These trays were incubated in growth room at a temperature of 18±2°C. After 7 days of inoculation, disease severity index (DSI) were recorded on the basis of visual appearance of percentage of leaf area affected of individual leaf in the scale of 0-6 as described by CHEN *et al*., (2008) and fixed the reaction against late blight accordingly.

Genotypic screening for disease resistance genes

The genomic DNA (deoxyribonucleic acid) of all the lines was isolated by the protocol suggested by DOYLE and DOYLE (1987). The nucleic acid/DNA quantification was done by using "Thermo Scientific NanoDrop™ 1000 Spectrophotometer". The 10µl PCR reactions were performed using PCR components {DNA (200ng/µl) 1.0µl + Double distilled autoclaved water 4.3µl + EmeraldAmp GT PCR Master Mix (2X Premix) 3.5µl + Farward and reverse primers (20pico mole) 0.6µl + 0.6µl}. The DNA from advance breeding lines were subjected to marker analysis using *Ty-1, Ty-2, Ty-3, Ph-2* and *Ph-3* gene specific marker (Table 2) to confirm the presence or absence of the resistance genes. The PCR amplified product of all the CAPS (Cleaved Amplified Polymorphic Sequence) markers were digested by adding of 2µl restriction enzyme mixture per tube (0.2µl enzyme, 0.3µl buffer and 1.5µl dH_2O). The fragments were separated in 2% agarose gel in 1X TAE (Tris-acetate-EDTA) buffer stained with ethidium bromide 5.0µl/100ml with 50bp ladder. The photograph was captured in ultra violet (UV) light through Alphamager® HP system.

Table 2. Targeted resistance genes and linked molecular markers used in marker assisted selection

Marker	Type of marker	Targeted gene	Restriction enzyme	Expected product size (S,R) ~bp	Forward primer sequence 5`–3`	Reverse primer sequence 5`–3`	Reference
JB-1	CAPS	*Ty-1*	TaqI	400, **500**	AACCATTATCC GGTTCACTC	TTTCCATTCCT TGTTTCTCTG	De Castro *et al.* (2007)
TG105	CAPS	*Ty-2*	TaqI	200, **350**	CTTCAGAATTC TGTTTTAGT	ATGTCACATTT GTTGCTTGGAC CATCC	Garcia *et al.* (2007)
FLUW-25	SCAR	*Ty-3*	-	450, **600**	CAAGTGTGCAT ATACTTCATA (T/G)TCACC	CCATAATATAA CCTCTGTTTCT ATTTCGAC	Ji *et al.* (2007)
dTG422	CAPS	*Ph-2*	HinfI	245, **275**	Dr. Martha Mutschler personal communication, College of Agriculture and Life Sciences, Cornell University, USA		

CAPS= Cleaved Amplified Polymorphic Sequence, SCAR= Sequence Characterized Amplified Region

RESULTS AND DISCUSSION

Mean values for all the horticultural traits studied (Table 3) in tomato observed great variation among the lines. Fruit weight is one of the important traits that were directly linked with total yield, for this high mean value is desirable. The mean of breeding lines for fruit weight ranged from 27.17 g in TW-14-12G-3 to 186.11 g in PR-DH-22-1-13, with the overall mean of 75.09 g. The lines PR-DH-22-1-13, TH-PR-22-2-10 and TW-4-3-4 were performed better for fruit weight. The total fruit yield is a basic objective in any crop breeding programme which deserves highest consideration. The fruit yield varied from 0.48 in TW-4-3-4 to 4.30 kg in TH-PR-58-5-8, with a mean of 2.15 kg plant^{-1}. The lines TH-PR-58-5-8, TH-PR-26-2-17 and TH-PR-22-2-10 recorded highest fruit yield. The number of locules directly correlated with fruit firmness like more number of locules indicated less fruit firmness and vice-versa. Number of locules ranged from 2.08 (TW-12-10E-2) to 6.09 (102-2-6E-1), with overall mean of 3.14 fruit^{-1}. The line 102-2-6E-1 showed more number of locules fruit^{-1} followed by TH-PR-22-2-10 and PR-DH-22-1-13. Pericarp thickness is the desirable for long distant transportation and also improves the shelf life. The pericarp thickness ranged from 2.55 (TH-PR-56-4E-8) to 6.49 mm (TW-4-6E-17) with overall mean of 4.68 mm and the lines TW-4-6E-17, TH-PR-57-2E-1 and PR-DH-14-5E-11 had more pericarp thickness. The ratio of polar and equatorial diameter is important trait to indicate the fruit shape index, whether it is round or long. The P/E ratio ranged from 0.87 (TW-12-1-9) to 1.36 (TH-PR-58-11G-10), with grand mean of 1.04 and the lines TH-PR-58-11G-10, TW-12-10-1 and TW-12-4G-5 showed more P/E ratio. Per cent of dry matter is directly associated with production of processed dry product, high dry matter content produce more processed dry product. Dry matter content ranged from 3.45% (TW-8-3G-5) to 6.41% (TH-PR-56-1E-13), with overall mean of 4.71%. The lines TH-PR-56-1E-13, PR-DH-15-8G-1 and TH-PR-23-13E-2 were noticed for high dry matter content. Tomato flavour is directly associated with the relative concentrations of sugars and acids in the fruit, mainly fructose and citric acid. BERRY and UDDIN (1991) reported that, an increase of one percent TSS increase 20 percent in processed dry product. TSS ranged from 2.62 (PR-DH-22-1-13) to 6.27°Brix (TW-14-12G-3), with overall mean of 4.69°Brix. The lines TW-14-12G-3, TH-PR-23-13E-2 and TH-PR-26-4-4

showed high total soluble solids. The lycopene is a major carotenoid pigment and responsible for red color in tomato. The lycopene content ranged from 1.93 (PR-DH-28-1-7) to 8.23 (TH-PR-56-4E-8) mg.100g^{-1} of fresh weight, with grand mean of 4.19 mg. The lines, TH-PR-56-4E-8, PR-DH-15-3E-8 and TH-PR-56-1E-13 were promising for high lycopene content. Titrable acidity below 0.5% is recommended for processing tomatoes. The titrable acidity ranged from 0.23 (TW-18-5G-9) to 0.77 (TH-PR-58-9-2) g.100 ml^{-1} of juice, with grand mean of 0.49 g. The lines TW-18-5G-9 exhibited minimum titrable acidity followed by PR-DH-28-2G-6 and 102-2-4E-10. There was significant variation observed among lines for all the studied traits indicating high degree of genetic variability in materials. Similar finding were also noticed by SINGH and CHEEMA (2005); GOLANI *et al.* (2007); DAR and SHARMA (2011); KAUSHIK *et al.* (2011); REDDY *et al.* (2013), and OLAKOJO and ADETULA (2014) in tomato genotypes for these studied traits.

Table 3.Mean performance of breeding lines for different horticultural traits

Lines	Fruit weight (g)	Fruit yield (kg plant^{-1})	No. of locules fruit^{-1}	Pericarp thickness (mm)	P/E ratio	Dry matter (%)	TSS (°Brix)	Lycopene content (mg.100 g^{-1})	Titrable acidity (g.100ml^{-1})
TH-PR-21-2-3	71.63	2.80	3.38	3.99	0.99	5.30	5.68	3.53	0.61
TH-PR-21-4G-6	67.07	2.75	2.68	4.14	0.96	4.79	5.10	3.60	0.50
TH-PR-21-5-3	70.38	2.61	2.41	4.03	1.08	5.50	5.04	4.12	0.39
TH-PR-21-6-4	72.90	2.78	2.95	5.63	0.99	4.84	5.05	2.82	0.28
TH-PR-21-10G-13	68.75	1.69	2.97	4.74	1.01	4.30	5.32	3.91	0.61
TH-PR-21-11-7	77.75	1.97	3.63	4.78	0.92	4.70	5.15	3.73	0.50
TH-PR-22-1-10	81.30	2.55	2.90	4.45	1.03	3.85	4.92	4.19	0.74
TH-PR-22-2-10	137.36	4.02	5.36	4.65	0.93	4.30	3.82	3.72	0.49
TH-PR-22-3-6	69.90	1.14	3.55	3.57	1.13	5.10	4.56	3.60	0.30
TH-PR-22-5-5	56.38	2.76	3.11	3.91	1.07	4.80	4.91	3.95	0.50
TH-PR-22-5G-1	66.26	1.69	2.52	5.31	1.04	4.20	5.09	4.54	0.53
TH-PR-22-6-13	84.77	2.58	3.66	5.24	0.95	3.80	4.26	3.05	0.37
TH-PR-22-6E-2	76.35	1.71	2.40	4.76	0.99	4.10	4.13	3.83	0.46
TH-PR-22-7-9	96.00	1.69	3.57	5.32	1.02	4.30	4.64	4.32	0.74
TH-PR-22-7E-9	76.63	2.02	2.25	5.76	1.01	4.05	4.32	4.32	0.43
TH-PR-22-12-1	86.35	2.70	2.33	5.53	1.04	4.10	4.59	3.14	0.39
TH-PR-22-14-7	69.80	2.99	3.10	4.68	0.96	4.70	4.52	4.35	0.40
TH-PR-23-10-10	81.14	1.80	3.48	4.85	1.03	5.25	5.08	4.44	0.65
TH-PR-23-11-8	77.87	2.90	2.46	4.68	1.10	4.40	4.40	4.31	0.59
TH-PR-23-13E-2	92.92	1.90	3.84	5.29	0.94	5.90	6.07	3.91	0.59
TH-PR-26-1-5	61.67	2.99	3.54	4.72	1.08	4.56	4.52	4.85	0.43
TH-PR-26-1E-1	59.30	3.80	3.30	4.68	0.97	5.05	4.56	6.20	0.46
TH-PR-26-2-17	95.20	4.15	3.78	4.19	1.04	4.65	3.98	3.80	0.63
TH-PR-26-3E-6	97.25	3.75	3.00	5.18	0.92	5.10	4.40	3.11	0.65
TH-PR-26-4-4	97.01	1.02	4.39	5.11	0.92	5.50	6.07	3.38	0.65
TH-PR-26-5-5	92.60	2.50	3.63	3.63	1.02	4.25	4.22	3.23	0.59
TH-PR-27-12-2	129.25	2.70	4.45	4.66	0.92	3.70	4.26	3.20	0.39
TH-PR-56-1E-13	72.96	2.34	2.80	3.69	1.05	6.41	5.99	7.27	0.35
TH-PR-56-2-1	64.17	1.99	3.11	4.74	0.96	4.03	4.26	6.85	0.39
TH-PR-56-4-2	46.80	0.86	2.47	3.49	0.94	5.10	4.76	3.95	0.56
TH-PR-56-4E-8	30.70	3.22	2.20	2.55	1.01	5.88	6.06	8.23	0.31
TH-PR-56-6-6	67.60	3.49	2.68	4.58	0.95	4.70	5.00	3.97	0.52
TH-PR-56-6E-8	50.25	1.39	3.10	4.63	1.02	4.63	5.30	3.13	0.50
TH-PR-56-9-5	63.25	3.26	2.34	4.95	0.92	4.79	4.43	5.20	0.37
TH-PR-56-9E-7	82.67	1.05	2.88	5.07	1.09	5.20	5.43	3.60	0.43

Lines	Fruit weight (g)	Fruit yield (kg plant^{-1})	No. of locules fruit^{-1}	Pericarp thickness (mm)	P/E ratio	Dry matter (%)	TSS (°Brix)	Lycopene content (mg.100 g^{-1})	Titrable acidity (g.100ml^{-1})
TH-PR-57-2E-1	89.82	1.51	3.10	6.37	1.00	5.03	5.60	3.91	0.49
TH-PR-57-4E-8	48.17	2.03	3.09	4.59	1.01	4.70	5.01	3.31	0.56
TH-PR-58-3-8	85.00	1.83	2.12	4.23	1.13	4.90	4.70	3.89	0.52
TH-PR-58-3G-10	95.88	2.61	2.10	5.76	1.16	4.70	5.53	3.04	0.66
TH-PR-58-4-3	60.70	2.37	2.30	4.20	0.92	4.41	5.28	5.89	0.33
TH-PR-58-5-8	64.03	4.30	2.60	4.07	1.03	4.65	4.87	6.51	0.44
TH-PR-58-7-12	44.47	3.30	2.70	3.71	1.11	4.85	4.24	3.65	0.60
TH-PR-58-7E-10	129.83	2.88	2.50	5.05	1.15	3.70	4.93	4.48	0.39
TH-PR-58-8-5	54.35	2.25	2.40	4.63	1.06	4.68	4.66	3.94	0.40
TH-PR-58-8E-6	51.70	1.75	3.05	4.46	1.02	4.51	4.20	4.48	0.33
TH-PR-58-9-2	71.67	2.35	2.46	4.53	1.13	4.14	4.12	3.53	0.77
TH-PR-58-9E-5	76.33	1.70	2.38	5.57	1.06	4.83	4.77	5.42	0.46
TH-PR-58-9G-8	76.66	2.55	2.68	5.79	1.05	5.22	4.13	2.98	0.51
TH-PR-58-11G-10	96.83	3.77	2.63	5.28	1.36	4.50	4.65	3.49	0.30
TH-PR-58-16-5	51.79	3.16	2.85	5.29	1.02	4.30	4.63	3.34	0.56
TW-4-3-4	134.81	0.48	3.52	4.80	1.02	5.55	4.14	4.14	0.45
TW-4-3E-5	66.60	1.53	4.30	5.06	1.00	3.90	4.34	3.64	0.56
TW-4-5G-12	92.44	3.14	3.07	4.40	1.19	4.20	4.89	3.53	0.33
TW-4-6E-17	82.96	1.24	3.26	6.49	1.09	4.62	3.99	3.32	0.33
TW-4-7G-16	77.14	1.28	2.88	5.47	1.04	4.44	4.12	4.20	0.43
TW-5-1-17	73.76	1.02	2.81	5.64	1.26	4.93	3.64	4.70	0.41
TW-5-1E-7	46.00	3.90	3.29	4.73	1.01	5.52	4.17	4.68	0.33
TW-5-1E-18	70.60	1.99	3.32	5.11	1.11	5.27	5.89	4.65	0.46
TW-5-6-5	51.60	1.87	2.28	5.03	1.06	4.38	4.24	4.37	0.72
TW-6-7-16	44.61	1.78	3.07	4.72	0.97	5.03	4.07	3.76	0.68
TW-6-7E-2	121.00	2.32	2.20	5.51	1.26	5.48	4.63	3.30	0.54
TW-6-7G-5	77.46	3.85	2.91	5.63	0.90	4.10	5.45	4.56	0.56
TW-6-8-15	45.88	3.46	3.64	4.59	1.02	4.25	3.91	3.43	0.73
TW-8-3-5	126.49	1.19	4.36	4.49	1.25	4.40	5.05	3.43	0.39
TW-8-3G-5	95.14	3.83	2.88	5.55	0.97	3.45	4.77	3.72	0.33
TW-8-4G-12	101.10	1.56	5.07	3.98	0.94	5.44	5.68	3.51	0.50
TW-12-1-9	92.46	2.35	4.60	4.55	0.87	4.11	3.88	4.72	0.63
TW-12-1E-8	97.47	2.56	3.50	5.61	1.00	4.15	4.68	4.69	0.43
TW-12-4G-5	51.17	1.52	2.91	4.86	1.31	4.13	4.45	5.41	0.33
TW-12-5-2	71.05	1.73	2.55	4.39	1.02	3.85	4.61	6.59	0.70
TW-12-6-7	74.80	1.26	3.90	5.17	1.00	4.08	4.12	3.42	0.37
TW-12-8E-13	58.84	0.89	4.07	4.17	0.96	4.49	5.52	5.05	0.61
TW-12-10-1	71.13	2.00	3.42	3.82	1.35	4.90	4.74	3.79	0.70
TW-12-10E-2	59.76	1.91	2.08	3.86	1.27	5.24	4.68	3.95	0.46
TW-14-3E-6	57.53	1.88	2.20	4.52	1.06	4.30	4.49	4.05	0.39
TW-14-6G-2	71.51	2.30	2.43	4.44	1.00	4.10	5.47	3.22	0.63
TW-14-12G-3	27.17	0.71	2.27	3.88	1.16	4.05	6.27	4.10	0.63
TW-18-2-8	52.74	1.79	3.92	4.40	1.00	4.27	4.71	3.17	0.50
TW-18-2G-9	64.80	2.69	3.50	4.78	1.05	5.00	4.50	3.76	0.67
TW-18-3G-7	57.95	2.89	2.45	5.32	1.00	5.30	5.98	4.30	0.63
TW-18-4G-4	81.95	3.01	4.56	5.74	0.90	4.31	5.16	3.96	0.59
TW-18-5G-9	59.70	2.22	4.16	3.97	0.87	5.50	4.76	5.65	0.23
TW-18-5E-4	64.29	1.81	2.46	4.38	1.03	4.70	4.47	4.79	0.49
TW-20-1 -2	60.66	0.63	3.43	5.11	0.98	4.49	4.98	5.20	0.68
TW-20-1E-5	67.67	1.70	3.50	4.99	0.95	5.10	5.06	3.95	0.58
TW-20-2G-9	82.36	2.36	3.38	5.43	1.10	3.80	5.34	4.58	0.43
TW-20-3-6	66.38	2.53	2.90	4.81	0.96	4.00	4.15	3.35	0.46
TW-22-1-7	86.82	1.31	3.41	4.81	1.20	5.50	5.05	3.38	0.43
PR-DH-7-4E-15	59.40	1.49	2.67	4.28	1.16	4.50	4.76	3.76	0.59

Lines	Fruit weight (g)	Fruit yield (kg plant^{-1})	No. of locules fruit^{-1}	Pericarp thickness (mm)	P/E ratio	Dry matter (%)	TSS (°Brix)	Lycopene content (mg.100 g^{-1})	Titrable acidity (g.100ml^{-1})
PR-DH-14-3-5	103.57	0.83	2.40	5.01	1.04	4.15	4.14	5.96	0.46
PR-DH-14-4G-7	77.50	0.88	2.50	4.28	1.27	4.40	4.86	5.34	0.37
PR-DH-14-5E-11	67.97	1.82	3.09	6.11	1.15	4.64	4.15	5.22	0.39
PR-DH-15-2-14	61.19	1.39	2.77	4.88	1.03	5.42	5.14	4.53	0.46
PR-DH-15-3-10	75.57	1.41	3.25	4.25	0.99	4.93	5.23	3.57	0.52
PR-DH-15-3E-8	75.24	0.73	3.09	4.45	0.97	5.43	4.52	7.72	0.62
PR-DH-15-4E-8	69.70	3.22	2.60	4.39	0.94	5.22	4.70	3.78	0.47
PR-DH-15-7-11	52.92	2.74	3.73	3.31	0.89	5.71	4.11	6.13	0.49
PR-DH-15-7E-3	53.47	1.82	2.90	4.17	0.91	5.50	4.92	3.24	0.61
PR-DH-15-8G-1	76.79	2.62	3.59	4.51	0.93	6.12	5.53	5.65	0.74
PR-DH-15-16G-4	62.80	1.84	2.20	4.43	1.17	4.50	5.43	3.51	0.52
PR-DH-22-1-13	186.11	1.73	5.09	5.23	1.00	4.31	2.63	2.24	0.45
PR-DH-28-1-7	100.80	1.12	3.74	4.70	1.15	3.79	4.69	1.93	0.72
PR-DH-28-1E-14	64.83	1.11	3.08	5.18	1.05	3.70	4.32	3.24	0.37
PR-DH-28-2-6	71.93	2.06	3.54	3.82	1.21	3.84	3.34	2.99	0.34
PR-DH-28-2G-6	83.30	1.10	2.45	4.53	1.26	5.10	4.99	2.86	0.26
PR-DH-28-3E-5	82.75	2.32	3.46	5.28	1.17	4.94	4.52	5.46	0.63
PR-DH-28-11E-17	50.00	1.29	3.88	3.89	1.04	4.95	3.97	4.13	0.72
PR-DH-28-11G-3	93.38	3.84	3.90	4.11	1.08	4.10	4.78	3.21	0.39
PR-DH-29-5G-9	88.21	1.53	3.26	5.39	1.08	5.82	3.91	3.47	0.45
PR-DH-29-11E-16	84.21	1.06	4.68	3.14	1.04	4.13	3.18	4.57	0.47
PR-DH-30-2G-10	39.92	3.10	2.37	2.78	1.11	4.80	4.14	3.19	0.37
PR-DH-30-6-1	96.57	3.19	2.50	5.03	1.00	4.50	5.03	3.63	0.56
PR-DH-33-1E-10	74.35	2.34	2.85	5.44	0.94	4.93	4.13	4.81	0.37
PR-DH-33-4E-19	71.50	1.93	3.09	3.74	0.95	5.34	4.35	6.22	0.68
PR-DH-33-6-9	59.10	3.47	3.00	4.77	1.06	4.95	4.30	6.89	0.33
PR-DH-33-6G-4	81.35	2.97	2.20	5.11	1.18	5.28	4.51	4.45	0.44
102-2-2E-1	85.57	2.22	3.34	4.35	0.97	5.27	4.41	2.80	0.36
102-2-4E-10	50.63	1.13	3.16	4.87	0.95	4.53	5.11	3.56	0.28
102-2-1-2	82.96	1.37	3.58	4.53	0.91	4.83	3.99	3.81	0.44
102-2-6E-1	95.71	1.18	6.09	4.16	0.89	5.37	4.31	4.99	0.37
I-3-2-1-17	37.11	1.13	4.08	3.45	0.90	5.01	4.46	4.07	0.45
102-3-12E-20	81.07	1.85	2.77	4.28	0.95	4.36	4.30	3.68	0.48
Punjab Chhuhara (Check)	78.82	2.18	3.97	6.15	1.71	3.72	4.71	2.75	0.79
PVB-4 (Check)	85.0	2.45	3.40	4.95	1.01	4.63	4.11	3.48	0.49
LBR-10 (Check)	112.42	1.78	3.01	5.87	0.95	3.74	4.51	1.02	0.87
Range	**27.17-186.11**	**0.48-4.30**	**2.08-6.09**	**2.55-6.49**	**0.87-1.36**	**3.45-6.41**	**2.62-6.27**	**1.93-8.23**	**0.23-0.77**
Mean	**75.09**	**2.15**	**3.14**	**4.68**	**1.04**	**4.71**	**4.69**	**4.19**	**0.49**
CD at 5%	**12.33**	**0.28**	**0.62**	**0.51**	**0.25**	**0.34**	**0.44**	**0.62**	**0.06**

Estimation of genetic parameters

The range of mean values based on the phenotypic expression denotes a rough estimate of variation among lines. In any crop-improvement programme the success of selection as a breeding method determined by the magnitude of genetic variability for yield and yield components. The extent of variability present in the lines (Table 4) was measured in terms of genotypic variance, phenotypic variance, genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), heritability in broad sense (h^2) and genetic advance as percent of mean (5%) of the population.

Table 4. Genetic parameters of advance breeding lines for different horticultural traits

Traits	GV	PV	GCV (%)	PCV (%)	h^2 (%)	GA (5 %)
Average fruit weight(g)	492.29	531.12	29.53	30.67	93.00	58.57
Total fruit yield (kg plant^{-1})	0.81	0.83	41.64	42.15	98.00	84.72
Number of locules fruit^{-1}	0.50	0.60	22.60	24.69	84.00	42.60
Pericarp thickness (mm)	0.44	0.51	14.17	15.21	87.00	27.20
P/E ratio	0.00	0.02	5.63	13.40	18.00	4.87
Dry matter (%)	0.36	0.39	12.69	13.20	92.00	25.14
Total soluble solids (°Brix)	0.35	0.40	12.67	13.52	88.00	24.47
Lycopene (mg.100ml^{-1})	1.14	1.24	25.41	26.48	92.00	50.24
Titrable acidity (g.100ml^{-1})	0.02	0.02	24.97	25.80	94.00	49.79

GV= Genotypic variance, PV= Phenotypic variance, GCV= Genotypic coefficient of variance, PCV= Phenotypic coefficient of variance, h^2= Heritability, GA= Genetic advance

The highest genotypic and phenotypic variance, respectively were recorded for fruit weight (492.29 and 531.12) followed by lycopene content (1.14 and 1.24) and fruit yield (0.81 and 0.83) whereas lowest for P/E ratio (0.00 and 0.02). High genotypic variance indicates more contribution of genetic component for the expression of total variation whereas high phenotypic variance indicating the strong influence of environmental factors for their expression. The genotypic coefficient of variation (GCV) ranged from 5.63% for P/E ratio to 41.64% for total fruit yield and phenotypic coefficient of variation (PCV) from 13.20 for P/E ratio to 42.15% for total fruit yield. The highest GCV and PCV were observed for fruit yield (41.64 and 42.15) followed by fruit weight (29.53 and 30.67) and lycopene content (25.4 and 26.48) while moderate GCV and PCV was observed for pericarp thickness (14.17 and 15.21), TSS (12.67 and 13.52) and dry matter (12.69 and 13.20), whereas the low GCV and PCV was observed for P/E ratio (5.63 and 13.40). The estimates of PCV were higher than GCV for all the studied traits which is an indicator of additive effect of the environmental factors on the expression of the traits. Similarly high PCV than GCV were also reported by MOHANTY (2003) and SINGH *et al.* (2015). Less difference between PCV and GCV indicated that, the low impact of environment on the expression of characters and hence, they could be improved by following different phenotypic selections like directional, disruptive and stabilized selections. The heritability measures the proportion to which the variability of a character is transmitted to offspring. BURTON and DE VANE (1953) suggested that genetic coefficients of variability, along with heritability estimates, would provide a reliable indication of expected degree of improvement through selection. The estimates of heritability varied from 18.00 to 98.00%. Among all the traits studied, fruit yield (98.00), titrable acidity (94.00), fruit weight (93.00), lycopene content (92.00), dry matter (92.00), TSS (88.00), pericarp thickness (87.00) and number of locules (84.00) were exhibited high heritability indicating that, these traits were controlled by additive gene action while P/E ratio (18.00) showed less heritability indicating that, it was controlled by non-additive gene action. Similar findings were also noticed by GOLANI *et al.* (2007); SINGH *et al.* (2015); RAI *et al.* (2016) for these traits. Highest genetic advance was observed for fruit yield (84.72%) followed by fruit weight (58.57%) and lycopene content (50.24%) while low genetic advance was observed for P/E ratio (4.87%). Similar findings were also reported by SINGH *et al.* (2001); SHASHIKANTH *et al.* (2010); CHERNET *et al.* (2013). The heritability along with genetic advance is more meaningful and helps in predicating the resultant

effect of selection on phenotypic expression. Low genetic advance coupled with less heritability was noticed for P/E ratio indicating that, this trait was highly affected by environmental factors and genetic improvement were difficult through selection (JINDAL and KHAN, 2015). Similar results for these traits were also noticed by RAI *et al.* (2016); PRAJAPATI *et al.* (2015).

Screening for tomato leaf curl virus disease

After 45 days of inoculation, mild to severe infection was noticed in among different lines. On the basis of coefficient of infection, among 122 lines screened (Table 4.10), 33 lines were found to be highly resistant and 11 were resistant while resistant check (PVB-4) showed no incidence of ToLCV but susceptible check (Punjab Chhuhara) showed 100% disease incidence. The lines TH-PR-22-6-13, TH-PR-23-10-10, TH-PR-56-4-2, TH-PR-56-4E-8, TH-PR-58-8E-6, TW-20-2G-9 and PR-DH-15-7-11 showed no incidence of ToLCV in artificial phenotypic screening.

Table 5.Phenotypic and genotypic screening of advance breeding lines of tomato against leaf curl virus and late blight

Lines	Tomato leaf curl virus						Late blight			
	Phenotypic screening			Genotypic screening			Phenotypic screening		Genotypic screening	
	PDI (%)	CI	Reaction	*Ty-1*	*Ty-2*	*Ty-3*	DSI	Reaction	*Ph-2*	*Ph-3*
TH-PR-21-2-3	20.45±1.88	5.11	R	+/–	–/–	+/+	5.50±0.50	HS	–/–	–/–
TH-PR-21-4G-6	47.31±7.06	23.65	MS	NA	NA	–/–	6.00±0.00	HS	–/–	–/–
TH-PR-21-5-3	19.29±0.72	4.82	R	+/–	–/–	+/+	4.50±0.50	S	–/–	–/–
TH-PR-21-6-4	7.86±2.15	5.89	R	+/+	+/+	–/–	5.50±0.50	HS	–/–	–/–
TH-PR-21-10G-13	26.46±3.32	14.55	MR	NA	+/+	–/–	4.50±0.50	S	–/–	–/–
TH-PR-21-11-7	18.97±0.40	4.74	R	+/–	+/+	–/–	5.00±1.00	S	–/–	–/–
TH-PR-22-1-10	15.16±2.30	3.79	HR	+/–	+/+	–/–	5.50±0.50	HS	–/–	–/–
TH-PR-22-2-10	19.29±0.72	4.82	R	+/–	+/+	–/–	5.50±0.50	HS	–/–	–/–
TH-PR-22-3-6	65.87±3.50	49.40	S	NA	NA	–/–	5.00±1.00	S	–/–	NA
TH-PR-22-5-5	10.72±0.72	2.68	HR	NA	+/+	+/+	5.00±1.00	S	–/–	–/–
TH-PR-22-5G-1	12.14±2.14	3.04	HR	NA	NA	+/+	4.50±0.50	S	–/–	NA
TH-PR-22-6-13	0.00±0.00	0.00	HR	+/+	+/+	+/+	5.50±0.50	HS	–/–	–/–
TH-PR-22-6E-2	60.92±7.54	45.69	S	–/–	NA	NA	4.50±1.50	S	–/–	–/–
TH-PR-22-7-9	13.75±0.53	3.44	HR	+/–	+/+	–/–	5.00±1.00	S	–/–	–/–
TH-PR-22-7E-9	64.26±3.92	48.19	S	NA	NA	–/–	5.00±0.00	S	–/–	–/–
TH-PR-22-12-1	34.31±4.31	17.15	MR	–/–	+/+	–/–	4.50±0.50	S	–/–	–/–
TH-PR-22-14-7	35.86±3.51	17.93	MR	–/–	+/+	–/–	5.00±1.00	S	–/–	–/–
TH-PR-23-10-10	0.00±0.00	0.00	HR	+/+	+/+	+/+	4.50±0.50	S	–/–	–/–
TH-PR-23-11-8	30.56±1.99	15.28	MR	–/–	+/+	–/–	5.00±0.00	S	–/–	–/–
TH-PR-23-13E-2	17.86±0.71	4.46	HR	+/–	+/+	NA	5.50±0.50	HS	–/–	–/–
TH-PR-26-1-5	44.88±4.50	22.44	MS	NA	NA	–/–	5.00±1.00	S	–/–	–/–
TH-PR-26-1E-1	6.43±0.71	1.61	HR	+/–	+/+	–/–	5.50±0.50	HS	–/–	–/–
TH-PR-26-2-17	78.50±1.86	78.50	HS	–/–	–/–	–/–	4.50±0.50	S	–/–	–/–
TH-PR-26-3E-6	90.42±4.25	90.42	HS	–/–	–/–	–/–	5.50±0.50	HS	–/–	–/–
TH-PR-26-4-4	42.84±2.24	21.42	MS	NA	NA	–/–	5.00±1.00	S	–/–	–/–
TH-PR-26-5-5	52.41±2.96	39.31	MS	–/–	NA	–/–	4.50±0.50	S	–/–	–/–
TH-PR-27-12-2	16.43±2.14	4.11	HR	+/–	+/+	–/–	6.00±0.00	HS	–/–	–/–
TH-PR-56-1E-13	20.76±3.62	5.19	R	NA	NA	+/–	5.50±0.50	HS	–/–	–/–
TH-PR-56-2-1	40.34±2.78	20.17	MS	–/–	+/–	–/–	5.50±0.50	HS	–/–	–/–
TH-PR-56-4-2	0.00±0.00	0.00	HR	+/–	+/+	+/+	2.50±0.00	MR	–/–	–/–

Lines	Tomato leaf curl virus						Late blight			
	Phenotypic screening			Genotypic screening			Phenotypic screening		Genotypic screening	
	PDI (%)	CI	Reaction	*Ty-1*	*Ty-2*	*Ty-3*	DSI	Reaction	*Ph-2*	*Ph-3*
TH-PR-56-4E-8	0.00±0.00	0.00	HR	+/+	+/+	+/+	5.50±0.50	HS	−/−	−/−
TH-PR-56-6-6	29.26±0.72	14.63	MR	NA	+/+	−/−	5.00±0.50	S	−/−	−/−
TH-PR-56-6E-8	12.14±2.14	3.04	HR	NA	NA	+/+	4.50±0.50	S	−/−	NA
TH-PR-56-9-5	7.86±2.15	1.96	HR	NA	+/−	+/+	5.00±0.50	S	−/−	−/−
TH-PR-56-9E-7	12.14±2.14	3.04	HR	+/−	NA	+/+	5.50±0.50	HS	−/−	−/−
TH-PR-57-2E-1	37.85±3.60	18.92	MR	+/+	−/−	−/−	4.50±0.50	S	NA	NA
TH-PR-57-4E-8	59.71±4.68	44.78	S	−/−	−/−	−/−	5.00±0.50	S	−/−	−/−
TH-PR-58-3-8	27.59±4.57	13.79	MR	+/−	NA	−/−	5.00±0.50	S	−/−	−/−
TH-PR-58-3G-10	38.01±2.38	19.00	MR	+/−	NA	−/−	5.00±0.50	S	−/−	−/−
TH-PR-58-4-3	61.42±2.95	46.06	S	NA	NA	−/−	5.50±1.00	HS	−/−	−/−
TH-PR-58-5-8	42.01±5.14	21.01	MS	+/−	−/−	−/−	5.00±0.50	S	−/−	−/−
TH-PR-58-7-12	31.36±2.00	15.68	MR	+/−	NA	−/−	5.00±0.50	S	−/−	−/−
TH-PR-58-7E-10	34.74±4.71	17.37	MR	NA	NA	−/−	5.00±0.50	S	−/−	−/−
TH-PR-58-8-5	10.72±0.72	2.68	HR	+/−	NA	+/+	4.50±0.50	S	−/−	−/−
TH-PR-58-8E-6	0.00±0.00	0.00	HR	NA	+/+	+/+	5.00±0.00	S	−/−	−/−
TH-PR-58-9-2	36.79±2.67	18.40	MR	+/−	−/−	−/−	4.50±0.50	S	−/−	−/−
TH-PR-58-9E-5	44.13±4.49	22.06	MS	+/−	NA	−/−	5.00±0.50	S	−/−	−/−
TH-PR-58-9G-8	49.78±4.56	24.89	MS	−/−	NA	NA	4.00±0.50	MS	−/−	−/−
TH-PR-58-11G-10	19.29±0.72	4.82	R	−/−	+/+	+/−	4.50±0.50	S	−/−	−/−
TH-PR-58-16-5	90.03±4.64	90.03	HS	−/−	−/−	−/−	5.00±0.50	S	−/−	−/−
TW-4-3-4	27.43±7.15	13.72	MR	NA	+/+	−/−	5.50±0.50	HS	−/−	−/−
TW-4-3E-5	68.37±9.98	51.28	S	−/−	NA	−/−	4.50±0.50	S	−/−	−/−
TW-4-5G-12	8.57±2.86	2.14	HR	+/−	NA	+/+	0.50±0.50	HR	+/+	+/+
TW-4-6E-17	12.14±2.14	3.04	HR	+/−	NA	+/+	3.50±0.00	MS	−/−	+/−
TW-4-7G-16	79.87±3.51	79.87	HS	−/−	NA	−/−	3.50±0.50	MS	+/+	−/−
TW-5-1-17	50.26±7.08	37.69	MS	NA	NA	NA	5.50±0.50	HS	−/−	NA
TW-5-1E-7	12.86±1.43	3.21	HR	+/−	+/+	+/−	1.50±0.50	R	+/+	+/−
TW-5-1E-18	31.16±3.22	15.58	MR	+/−	NA	+/−	1.50±0.50	R	+/−	+/+
TW-5-6-5	2.86±2.86	0.71	HR	+/−	NA	+/+	5.50±0.50	HS	−/−	−/−
TW-6-7-16	77.44±3.90	77.44	HS	−/−	NA	−/−	0.50±0.50	HR	+/+	+/+
TW-6-7E-2	19.29±0.72	4.82	R	+/−	+/+	NA	4.50±0.50	S	−/−	−/−
TW-6-7G-5	26.83±2.55	13.41	MR	−/−	+/+	−/−	5.00±0.00	S	−/−	−/−
TW-6-8-15	92.14±3.50	92.14	HS	−/−	−/−	−/−	1.50±0.50	R	+/+	+/−
TW-8-3-5	31.51±2.94	15.75	MR	NA	+/+	−/−	2.50±0.50	MR	+/+	−/−
TW-8-3G-5	61.24±2.89	45.93	S	+/−	−/−	−/−	5.50±0.50	HS	−/−	−/−
TW-8-4G-12	23.88±2.46	5.97	R	+/+	NA	−/−	3.50±0.00	MS	+/+	−/−
TW-12-1-9	57.76±6.61	43.32	MS	NA	NA	−/−	1.50±0.00	R	NA	+/+
TW-12-1E-8	72.51±4.17	54.38	S	NA	NA	−/−	4.50±0.00	S	−/−	NA
TW-12-4G-5	28.67±3.72	14.33	MR	NA	+/−	−/−	4.50±0.50	S	−/−	−/−
TW-12-5-2	30.71±2.34	15.35	MR	NA	+/−	−/−	3.50±0.50	MS	+/+	−/−
TW-12-6-7	89.50±4.84	89.50	HS	−/−	−/−	−/−	5.50±0.00	HS	−/−	−/−
TW-12-8E-13	22.11±3.54	5.53	R	+/−	+/+	−/−	0.50±0.50	HR	+/+	+/+
TW-12-10-1	18.49±1.51	4.62	R	+/−	+/+	−/−	3.50±0.50	MS	+/+	−/−
TW-12-10E-2	38.07±4.26	19.04	MR	NA	NA	NA	2.50±0.50	MR	NA	+/−
TW-14-3E-6	7.86±2.15	1.96	HR	+/−	−/−	+/+	1.00±0.50	HR	+/+	+/+
TW-14-6G-2	57.81±3.95	43.36	S	+/−	−/−	−/−	2.50±1.00	MR	+/+	−/−
TW-14-12G-3	42.14±2.14	21.07	MS	NA	+/+	−/−	1.50±0.50	R	+/+	+/−
TW-18-2-8	64.31±5.63	48.23	S	+/−	NA	−/−	4.50±0.50	S	−/−	−/−
TW-18-2G-9	82.03±2.58	82.03	HS	+/−	−/−	−/−	3.50±0.00	MS	+/+	−/−
TW-18-3G-7	49.50±7.19	24.75	MS	NA	+/−	−/−	1.50±0.50	R	+/+	+/+
TW-18-4G-4	28.57±1.43	14.28	MR	−/−	+/+	−/−	1.50±0.50	R	+/+	+/+

Lines	Tomato leaf curl virus						Late blight			
	Phenotypic screening			Genotypic screening			Phenotypic screening		Genotypic screening	
	PDI (%)	CI	Reaction	*Ty-1*	*Ty-2*	*Ty-3*	DSI	Reaction	*Ph-2*	*Ph-3*
TW-18-5G-9	43.25±5.39	21.63	MS	–/–	+/–	–/–	0.50±0.50	HR	+/+	+/+
TW-18-5E-4	7.62±1.91	1.90	HR	NA	+/–	+/+	1.00±0.00	HR	+/+	+/+
TW-20-1 -2	28.57±1.43	14.28	MR	NA	+/+	NA	2.50±0.50	MR	+/+	–/–
TW-20-1E-5	32.86±1.43	16.43	MR	NA	+/+	NA	1.50±0.00	R	+/+	+/+
TW-20-2G-9	0.00±0.00	0.00	HR	+/+	NA	+/+	1.50±0.50	R	+/+	+/+
TW-20-3-6	91.02±5.65	91.02	HS	–/–	–/–	–/–	1.00±0.50	HR	+/+	+/+
TW-22-1-7	12.86±1.43	3.21	HR	–/–	–/–	+/+	4.50±0.00	S	–/–	–/–
PR-DH-7-4E-15	15.86±1.57	3.96	HR	+/–	+/+	–/–	1.00±0.50	HR	+/+	+/+
PR-DH-14-3-5	53.54±5.23	40.15	S	–/–	–/–	–/–	1.00±0.50	HR	+/+	+/+
PR-DH-14-4G-7	10.00±4.29	2.50	HR	+/–	NA	+/+	4.50±0.50	S	–/–	–/–
PR-DH-14-5E-11	31.57±3.00	15.78	MR	–/–	+/+	–/–	0.50±0.50	HR	+/+	+/+
PR-DH-15-2-14	10.00±4.29	2.50	HR	+/+	+/+	–/–	1.50±1.00	R	+/+	+/+
PR-DH-15-3-10	41.89±6.53	20.94	MS	–/–	–/–	NA	2.00±0.50	R	NA	NA
PR-DH-15-3E-8	50.23±5.94	25.11	MS	NA	NA	NA	2.50±0.50	MR	NA	+/+
PR-DH-15-4E-8	29.73±5.37	14.87	MR	+/+	NA	–/–	1.50±0.50	R	NA	NA
PR-DH-15-7-11	0.00±0.00	0.00	HR	+/+	+/+	+/+	1.50±0.50	R	+/+	+/+
PR-DH-15-7E-3	4.96±0.75	1.24	HR	–/–	+/+	+/+	1.50±1.00	R	+/+	+/+
PR-DH-15-8G-1	16.43±2.14	4.11	HR	+/–	+/+	–/–	5.00±0.50	HS	NA	–/–
PR-DH-15-16G-4	53.07±3.31	39.80	S	+/–	–/–	–/–	4.50±1.00	S	–/–	–/–
PR-DH-22-1-13	77.44±3.90	77.44	HS	–/–	NA	–/–	2.50±0.50	MR	NA	NA
PR-DH-28-1-7	35.00±3.57	17.50	MR	–/–	+/+	–/–	1.00±0.50	HR	NA	+/+
PR-DH-28-1E-14	38.482.91	19.24	MR	–/–	+/+	–/–	1.50±1.00	R	+/+	+/+
PR-DH-28-2-6	27.14±2.86	13.57	MR	–/–	+/+	–/–	1.50±0.00	R	+/+	+/+
PR-DH-28-2G-6	59.23±3.52	44.42	S	+/–	NA	–/–	1.50±1.00	R	+/+	+/+
PR-DH-28-3E-5	12.14±2.14	3.04	HR	+/–	+/+	–/–	2.00±0.50	R	+/+	+/–
PR-DH-28-11E-17	25.72±4.29	12.86	MR	NA	+/+	–/–	2.50±0.00	MR	NA	NA
PR-DH-28-11G-3	2.11±2.11	0.53	HR	+/+	+/+	–/–	1.00±1.00	HR	+/+	+/+
PR-DH-29-5G-9	61.42±2.95	46.06	S	NA	NA	–/–	1.50±1.00	R	NA	NA
PR-DH-29-11E-16	42.14±0.71	21.07	MS	NA	+/+	NA	1.50±0.50	R	+/+	+/+
PR-DH-30-2G-10	30.33±1.76	15.16	MR	–/–	+/+	–/–	2.00±1.00	R	+/+	+/–
PR-DH-30-6-1	64.86±3.52	48.64	S	NA	–/–	NA	3.50±0.50	MS	+/+	–/–
PR-DH-33-1E-10	90.91±3.26	90.91	HS	–/–	NA	–/–	2.50±1.00	MR	+/+	–/–
PR-DH-33-4E-19	82.02±2.34	82.02	HS	NA	–/–	–/–	5.50±1.00	HS	NA	NA
PR-DH-33-6-9	37.85±3.60	18.92	MR	+/–	NA	–/–	3.50±0.50	MS	+/+	–/–
PR-DH-33-6G-4	92.14±3.50	92.14	HS	NA	NA	–/–	4.00±1.00	MS	+/+	–/–
102-2-2E-1	80.53±6.14	80.53	HS	–/–	NA	NA	4.00±1.00	MS	NA	–/–
102-2-4E-10	10.00±1.43	2.50	HR	+/–	+/+	+/–	4.50±0.50	S	–/–	–/–
102-2-1-2	54.91±0.19	41.19	S	–/–	NA	–/–	5.50±0.50	HS	–/–	–/–
102-2-6E-1	91.07±3.31	91.07	HS	–/–	–/–	NA	4.50±0.50	S	–/–	–/–
I-3-2-1-17	68.78±7.56	51.59	S	NA	NA	–/–	5.50±0.50	HS	NA	NA
102-3-12E-20	42.14±5.00	21.07	MS	–/–	+/+	–/–	3.50±0.50	MS	NA	NA
Punjab Chhuhara (SC)	**100.00±0.00**	**100.00**	**HS**	**–/–**	**–/–**	**–/–**	**6.00±0.00**	**HS**	**–/–**	**–/–**
PVB-4 (RC)	**0.00±0.00**	**0.00**	**HR**	**+/+**	**+/+**	**+/+**	**-**	**-**	**–/–**	**–/–**
LBR-10 (RC)	**-**	**-**	**-**	**–/–**	**–/–**	**–/–**	**1.00±0.50**	**HR**	**+/+**	**+/+**

CI= Coefficient of infection, PDI= Percent disease index, DI= Disease incidence, HR= Highly resistant, R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S= Susceptible, HS= Highly susceptible, DAI= Days after inoculation, RC= Resistant check, SC= Susceptible check, +/+ = Homozygous resistant, +/– = Heterozygous, –/– = Homozygous susceptible, NA= Not amplified

All the lines were also subjected to marker analysis for confirmation of resistance genes. Among 122 lines evaluated (Table 5), 50 lines were carried *Ty-1* gene, 54 lines were carried *Ty-2* gene and 28 lines were carried *Ty-3* gene in homozygous/heterozygous conditions while among them, the lines TH-PR-22-6-13, TH-PR-23-10-10, TH-PR-56-4-2, TH-PR-56-4E-8, TW-5-1E-7, PR-DH-15-7-11 and 102-2-4E-10 were carried *Ty-1+Ty-2+Ty-3* genes and found to be highly resistant during phenotypic screening against ToLCV disease. Among all the three genes (*Ty-1, Ty-2* and *Ty-3*) screened for ToLCV disease, most of the breeding lines had *Ty-1* gene in heterozygous condition in the population. The lines possess only *Ty-1* gene was showing moderately resistant reaction in phenotypic screening. The lines which carried only *Ty-2* gene were showed moderate level of resistance in phenotypic screening. Similarly, KALLOO and BANERJEE (2000) also reported that, lines of tomato which carried *Ty-2* gene showed moderate level of resistant reaction against ToLCV. The lines which were carried only *Ty-3* gene showed resistant reaction under in phenotypic screening with less than 4 coefficients of infection (CI). PRASANNA *et al.* (2015) were also reported that, lines carried *Ty-3* gene showed high degree of resistance against both viz. monopartite and bipartite begomoviruses in their studied genotypes. The presence of *Ty-1* gene with *Ty-2*/*Ty-3* gene was also providing sufficient level of resistance reaction under phenotypic screening. Similar results were also noticed by ELBAZ *et al.* (2016) and TABEIN *et al.* (2017) for confirmation the presence of *Ty-1, Ty-2* and *Ty-3* genes among different genotypes of tomato through MAS and artificial screening.

Screening for late blight disease

In artificial phenotypic screening against late blight, none of the lines was found to be completely free from disease symptoms in detached leaf assay at seven days after inoculations indicating that, there was presence of high and uniform inoculums with favourable environment. The results revealed that, out of 122 lines screened (Table 4), 22 lines were found to be highly resistant and 21 were resistant. The lines, TW-4-5G-12, TW-6-7-16, TW-12-8E-13, TW-18-5G-9 and PR-DH-14-5E-11 showed minimum disease severity index (DSI=0.5). Resistant check (LBR-10) also showed disease infection in very less symptom severity (DSI=1) while Punjab Chhuhara showed severe infestation and categorized to highest disease severity index (DSI=6). The two gene specific markers viz. dTG422 and TG328 were associated with *Ph-2* and *Ph-3* gene locus confirming resistance to late blight, respectively. The main principle behind MAS is the tightly linkage of primers to the target character/trait from the cloned target gene (TACCONI *et al.,* 2010). Among 122 lines screened (Table 4), 40 lines carried *Ph-2* gene and 33 lines carried *Ph-3* gene in homozygous/heterozygous conditions. The lines carried only *Ph-2* gene was showed moderate level of resistant reaction during phenotypic screening while those lines carried only *Ph-3* gene was provide sufficient level of resistant reaction during phenotypic screening. Twenty three lines carried both viz. *Ph-2+Ph-3* genes and showed resistant to highly resistant reaction during phenotypic screening. ARAFA *et al.* (2017) and HANSON *et al.* (2016) also reported that, lines carried both *Ph-2+Ph-3* genes showed highly resistant reaction during phenotypic screening.

CONCLUSIONS

Out of 122 breeding lines evaluated, 12 lines viz. TW-4-5G-12, TW-5-1E-7, TW-12-8E-13, TW-14-3E-6, TW-18-5E-4, TW-20-2G-9, PR-DH-7-4E-15, PR-DH-15-2-14, PR-DH-15-

7-11, PR-DH-15-7E-3, PR-DH-28-3E-5 and PR-DH-28-11G-13 were found to be resistant against both the diseases during phenotypic screening and also confirmed the presence of resistance genes of leaf curl virus and late blight diseases. The lines TW-4-5G-12, TW-5-1E-7, PR-DH-15-7-11 and PR-DH-28-11G-13 were also more yielder than all the three checks (Punjab Chhuhara, PVB-4 and LBR-10) along with other horticultural traits. These resistant lines could be evaluated in multi-locations for their commercial exploitation and release as a variety.

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EVALUACIJA LINIJA PARADAJZA (*Solanum lycopersicum* L.) KOJE POSEDUJU RAZLIČITE KOMBINACIJA *Ty* I *Ph* GENA

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Izvod

Prema agro-statistici, Indija je drugi najveći proizvođač paradajza tek u svetu, posle Kine. Paradajz se gaji tokom cele godine zbog šire prilagodljivosti, ali na proizvodnju negativno utiču pojave virusa kovrdžavosti lista i bolesti truleži. Ovi patogeni imaju ogroman kapacitet za stvaranje novih oblika i kontrola ovih patogena uglavnom se postiže hemijskim pristupima koji nisu bezbedni ni za životnu sredinu, ni za ljude i takođe prave dodatne troškove u proizvodnji paradajza. Stoga je stvaranje otpornih genotipova najbolji pristup za upravljanje ovim vrstama bolesti, uz poboljšanje prinosa i parametara kvaliteta. Pet gena *Ty-1, Ty-2, Ty-3, Ph-2* i *Ph-3* je odabrano od različitih roditelja hibridizacijom kako bi se postigla otpornost protiv virusa kovrdžavosti lista i bolesti truleži. U ovoj studiji, ocenjeno je 122 poboljšanih linija paradajza tokom 2016-17 i 2017-18, fenotipskim i genotipskim skriningom da bi se identifikovale najbolje linije koje imaju otpornost na obe bolesti sa boljim hortikulturnim osobinama. Punjab Chhuhara sorta je korišćena kao zajednički standard za proveru osetljivosti na obe bolesti, dok su PVB-4 i LBR-10 korišćeni kao otporni standardi za proveru virusa kovrdžavosti lista paradajza i bolesti truleži. Od 122 procenjene linije, nađeno je da 12 linija ima otpornost i na bolesti sa različitom kombinacijom *Ty* i *Ph* gena. Među njima su i četiri linije TV-4-5G-12, TV-5-1E-7, PR-DH-15-7-11 i PR-DH-28-11G-13 koji su otporni na obe bolesti i daju prinos od 3.14, 3.90, 2.74 i 3.84 kg biljci-1 što je više od sva tri standarda. Ove otporne linije potrebno je ocenjivati na više lokacija pre njihove komercijalne eksploatacije.

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