Vegetable Science (2025) 52(2): 358-367
doi: 10.61180/vegsci.2025.v52.i2.17
ISSN- 0970-6585 (Print), ISSN- 2455-7552 (Online)
www.isvsvegsci.in

RESEARCH ARTICLE

Harnessing resistance sources to develop ToLCV-tolerant hybrids of tomato (*Solanum lycopersicum* L.)

Athira Sebastian[1], Deepthy Antony P[2*], Jiji Joseph[1], Vimi Louis[3] and M. Sangeeta Kutty[1]

Abstract

Tomato leaf curl virus disease (ToLCD) is a major constraint in tomato cultivation, and host plant resistance is the best strategy to manage ToLCD. Seven lines reported as field resistant or known to carry *Ty* genes were crossed with three commercial varieties of Kerala, and the evaluation of seventeen hybrids obtained revealed that the hybrids Vellayani Vijai×EC519806, Akshaya×AVTO1726, Akshaya×EC519806 and Akshaya×AVTO1707 showed high field resistance, while hybrids of EC519806 and Akshaya×AVTO1726 recorded high yield. High glandular to non-glandular trichome density ratio on the abaxial leaf surface was found to be an important factor in determining the tolerance of genotypes under field screening. Markers linked to *Ty* genes were detected in crosses with AVTO lines, while none of the *Ty* gene-linked markers were detected in the accession EC519806, which displayed field resistance. *Ty 6* was detected in Local Collection (Idukki) and its hybrids. Thus, *per se* performance, heterosis and disease response of the hybrids in the present study can be utilised for the selection of superior tomato genotypes for future ToLCV resistance breeding programmes.

Keywords: Molecular marker, Resistance breeding, ToLCV, Tomato hybrids, Trichome density, *Ty* genes.

[1]College of Agriculture, KAU PO, Vellanikkara, Thrissur-680656, Kerala, India

[2]Rice Research Station, Vyttila PO, Kerala Agricultural University, Ernakulam-682019, Kerala, India

[3]Banana Research Station, Kannara, Thrissur, Kerala, India

[*]**Corresponding author;** Email: deepthy.a@kau.in

Citation: Sebastian, A., Antony, D.P., Joseph, J., Louis, V., & Kutty, M.S. (2025). Harnessing resistance sources to develop ToLCV-tolerant hybrids of tomato (*Solanum lycopersicum* L.). Vegetable Science 52(2), 358-367.

Source of support: Nil

Conflict of interest: None.

Received: 04/08/2025 **Revised:** 20/12/2025 **Accepted:** 22/12/2025

Introduction

Tomato (*Solanum lycopersicum* L.) of the Solanaceae family is cultivated worldwide under wide climatic conditions, primarily for its edible fruits and is preferred for its taste and nutritional value. Medicinal and antioxidant properties, and processing potential of tomato fruits increase the importance of the crop (Achari et al., 2019). Among the major production constraints, *viz.,* abiotic and biotic stresses, tomato leaf curl virus disease (ToLCD) is a major factor causing crop loss in all the major tomato growing regions of India (Lapidot, 2007). ToLCD is caused by Tomato leaf curl virus, belonging to the Geminiviridae family (Begomovirus genus) and is transmitted by the whitefly vector (*Bemisia tabaci*), in a persistent and circulative manner. Tomato is susceptible to ToLCV at all stages of its growth and the symptoms appear within two to three weeks after exposure to a viruliferous whitefly. Severe curling, cupping, yellowing of top leaves and stunting are seen, and may lead to yield loss up to 100% (Lapidot, 2007; Tipu et al., 2021). Currently, the disease is managed by pesticide application for vector control, which negatively impacts the environment. Changing climate, rising whitefly populations, and increasing strain virulence have necessitated an environmentally friendly alternative approach, like the adoption of resistant cultivars. The wild tomato species (*Solanum pimpinellifolium*, *S. peruvianum, S. chilense* and *S. habrochaites*) are potential sources of

resistance to ToLCV, and have been used in the introgression of resistance genes (*Ty 1–Ty 6* genes) in tomato breeding (Srivastava et al., 2020). ToLCV-tolerant hybrids and varieties show varying levels of susceptibility due to partial resistance conferred by *Ty* genes. Therefore, identifying high-yielding genotypes with stable and durable resistance to ToLCV remains a priority. The present study utilized known donors for ToLCV resistance and yield contributing traits to develop ToLCV tolerant high yielding tomato hybrids.

Materials and Methods

The experiment was conducted during 2021-23 at Thrissur, Kerala, India, situated at latitude 10° 32′ 46″ N, longitude 76° 16′ 44″ E, at an altitude of 26 m above MSL.

Selection of parents

Agronomically superior varieties released by Kerala Agricultural University (KAU), *viz.,* Akshaya, Manuprabha and Vellayani Vijai, were used as female parents, and seven reported ToLCV-resistant or *Ty* gene-harbouring lines (AVTO1707, EC519806, Local collection (Idukki) (LC), AVTO1314, AVTO1726, AVTO0301 and AVTO0922) as male parents (Anjitha et al., 2023). KAU variety Anagha was used as a control variety in the experiment.

Development of hybrids

The seeds were sown (staggered sowing at seven days interval) in trays after seed treatment with *Pseudomonas fluorescence* @ 10 g/Kg seeds for 8 hours. Thirty-day-old seedlings dipped in streptocyclin solution (6 g/60 L) were transplanted in grow bags (40×24×24 cm) and managed as per the Package of Practices Recommendations: Crops (KAU 2016). Female parents (Akshaya, Manuprabha and Vellayani Vijai) were emasculated between 4 and 6 pm. Pollen from freshly opened flowers of male parents was collected between 6 and 8 am for hand pollination. Fruits were harvested at maturity from 20 successful crosses (except Akshya×AVTO030), seeds were extracted, dried and stored in a refrigerator for sowing in the next season. Among the 20 crosses, three hybrids failed to germinate and the remaining 17 hybrids were used in the subsequent experiments for natural screening for ToLCV tolerance.

Screening for ToLCV tolerance

The summer season is optimum for screening for ToLCV tolerance, when the natural ToLCV inoculation pressure is at its peak in tropical regions (Achari et al., 2019). One-month-old seedlings of 17 crosses, along with parents and control variety (Anagha), were transplanted in a Randomized Block Design (RBD) with two replications, each with ten plants at 60 cm×60 cm spaced grow bags during the summer season (April-May). Natural incidence of whitefly population in the field was observed during the screening period. The hybrids selected based on growth and yield traits and natural disease response were subjected to whitefly-mediated artificial inoculation. Whiteflies were reared on 50-day-old brinjal plants inside insect proof net cage, and were allowed to feed on the ToLCV-infected tomato plants for 24 hours, and 30 to 50 viruliferous whiteflies per seedling were released to 20 days old healthy hybrid tomato seedlings and kept in an inoculation cage for 48 hours for inoculation feeding. Subsequently, cages were removed after spraying insecticide on inoculated plants to kill the whiteflies. The symptoms in inoculated plants were recorded at regular intervals up to 30 days as per Banerjee and Kalloo (1987).

Observations on morphological traits

Observations on plant height (cm), spread of the plant (cm), growth habit and number of primary branches per plant were recorded at 60 days after transplanting (DAT); while, yield related traits like days to flowering (days), number of fruits per plant, fruit weight (g) and fruit yield per plant (g) were recorded during the fruiting period; and fruit characters like equatorial diameter (cm), polar diameter (cm) and fruit colour and fruit size (cm^3) of parents and hybrids were recorded at maturity.

Trichome density on leaf surfaces

Trichome density (including both glandular and non-glandular trichomes) was assessed by manually counting the number of trichomes per square centimetre on the adaxial and abaxial surfaces of leaves with three biological replications using a stereoscopic microscope. Fully expanded leaves from the third node below the apex were sampled at the vegetative stage.

Molecular marker analysis

DNA was extracted from young leaves of parents and hybrids as described by Aboul-Maaty and Oraby (2019). PCR amplification of *Ty* genes was performed using an Eppendorf Mastercycler Nexus Gradient thermocycler (Eppendorf, Germany). The thermal cycling conditions consisted of initial denaturation (94°C-4 minutes), followed by 35 cycles of denaturation (94°C-30 seconds), annealing (primer-specific temperature-1 minute), and extension (72°C-2 minutes), with a final extension (72°C-minutes (Table 1).

Statistical analysis

Statistical analysis of the performance of 10 parents and 17 hybrids was done in KAU Grapes software (Gopinath et al., 2021). Standard heterosis was calculated using the control variety Anagha, and the significance of F_1 heterosis values was assessed based on Critical Difference (CD) values as suggested by Fonseca and Patterson (1968).

Percent heterosis over standard check= (F1-Standard check) x 100/Standard check

Disease scoring

Under natural field screening, disease symptoms were recorded at 15, 30, 60 and 90 days after transplanting (DAT)

Table 1: Primers used for marker analysis for *Ty* genes

Gene	*Marker*	*Amplicon (bp)**		*Annealing temperature (°C)*	*Reference*
		R	*S*		
Ty 1/3	TY-1/3_K	102	114	60	Chen et al. (2015)
Ty 2	SCAR2	800	900	60	Nevame et al. (2018)
Ty 4	C2_AT5g51110	325	430	55	Patel et al. (2021)
Ty 6	SLM 10-46	255	230	60	Kadirvel et al. (2013)

*R: Resistant; S: Susceptible

and were scored as described by Banerjee and Kalloo (1987) on a scale from 0 to 4. Disease Severity Index (DSI) and Per cent Disease Incidence (PDI) were calculated as given by McKinney (1923) and Sharma and Sharma (1984), respectively. Coefficient of Infection (CI), calculated as the product of PDI and DSI, was used to categorize into six groups ranging from highly susceptible to highly resistant (PDVR, 1997).

Results and Discussion

Performance of parents and hybrids

Evaluation of 10 parental lines and 17 hybrids revealed significant differences in performance for vegetative and reproductive traits compared to the control variety Anagha (Table 2). For growth habit, if any one of the parents had an indeterminate growth habit, their F1 hybrid showed an indeterminate growth habit. It has been shown that the major recessive gene *sdt* modifies the determinate growth habit expression of the sp/sp (recessive) genotype; the *sdt* gene is not expressed in the presence of the dominant allele sp+ and the plant shows indeterminate growth (Elkind et al., 1991). Early flowering is a preferred trait as it ensures early harvest and a longer yielding period (Nadkarni et al., 2017). All female lines had significantly longer days to flowering, while, among the male parents, LC (27.8) had significantly lower days to flowering, and EC519806 had comparable days to flowering to Anagha (31.5 days). Hybrids of EC519806 or LC had either significantly lower or comparable days to flowering compared to Anagha. Akshaya×LC (27.2 days) had the lowest number of days to flowering among all the parents and hybrids tested.

Among the parents, Akshaya, LC and AVTO0301 had a significantly higher number of primary branches, while all the hybrids of LC exhibited significantly higher spread of the plant compared to Anagha. The hybrids Manuprabha×AVTO1707, Akshaya×EC519806, Vellayani Vijai×AVTO1314, and Vellayani Vijai×AVTO0922 exhibited significant superiority for the number of primary branches per plant. Rojalin et al. (2018) reported a positive and significant correlation between the number of primary branches and yield per plant. All the hybrids and parents showed higher plant height than the control variety, Anagha, except AVTO1707, which was on par with the control variety. The highest spread of the plant was observed in Akshaya×LC (62.6 cm) and the lowest in Vellayani Vijai×AVTO1707 (15.9 cm). All the female lines had significantly higher plant spread, while male lines, except EC519806 and LC, had significantly lower plant spread compared to Anagha. Male lines EC519806 and LC, and their progenies, exhibited significantly superior plant height than Anagha (33.1 cm). LC and its hybrids were significantly superior to Anagha for all the yield contributing vegetative traits. Lekshmi and Celine (2020) reported that fruit yield per plant had a significant and positive correlation with fruits per plant, fruit weight, polar diameter and equatorial diameter. Compared to Anagha, all AVTO lines and their crosses (except Akshaya×AVTO1707) had significantly higher equatorial diameter, polar diameter, fruit size and fruit weight. Although AVTO lines had larger fruits, they displayed lower fruit number (6.5–8.6 and 5.4–9.9 fruits per plant in parents and hybrids, respectively). Although the number of fruits per plant was higher in EC519806 (29.8) and LC (52.6) and their hybrids, they exhibited significantly lower equatorial diameter, polar diameter, fruit size and fruit weight compared to Anagha. Significantly higher yield per plant than Anagha (383.3 g/plant) was observed in Vellayani Vijai×EC519806 (652.9 g/plant), followed by Akshaya×EC519806 (477.2 g/plant). Higher yield per plant in these crosses was due to the significantly higher number of fruits per plant, along with a moderate fruit weight in the hybrids of EC519806.

Heterosis in hybrids

Most hybrids had significant positive standard heterosis for plant height (Table 3). Bhattarai et al. (2018) reported that in the case of the determinate type, plant height is a desirable trait, while it is undesirable for the indeterminate type. Akshaya×AVTO1726, Manuprabha×EC519806, Manuprabha×LC and Vellayani Vijai×AVTO0301 were desirable crosses for plant height. For the trait days to flowering, Akshaya and Vellayani Vijai crosses with EC519806 and LC showed significant negative standard heterosis. Nadkarni et al. (2017) had also suggested that early flowering is a preferred trait in tomato. Spread of the plant and number of primary branches per plant of most of the crosses of EC519806 and LC were superior to Anagha. Hybrids of EC519806 and LC exhibited significant heterosis for the

Table 2: Mean performance of tomato hybrids and their parents

Genotype	*Hab*	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
Akshaya	ID	37.15	3.40*	123.64*	65.44*	8.15*	3.44	38.23*	11.4	24.10*	273.55
Manuprabha	DT	40.75	3.25	122.80*	65.17*	7.70*	4.32*	42.77*	14.1	21.68	304.57
Vellayani Vijai (VV)	DT	37.70	3.25	86.80*	37.07*	5.11	3.91*	17.10*	15.0	20.30	304.50
AVTO1707	DT	45.70	2.30	75.00	30.91	6.91*	5.72*	45.85*	6.5	33.91*	220.44
EC519806	ID	29.65	3.35	111.01*	34.39*	3.73	2.96	6.90	29.8*	7.78	231.44
Local collection (Idukki) (LC)	ID	27.80*	4.30*	127.60*	56.79*	3.91	1.90	4.87	52.6*	5.34	280.62
AVTO1314	DT	30.60	2.75	126.43*	31.08	6.73*	5.25*	39.88*	8.6	29.77*	254.52
AVTO1726	DT	44.40*	2.20	164.89	25.38	6.82*	6.04*	47.03*	7.8	41.23*	319.54
AVTO0301	SD	39.25*	4.25*	98.72	26.32	7.03*	5.4*	44.50*	7.1	30.03*	211.73
AVTO0922	DT	35.25*	3.10	115.06	28.44	5.88*	5.12*	29.65*	6.8	33.94*	229.09
Akshaya×AVTO1707	ID	41.15	2.50	114.69*	30.28	6.32*	3.5	23.38*	7.9	35.98*	282.45
Manuprabha×AVTO1707	DT	33.60	3.65*	95.50*	43.32*	7.73*	4.63*	46.28*	7.2	34.29*	245.18
VV×AVTO1707	DT	45.10	2.30	86.95*	15.92	6.71*	5.55*	41.81*	6.9	33.84*	231.84
Akshaya×EC519806	ID	28.55	4.20*	127.01*	35.33*	4.72	2.9	10.83	29.0*	16.48	477.15*
Manuprabha×EC519806	ID	32.90	3.35	91.16*	57.63*	4.81	2.29	8.86	25.2*	16.12	405.45
VV×EC519806	ID	27.90*	2.65	99.96*	38.22*	3.33	3.26	6.08	38.6*	16.91	652.89*
Akshaya×LC	ID	27.15*	3.85*	135.75*	62.63*	4.43	2.47	8.18	34.9*	7.00	244.39
Manuprabha×LC	ID	30.15	4.25*	108.68*	35.51*	3.89	2.17	5.62	30.7*	6.50	199.19
VV×LC	ID	27.25*	3.50*	133.90*	61.72*	4.49	2.2	7.49	32.2*	6.62	212.91
Akshaya×AVTO1314	DT	45.55	2.80	99.51	30.71	7.37*	5.09*	46.39*	8.4	23.09*	192.82
VV×AVTO1314	DT	28.40	3.80*	87.63	34.31*	6.42*	5.28*	36.31*	7.8	25.61*	199.75
Akshaya×AVTO1726	ID	33.30	2.70	133.80*	41.68*	7.63*	5.31*	51.71*	9.4	33.37*	312.02
VV×AVTO1726	SD	41.85	2.60	130.11*	25.88	7.56*	5.76*	55.09*	8.4	29.19*	243.79
Manuprabha×AVTO0301	ID	45.50*	3.20	118.40*	44.38*	6.99*	5.21*	42.66*	7.1	34.78*	245.21
VV×AVTO0301	ID	41.65*	2.80	83.98	37.81*	7.36*	5.22*	47.31*	5.4	38.33*	206.99
Manuprabha×AVTO0922	DT	47.75*	2.90	120.39*	35.11*	7.25*	4.94*	43.28*	7.1	23.99*	170.36
VV×AVTO0922	DT	35.15*	4.30*	101.44*	33.08*	5.46*	5.09*	25.58*	9.9	22.38*	221.59
Anagha	DT	31.45	2.80	75.57	32.72	5.07	3.45	14.94	18.0	21.35	383.26
CV		18.92	21.40	19.28	33.63	23.74	30.75	57.89	77.33	44.20	36.42
SE		1.112	0.204	2.736	0.188	0.076	0.123	0.887	0.443	0.341	9.596
C.D at 5 %		3.228	0.593	7.938	0.546	0.221	0.356	2.574	1.284	0.988	27.845

*Significant at 5%; Hab-Growth Habit, ID-Indeterminate, DT-Determinate, SD-Semi determinate,
A-Days to flowering, B-Number of primary branches per plant, C-Plant height (cm), D-Spread of the plant (cm), E-Equatorial diameter (cm), F-Polar diameter (cm), G-Fruit size (cm3), H-Number of fruits per plant, I-Fruit weight (g) and J-Fruit yield per plant (g)

number of fruits per plant, irrespective of the female parent. In hybrids of Vellayani Vijai, significant standard heterosis was observed for fruit characters *viz.,* equatorial diameter, polar diameter, fruit size and fruit weight, except in Vellayani Vijai×EC519806 and Vellayani Vijai×LC. For fruit size and average fruit weight, significant heterosis was observed for hybrids of AVTO1707 with all female parents, AVTO1314 with Akshaya and Vellayani Vijai, and AVTO0301 and AVTO0922 with Manuprabha and Vellayani Vijai. Hybrids of EC519806 and LC exhibited significant negative standard heterosis, as EC519806 and LC had smaller fruits compared to Anagha. Vellayani Vijai×EC519806 was superior to Anagha for yield per plant, and exhibited significant standard heterosis.

ToLCV disease incidence

PDI is the percentage of diseased plants in the sample or population, whereas DSI is the extent of infection. Coefficient of Infection (CI) combines disease severity

Table 3: Standard heterosis of hybrids for growth and yield characters

Male parent	*Female parent*	*Plant height (cm)*	*No. of primary branches per plant*	*Spread of the plant (cm)*	*Days to flowering (days)*	*Number of fruits per plant*	*Equatorial diameter (cm)*	*Polar diameter (cm)*	*Fruit size (cm^3)*	*Average fruit weight (g)*	*Yield per plant (g)*
	Akshaya	51.76**	-10.71	-7.47**	30.84**	-56.39**	24.65**	1.45	56.49**	68.52**	-26.3
AVTO1707	Manuprabha	26.37**	30.36**	32.40**	6.84	-60.28**	52.47**	34.20**	209.77**	60.61**	-36.23*
	Vellayani Vijai	15.06**	-17.86*	-51.34**	43.40**	-61.94**	32.35**	60.87**	179.85**	58.50**	-39.51*
	Akshaya	68.06**	50.00**	7.96**	-9.22*	61.11**	-6.90**	-15.94**	-27.51**	-22.81**	24.49
EC519806	Manuprabha	20.63**	19.64*	76.12**	4.61	39.72**	-5.13*	-33.62**	-40.76**	-24.50**	5.79
	Vellayani Vijai	32.27**	-5.36	16.81**	-11.29*	114.44**	-34.32**	-5.51	-59.30**	-20.80**	70.35**
	Akshaya	79.63**	37.50**	27.37**	-13.67**	94.44**	-12.62**	-28.41**	-45.25**	-67.21**	-36.23*
Local collection (Idukki)	Manuprabha	43.80**	51.78**	8.51**	-4.13	72.22**	-23.27**	-37.10**	-62.38**	-69.56**	-48.03**
	Vellayani Vijai	77.19**	25.00*	88.62**	-13.35**	77.78**	-11.44**	-36.23**	-49.87**	-68.99**	-44.45*
	Akshaya	31.68**	0	-6.14**	44.83**	-53.61**	45.36**	47.54**	210.51**	8.15**	-49.69**
AVTO1314	Vellayani Vijai	15.95**	35.71**	4.86**	-9.70*	-56.67**	26.63**	53.04**	143.04**	19.95**	-47.88**
	Akshaya	77.05**	-3.57	27.37**	5.88	-50.00**	50.49**	53.91**	246.12**	56.30**	-18.59
AVTO1726	Vellayani Vijai	72.17**	-7.14	-20.92**	33.07**	53.61**	49.11**	66.96**	268.74**	36.77**	-36.39*
	Manuprabha	56.67**	14.28	35.64**	44.67**	-60.83**	37.87**	51.01**	185.61**	62.90**	-36.02*
AVTO0301	Vellayani Vijai	11.13*	0	15.56**	32.43**	-70.00**	45.17**	51.30**	216.73**	79.53**	-45.99**
	Manuprabha	59.31**	3.57	7.30**	51.83**	-60.56**	43.00**	43.19**	189.69**	12.37**	-55.55**
AVTO0922	Vellayani Vijai	34.23**	53.57**	1.1	11.76*	-45.00**	7.69**	47.54**	71.22**	4.82*	-42.18*
Mean performance of Anagha		75.57	2.8	32.72	31.45	18	5.07	3.45	14.94	21.35	383.26

* Significant at 5% , ** Significant at 1%

with the response of the host plant. Upto 15 DAT, most of the plants remained symptomless and belonged to the highly resistant to moderately resistant category. Initially, symptoms like slight crinkling and puckering of the leaves were observed. Subsequently, vein clearing, crinkling and puckering of the leaves, stunting and bushy growth were observed at 60 and 90 DAT. At 60 DAT, DSI ranged from 1.25 to 46.25 and PDI ranged from 5 to 85 (Fig. 1). Maximum PDI, DSI and CI were recorded for Akshaya, whereas minimum was for Akshaya×AVTO1707. CI ranged from 0.06 to 39.31. Hybrids of Akshaya (Akshaya×AVTO1707, Akshaya×EC519806 and Akshaya×AVTO1726) and Vellayani Vijai (Vellayani Vijai×AVTO1707, Vellayani Vijai×EC519806 and Vellayani Vijai×LC) and the male parents AVTO1707, EC519806, AVTO1726, AVTO0301 and AVTO0922 were highly resistant, while Akshaya was susceptible under field screening. Anagha was reported as highly susceptible, and Manuprabha as susceptible to ToLCV in Kerala (Yadav, 2011). In the present study, Akshaya was more susceptible compared to all the parents and hybrids studied. However, all the hybrids of Vellayani Vijai and Akshaya as female parents were highly resistant or resistant, whereas all hybrids with Manuprabha as female parent displayed moderate resistance to ToLCD under field screening.

Earlier studies showed that flower initiation and fruit set were reduced by ToLCV incidence (Srivastava et al., 2020). In this study, the number of fruits per plant, fruit weight and fruit yield per plant were high in highly resistant Akshaya×AVTO1726 and Akshaya×AVTO1707 hybrids, when compared to the hybrids of AVTO1726 and AVTO1707 with other female parents. Similarly, Vellayani Vijai×EC519806, with low CI, showed a high number of fruits per plant, fruit weight and fruit yield per plant compared to hybrids of EC519806 with Akshaya and Manuprabha.

Under field conditions, the whitefly pressure, inoculum intensity and plant stage at the time of infection are unpredictable (Lapidot, 2007). Susceptible plants sometimes escape infection and selection based on the absence of symptoms in the field alone could be misleading (Vidavsky et al., 1998). Lapidot (2007) found that whitefly-mediated screening of ToLCV in tomato was a reliable technique for screening for virus resistance. Selected hybrids (crosses of AVTO1707, EC519806, LC and AVTO1726) along with Anagha (Control) and Akshaya (Susceptible), were subjected to artificial screening (Table 4). Akshaya×AVTO1726 was highly resistant and Manuprabha×AVTO1707 and Akshaya×EC519806 were moderately resistant. Vellayani Vijai×EC519806, followed by Manuprabha×EC519806, showed high susceptibility to ToLCV. Although the hybrids of LC were either highly resistant, resistant, or moderately resistant under field screening, they were susceptible under artificial screening, in agreement with Yan et al. (2018), who reported that symptomless plants in natural field

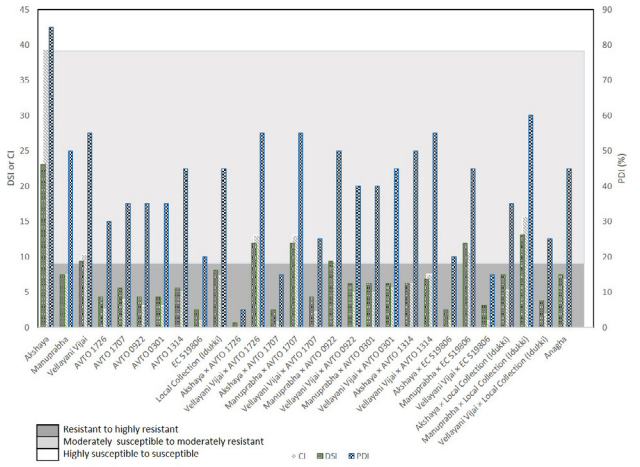

Fig. 1: Disease response of parents and hybrids under field screening based on CI (Coefficient of Infection) of genotypes at 60 DAT; HR (Highly Resistant, CI:0-4); R (Resistant, CI:4.1-9); MR (Moderately Resistant, CI:9.1-19); MS (Moderately Susceptible, CI:19.1-39)

Table 4: ToLCD response of selected tomato hybrids under artificial screening and molecular marker screening

Genotype	*Artificial screening*				*Ty genes*
	PDI (%)	*DSI (%)*	*CI*	*DR*	
Akshaya×AVTO1726	20	5	1	HR	*Ty 2, Ty 1/3*
Vellayani Vijai×AVTO1726	80	30	24	MS	*Ty 2, Ty 1/3*
Akshaya×AVTO1707	80	35	28	MS	*Ty 2, Ty 1/3*
Manuprabha×AVTO1707	60	20	12	MR	*Ty 2, Ty 1/3*
Vellayani Vijai×AVTO1707	80	45	36	MS	*Ty 2, Ty 1/3*
Akshaya×EC519806	60	30	18	MR	-
Manuprabha×EC519806	100	80	80	HS	-
Vellayani Vijai×EC519806	100	100	100	HS	-
Akshaya×LC	100	45	45	S	*Ty 6*
Manuprabha×LC	80	50	40	S	*Ty 6*
Vellayani Vijai×LC	100	60	60	S	*Ty 6*
Anagha	100	65	65	S	-
Akshaya	100	90	90	HS	-

*PDI - Per cent Disease Incidence, DSI-Disease Severity Index, CI-Coefficient of Infection, DR-Disease Reaction, HR-Highly resistant, MR-Moderately resistant, MS-Moderately susceptible, S-Susceptible, HS-Highly susceptible

screening may have occurred due to escape of whitefly infection.

Trichome density

Trichome density on abaxial and adaxial leaf surfaces varied significantly for both glandular and non-glandular trichomes. Glandular trichome density on adaxial leaf surface ranged from 0-87 No.s/cm^2, and was highest in

EC519806 (87 No.s/cm^2), while on the abaxial surface it ranged from 8-302 No.s/cm^2 and LC (302 No.s/cm^2) had the highest density, and both were resistant in the field (Fig. 2). Glandular trichome density has a negative correlation with disease incidence, as whiteflies will be repelled from landing, feeding and egg laying on leaves. Significant negative and positive correlations of disease incidence with glandular and non-glandular trichome density, respectively, were observed. Whiteflies prefer hairy abaxial leaf surfaces to adaxial for landing, survival and oviposition (Firdaus et al., 2012). Negative effect of glandular trichomes on whitefly survival and oviposition may be due to acyl-sugars (Mutschler et al., 1996), monoterpenes and sesquiterpenes (Firdaus, 2012) from glandular trichomes. Thus, based on the high glandular to non-glandular trichome density ratio, as well as the response of the genotypes, it can be concluded that AVTO1726, EC519806, LC, Akshaya×AVTO1726 and Akshaya×AVTO1707 are highly resistant to ToLCV.

Molecular marker screening for Ty genes

Genomic DNA isolated from parents and hybrids was analysed for the reported *Ty* genes (Fig. 3, 4, 5, 6). Earlier studies have shown that a single *Ty* gene cannot impart resistance to the plant, and hence, gene pyramiding of more than one *Ty* gene is required to develop ToLCV-resistant genotypes (Chandel et al., 2019).

Ty 1 and *Ty 3* were reported as partially dominant genes, and *Ty 3*, which is allelic with *Ty 1*, codes for RNA-dependent RNA polymerase (RDR) (Verlaan et al., 2013). AVTO1726, AVTO1707 and their crosses showed a resistant allele for *Ty 1/3*. All the AVTOlines and their crosses, except AVTO1314 and its crosses, carried the resistant allele for *Ty 2*. All the crosses of Akshaya with AVTO lines, except Akshaya×AVTO1314, harbour *Ty 2* and *Ty 1/3* genes in a heterozygous condition and showed high resistance under field screening. Akshaya×AVTO1314, Akshaya×EC519806 and Akshaya×LC, which were lacking *Ty 2* and *Ty 1/3*, were moderately resistant or susceptible.

Ty 2 and *Ty 1/3* genes can be regarded as reliable sources of resistance against ToLCV, as all crosses of AVTO1707 and AVTO1726 showed the presence of *Ty 2* and *Ty 1/3* and were highly to moderately resistant under field and artificial screening. Hybrids of AVTO0922 and AVTO0301 had *Ty 2* and were resistant under field screening. Only LC and its hybrids

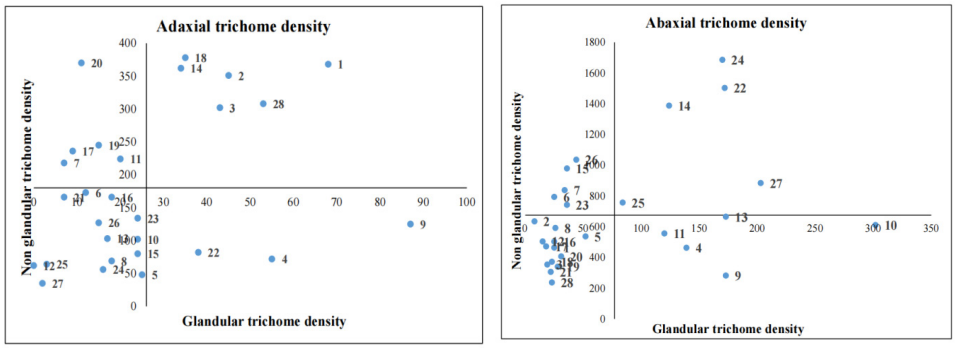

Fig. 2: Distribution of trichomes on the adaxial and abaxial leaf surface of parents and hybrids (1-Akshaya (Ak), 2-Manuprabha (MP), 3-Vellayani Vijai (VV), 4-AVTO1726, 5-AVTO1707, 6-AVTO0922, 7-AVTO0301, 8-AVTO1314, 9-EC519806 (EC), 10-LC, 11- AkxAVTO1726, 12-VVxAVTO1726, 13-AkxAVTO1707, 14-MPxAVTO1707, 15, VVxAVTO1707, 16-MPxAVTO0922, 17-VVxAVTO0922, 18-MPxAVTO0301, 19-VVxAVTO0301, 20-AkxAVTO1314, 21-VVxAVTO0314, 22-AkxEC, 23-MPxEC, 24-VVxEC, 25-AkxLC, 26-MPxLC, 27-VVxLC, 28-Anagha

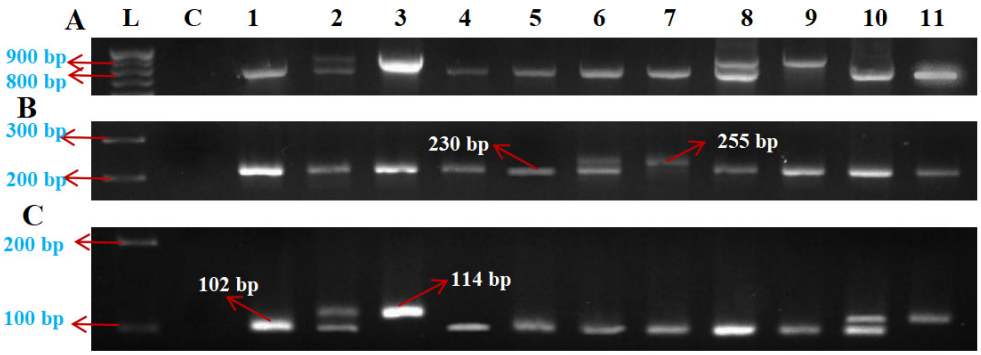

Fig. 3: Amplification of genomic DNA in hybrids with Akshaya as female parent (**A**) SCAR2 primer for *Ty 2* (R-900 bp & S-800 bp), (**B**) SLM 10-46 primer for *Ty 6* (R-255 bp & S-230 bp), (**C**) TY-1/3_K primer for *Ty 1/3* (R-114 bp & S-102 bp). Lanes: L-Ladder, C-Control, 1-Akshaya, 2-Akshaya×AVTO1707, 3-AVTO1707, 4-Akshaya×EC519806, 5-EC519806, 6-Akshaya×LC, 7-LC, 8-Akshaya×AVTO1726, 9-AVTO1726, 10-Akshaya×AVTO1314, 11-AVTO1314. Lane for C: Lanes: L-Ladder, C-Control, 1-Akshaya, 2-Akshaya×AVTO1707, 3-AVTO1707, 4-Akshaya×EC519806, 5-EC519806, 6-Akshaya×LC, 7-LC, 8-Akshaya×AVTO1314, 9-AVTO1314, 10- Akshaya×AVTO1726, 11-AVTO1726. * R-resistant allele, S-susceptible allele

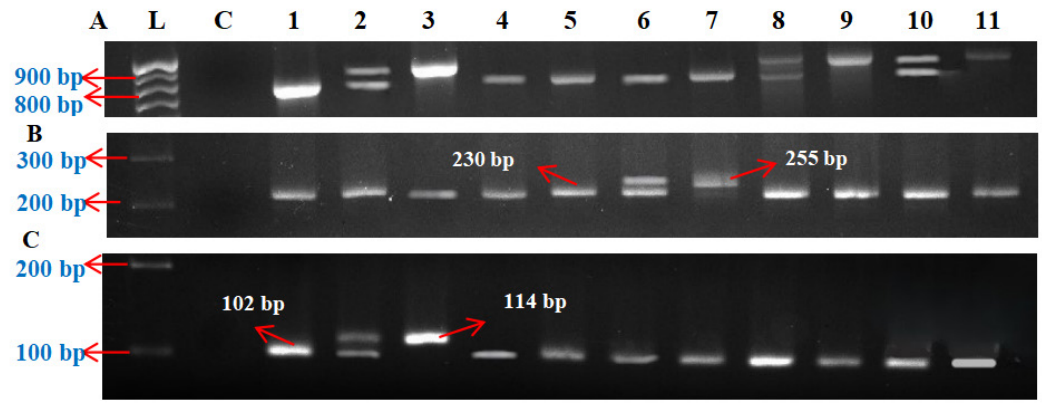

Fig. 4: Amplification of genomic DNA in hybrids with Manuprabha as female parent (**A**) SCAR2 primer for *Ty 2* (R-900 bp & S-800 bp), (**B**) SLM 10-46 primer for *Ty 6* (R-255 bp & S-230 bp), (**C**) TY-1/3_K primer for *Ty 1/3* (R-114 bp & S-102 bp). Lanes: L-Ladder, C-No Template Control, 1-Manuprabha, 2-Manuprabha×AVTO1707, 3-AVTO1707, 4-Manuprabha×EC519806, 5-EC519806, 6-Manuprabha×LC, 7-LC, 8-Manuprabha×AVTO0922, 9-AVTO0922, 10-Manuprabha×AVTO0301, 11-AVTO0301.* R-resistant allele, S-susceptible allele

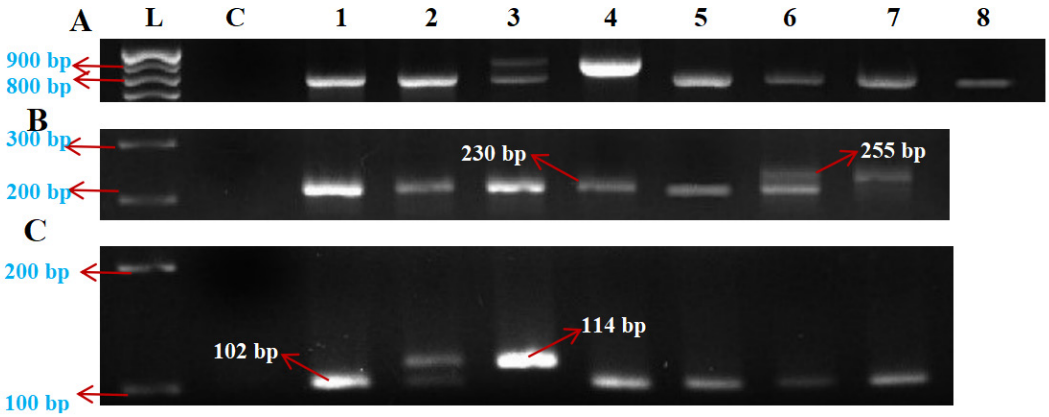

Fig. 5: Amplification of genomic DNA in hybrids with Vellayani Vijai (VV) as female parent (**A**) SCAR2 primer for *Ty 2* (R-900 bp & S-800 bp), (**B**) SLM 10-46 primer for *Ty 6* (R-255 bp & S-230 bp), (**C**) TY-1/3_K primer for *Ty 1/3* (R-114 bp & S-102 bp). Lanes#: L-Ladder, C-Control, 1-VV, 2-VV, 3-VV×AVTO1707, 4-AVTO1707, 5-VV×EC519806, 6-EC519806, 7-VV×LC, 8-LC. #Lanes for B & C: L-Ladder, C-Control, 1-VV, 2-VV×AVTO1707, 3-AVTO1707, 4-VV×EC519806, 5-EC519806, 6-VV×LC, 7-LC. * R-resistant allele, S-susceptible allele

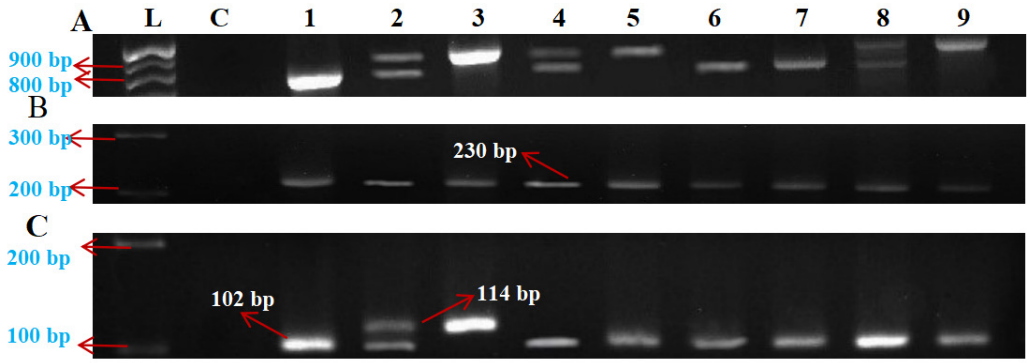

Fig. 6: Amplification of genomic DNA in hybrids with Vellayani Vijai (VV) as female parent (**A**) SCAR2 primer for *Ty 2* (R-900 bp & S-800 bp), (**B**) SLM 10-46 primer for *Ty 6* (R-255 bp & S-230 bp), (C) TY-1/3_K primer for *Ty 1/3* (R-114 bp & S-102 bp). Lanes: L-Ladder, C-Control, 1-VV, 2-VV×AVTO1726, 3-AVTO1726, 4-VV×AVTO0922, 5-AVTO0922, 6-VV×AVTO1314, 7-AVTO1314, 8-VV×AVTO0301, 9-AVTO0301. * R-resistant allele, S-susceptible allele

had *Ty 6,* and were resistant under field screening, but were susceptible under artificial screening, indicating the need for additional *Ty* genes for stable resistance. Gill et al. (2019) reported that *Ty 6* of chromosome 10 confers resistance to ToLCV in the presence of the *Ty 3* and *ty 5* genes.

Conclusion

Evaluation of 17 hybrids along with their parents revealed that hybrids of EC519806 and Local collection (Idukki) exhibited significant heterosis for the number of fruits per plant, and hybrids with AVTO lines as male parent exhibited significant heterosis for fruit size and weight, and hence can be utilised for breeding programmes for improving fruit number and size, respectively. High glandular to non-glandular trichome density ratio on the abaxial leaf surface was an important factor in determining the ToLCD tolerance, as evidenced by the performance of AVTO1726, EC519806, Local Collection (Idukki), Akshaya×AVTO1726 and Akshaya×AVTO1707. AVTO1707 and AVTO1726 and their hybrids having *Ty 2*and *Ty 1/3* showed high to moderate resistance under field and artificial screening. *Ty 6* was detected only in the local collection (Idukki) and its crosses, and the hybrids with *Ty 6* were resistant under field screening, but susceptible under artificial screening, indicating the need for additional Ty genes for stable resistance. Although none of the reported *Ty* genes could be detected in EC519806, the parent as well as its hybrids displayed resistance and further studies are required to decipher its mechanism. The genotypes identified in this study that contributed to various agronomic traits and ToLCD resistance can be effectively utilised for resistance breeding in tomato.

Acknowledgments

We are thankful to the World Vegetable Center, Taiwan and the National Bureau of Plant Genetic Resources, New Delhi, for providing the seeds of genotypes used in the study. Authors also acknowledge the financial support received from Kerala Agricultural University, Thrissur, Kerala, during the study.

References

Aboul-Maaty, N. A. F., & Oraby, H. A. S. (2019). Extraction of high-quality genomic DNA from different plant orders by applying a modified CTAB-based method. Bulletin of the National Research Centre, 43(1), 1-10. https://doi.org/10.1186/s42269-019-0066-1.

Achari, R., Fakruddin, B., Rangaswamy, K. T., & Savithramma, D. L. (2019). Screening for identification of resistance sources of tomato against tomato leaf curl virus (ToLCV) from segregating population and private sector hybrids under natural disease incidence. International Journal of Current Microbiology and Applied Sciences, 8(8), 844-852. https://doi.org/10.20546/ijcmas.2019.808.097.

Anjitha, A. R., Antony, P. D., Joseph, J., Louis, V., & Kutty, M. S. (2023). Evaluation of Tomato Genotypes for Tomato Leaf Curl Virus (ToLCV) Resistance. Journal of Tropical Agriculture, 61(1), 68-77. https://jtropag.kau.in/index.php/ojs2/article/view/1218.

Banerjee, M. K., & Kalloo, G. (1987). Sources and inheritance of resistance leaf curl virus in *Lycopersicon*. Theoretical and Applied Genetics, 73, 707-710. https://doi.org/10.1007/BF00260780.

Bhattarai, K., Sharma, S., & Panthee, D. R. (2018). Diversity among modern tomato genotypes at different levels in fresh-market breeding. International Journal of Agronomy. https://doi.org/10.1155/2018/4170432.

Chandel, R., Sadashiva, A. T., & Singh, T. H. (2019). Evaluation of tomato parental lines for leaf curl disease resistance and its validation through molecular markers. Indian Journal of Horticulture, 76(4), 678-682.

Chen, H., Lin, C., Yoshida, M., Hanson, P., & Schafleitner, R. (2015). Multiplex PCR for detection of tomato yellow leaf curl disease and root-knot nematode resistance genes in tomato (*Solanum lycopersicum* L.). International Journal of Plant Breeding and Genetics, 9(2), 44-56. https://scialert.net/abstract/?doi=ijpbg.2015.44.56.

Elkind, Y., Gurnick, A., & Kedar, N. (1991). Genetics of semi determinate growth habit in tomato. Horticultural Science, 26(8), 1074-1075. https://doi.org/10.21273/HORTSCI.26.8.1074.

Firdaus, S. (2012). Identification of whitefly resistance in tomato and hot pepper. PhD thesis, Wageningen University, Wageningen.

Firdaus, S., Heusden, A. W. V., Hidayati, N., Supena, E. D. J., Visser, R. G., & Vosman, B. (2012). Resistance to Bemisia tabaci in tomato wild relatives. Euphytica, 187, 31-45. https://doi.org/10.1007/s10681-012-0704-2.

Fonseca, A., & Patterson, F. L. (1968). Hybrid vigour in a seven parent diallel cross in common winter wheat (*T. aestivum* L.).Crop Science, 8(1), 85-88. https://doi.org/10.2135/cropsci1968.0011183X000800010025x.

Gill, U., Scott, J. W., Shekasteband, R., Ogundiwin, E., Schuit, C., Francis, D. M., Sim, S. C., Smith, H., & Hutton, S. F. (2019). Ty-6, a major begomovirus resistance gene on chromosome 10, is effective against Tomato yellow leaf curl virus and Tomato mottle virus. Theoretical and Applied Genetics, 132, 1543-1554. https://doi.org/10.1007/s00122-019-03298-0.

Gopinath, P. P., Parsad, R., Joseph, B., & Adarsh, V. S. (2021). grapesAgri1: Collection of shiny apps for data analysis in agriculture. Journal of Open Source Software. https://doi.org/10.21105/joss.03437.

Kadirvel, P., De la Peña, R., Schafleitner, R., Huang, S., Geethanjali, S., Kenyon, L., Tsai, W., & Hanson, P. (2013). Mapping of QTLs in tomato line FLA456 associated with resistance to a virus causing tomato yellow leaf curl disease. Euphytica,190, 297-308. https://doi.org/10.1007/s10681-012-0848-0.

KAU. (2016). Package of Practices Recommendations: Crops 2016 (15th Ed.). Kerala Agricultural University, Thrissur.

Lapidot, M. (2007). Screening for TYLCV-resistance plants using whitefly-mediated inoculation. In: Czosnek H (ed.) Tomato yellow leaf curl virus disease: management, molecular biology, breeding for resistance. (pp. 329-342). Springer, Dordrecht.

Lekshmi, S. L., & Celine, V. A. (2020). Correlation and path analysis of tomato crop (*Solanum lycopersicum* L.) under protected conditions. The Journal of Research ANGRAU, 48(4), 53-58. https://epubs.icar.org.in/index.php/TJRA/article/

view/133597.

McKinney, H. (1923). Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Journal of Agricultural Research, 26, 195.

Mutschler, M. A., Doerge, R. W., Liu, S. C., Kuai, J. P., Liedl, B. E., & Shapiro, J. A. (1996). QTL analysis of pest resistance in the wild tomato *Lycopersicon pennellii*: QTLs controlling acylsugar level and composition. Theoretical and Applied Genetics, 92, 709-718. https://doi.org/10.1007/BF00226093.

Nadkarni, S. R., Jayalekshmy, V. G., Umamaheshwaran, K., Harikrishnan, P. J. (2017) Evaluation of tomato and allied species for tomato leaf curl virus (ToLCV) resistance (*Solanum lycopersicum* L.). International Journal of Pure & Applied Bioscience, 5(3), 271-277.

Nevame, A. Y. M., Xia, L., Nchongboh, C. G., Hasan, M. M., Alam, M., Yongbo, L., Wenting, Z., Yafei, H., Emon, R. M., Ismail, M. R., & Efisue, A. (2018). Development of a new molecular marker for the resistance to tomato yellow leaf curl virus. BioMed Research International. Hindawi. https://doi.org/10.1155/2018/8120281.

Patel, R., Parihar, A., Makwana, M. A., & Kulshreshtha, K. (2021). Validation of known ToLCV markers associated with ToLCV resistance in tomato through Bulked Segregant Analysis. IndianJournal of Genetics andPlant Breeding, 81(3), 466-468. https://doi.org/10.31742/IJGPB.81.3.15.

PDVR. (1997). Resistant varietal trials. In: Proceedings of XVI group meeting on vegetable research, Project Director of Vegetable Research, Varanasi.

Rojalin, M., Tripathy, P., Sahu, G. S., Dash, S. K., Lenka, D., Tripathy, B., & Sahu, P. (2018). Character association and path analysis study in determinate tomato (*Solanum lycopersicum* L.). International Journal of Current Microbiology and Applied Sciences, 7, 863-870.

Sharma, B. R., & Sharma, O. P. (1984). Field reaction of some root-knot resistant tomato varieties and hybrids to tobacco mosaic and tobacco leaf curl viruses.Indian Journal of Mycology and Plant Pathology, 14(2), 48-150.

Srivastava, R., Prasanna, H. C., Prasad, V. M., Singh, D., & Bahadur, V. (2020). Molecular assisted TLCNDV resistant breeding in tomato (*Solanum lycopersicum* L.). Journal of Pharmacognosy and Phytochemistry, 9(6), 105-109.

Tipu, M. M. H., Jahan, R., Rahman, J., Riad, M. I., Rahman, M. M., & Nabi, K. E. (2021). Status of major diseases of brinjal and tomato in charland of Jamalpur and Sherpur districts of Bangladesh. Plant Science Today, 8, 161-165. https://doi.org/10.14719/pst.2021.8.1.988.

Verlaan, M. G., Hutton, S. F., Ibrahem, R. M., Kormelink, R., Visser, R. G., Scott, J. W., Edwards, J. D., & Bai, Y. (2013). The tomato yellow leaf curl virus resistance genes Ty-1 and Ty-3 are allelic and code for DFDGD-class RNA– dependent RNA polymerases. PLoS genetics. https://doi.org/10.1371/journal.pgen.1003399.

Vidavsky, F., Leviatov, S., Milo, J., Rabinowitch, H. D., Kedar, N., & Czosnek, H. (1998). Response of tolerant breeding lines of tomato, *Lycopersicon esculentum*, originating from three different sources (*L. peruvianum*, *L. pimpinellifolium* and *L. chilense*) to early controlled inoculation by tomato yellow leaf curl virus (TYLCV). Plant Breeding, 117(2), 165-169. https://doi.org/10.1111/j.1439-0523.1998.tb01472.x.

Yadav, K. (2011). Incorporation of tomato leaf curl virus (ToLCV) resistance in bacterial wilt resistant tomato. PhD (Ag) thesis, Kerala Agricultural University, Thrissur.

Yan, Z., Pérez-de-Castro, A., Díez, M. J., Hutton, S. F., Visser, R. G., Wolters, A. M. A., Bai, Y., & Li, J. (2018). Resistance to tomato yellow leaf curl virus in tomato germplasm. Frontiers in Plant Science, 9, 1198. https://doi.org/10.3389/fpls.2018.01198.

सारांश

टमाटर लीफ कर्ल वायरस रोग (टी.ओ.एल.सी.डी) टमाटर की खेती में एक प्रमुख बाधा है, और इस रोग के प्रबंधन के लिए मेज़बान पौधों में प्रतिरोध सबसे प्रभावी रणनीति मानी जाती है। खेत में प्रतिरोधी बताई गई अथवा टी-वाई जीन धारण करने वाली सात लाइनों को केरल की तीन वाणिज्यिक किस्मों के साथ संकरण किया गया। प्राप्त सतह संकरों के मूल्यांकन से यह ज्ञात हुआ कि वेल्लायनी विजई× ई सी 519806, अक्षय × ए वी टी ओ 1726, अक्षय × ई सी 519806 तथा अक्षय × ए वी टी ओ 1707 संकरों में खेत की परिस्थितियों में उच्च स्तर का प्रतिरोध पाया गया, जबकि ई सी 519806 के संकर तथा अक्षय × ए वी टी ओ 1726 में अधिक उपज दर्ज की गई। पत्ती की निचली (एबैक्शियल) सतह पर ग्रंथियुक्त और अ-ग्रंथिय ट्राइकोम के घनत्व का उच्च अनुपात, खेत में स्क्रीनिंग के दौरान जीनोटाइप की सहनशीलता निर्धारित करने का एक महत्वपूर्ण कारक पाया गया। ए वी टी ओ लाइनों के साथ किए गए संकरणों में टी-वाई जीन से संबद्ध मार्कर पाए गए, जबकि खेत में प्रतिरोध प्रदर्शित करने वाली अभिग्रहण ई सी 519806 में टी-वाई जीन से जुड़े कोई भी मार्कर नहीं पाए गए। स्थानीय संग्रह (इडुक्की) तथा उसके संकरों में टी-वाई 6 की उपस्थिति पाई गई। अतः, वर्तमान अध्ययन में संकरों का स्व-प्रदर्शन, हेटेरोसिस तथा रोग प्रतिक्रिया भविष्य के टी ओ एल सी वी प्रतिरोधी प्रजनन कार्यक्रमों के लिए श्रेष्ठ टमाटर जीनोटाइप के चयन में उपयोगी सिद्ध हो सकती है।