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Original Research Paper

Hybrid analysis for ToLCV resistance in tomato (*Solanum lycopersicum* L.) and molecular validation of *Ty-3* gene

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ABSTRACT

Tomatoes (*Solanum lycopersicum* L.) are widely recognized as a nutritious food due to their rich content of essential nutrients. Their growing popularity in recent years is largely attributed to lycopene, a powerful antioxidant known for its anti-cancer properties. However, the tomato leaf curl virus (ToLCV) represents a substantial risk to tomato crops at global level resulting in 100% crop loss. Therefore, the aim of this study was to develop and screen the tomato hybrids for ToLCV resistance and validate the presence of *Ty-3* gene. The study involved evaluating 12 parents, 8 hybrids and 7 double cross hybrids carrying *Ty-3* genes, which underwent screening at both phenotypic and genotypic levels. Assorted array of co-dominant sequence characterized amplified region (SCAR) markers were utilised to assess the resistance genes linked with tomato leaf curl virus (ToLCV), focusing specifically on *Ty-3*. The chosen markers P6-25, FLUW-25F and SCAR-1 demonstrated high reliability in distinguishing susceptible and resistant lines, facilitating the efficient identification of homozygous or heterozygous alleles relevant to ToLCV resistance. The screening resulted in the identification of three resistant parents (CBESL159, CBESL162 and CBESL169), two hybrids (H5-CBESL133×CBESL169 and H7-CBESL146×CBESL162) and two double hybrids (H5xH7 and H4xH5). These promising parents hold potential as parental materials for developing lines or hybrids with genes providing strong and enduring resistance against ToLCV and yield improvement. The double hybrids can be used for development of breeding lines.

Keywords: Molecular screening, SCAR marker, *Solanum lycopersicum* L., ToLCV, *Ty-3* gene, yield

INTRODUCTION

Tomatoes are esteemed as a protective food due to their rich content of nutrients like lycopene, beta-carotene, vitamin C, and flavonoids. Particularly, the surge in popularity of tomatoes in recent years is attributed to the antioxidative and anticancer properties of lycopene (Fentik, 2017). Nonetheless, the tomato leaf curl virus (ToLCV) represents a significant risk to tomato crops worldwide. It stands as one of the most destructive global diseases disseminated by the whitefly. It induces severe symptoms including cupping, leaf chlorosis, stunting and diminished flower and fruit development, resulting in substantial yield losses that can reach up to 100% (Pico et al., 1996; Singh et al., 2018). As a result, it serves as a constraining factor in tomato cultivation, impacting both open-field and protected cultivation systems. Consequently, the identification of resistant genotypes to ToLCV holds significant value for utilization in breeding programs aimed at developing new, high-yielding, and resilient genotypes

Plant breeders are extensively exploring various germplasm sources, from cultivated to wild, to identify resistance traits against tomato leaf curl virus. Understanding the phenotypic and morphological characteristics of resistant lines is pivotal for commercial production of disease-resistant varieties (Gharib et al., 2021). In addressing the challenge of leaf curl disease in tomatoes, researchers have pinpointed six independently inherited tomato leaf curl disease resistance genes across various wild tomato species. Of the six introgressions (*Ty-1*, *Ty-2*, *Ty-3*, *Ty-4*, *Ty-5* and *Ty-6*) providing resistance to tomato leaf curl disease, the partially dominant *Ty-3* genes hold particular importance for the development of resistant hybrids because of their gene action (Prasanna et al., 2015).

The primary objective of this endeavour was to meticulously screen parents, hybrids, and double crosses with a specific focus on evaluating their resistance to tomato leaf curl disease (ToLCV) at both phenotypic and genotypic level.

MATERIALS AND METHODS

The present study was carried out at the University orchard of the Department of Vegetable Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, from March 2022 to 2024 geographically situated at 11°N latitude and 77°E longitude.

Plant materials

Twelve parents, which includes three lines from repository of Department of Vegetable Science at TNAU, Coimbatore (CBESL129, CBESL133 and CBESL154), three lines from IIHR (CBESL142, CBESL143 and CBESL146) and six lines (CBESL159, CBESL160, CBESL162, CBESL164, CBESL168 and CBESL169) from the World Vegetable Centre, Taiwan, along with 8 F_1 hybrids and 7 double cross hybrids developed using above parents in the previous study through MAGIC population (Purushothaman et al., 2022) were evaluated in randomized block design (RBD) with three replications. The evaluation was done in two seasons during early summer (March) of 2022 and 2023. F_1 hybrids, Arka Vishesh and Arka Rakshak, were used as check.

Screening for disease resistance

Field and artificial screening of parents and hybrids were done based on method suggested by Banerjee & Kalloo (1987). In artificial screening the insect vector transmission technique was employed to inoculate healthy tomato seedlings of parents and hybrids along with two check hybrids. Whiteflies were then transferred from the brinjal plants to tomato plants using an aspirator at a rate of 30-50 per plant. After a 48-hour acquisition access feeding period, these viruliferous whiteflies were used for inoculation. Symptoms and observations were recorded 45 days after inoculation. For molecular confirmation the genomic DNA was isolated from young tomato plant leaves following the established CTAB (cetyl-triethyl-ammonium-bromide) method outlined by Doyle & Doyle (1987). For the validation of *Ty-3* gene SCAR markers P6-25, FLUW-25F and SCAR-1 were employed (Table 1).

Observations were recorded on growth and yield parameters. The quality parameters such as T.S.S. (°Brix), lycopene and β-carotene (mg/100 g) (Rangana, 1979) and ascorbic acid content (mg/100 g) was calculated using the A.O.A.C. (1975) technique.

RESULTS AND DISCUSSION

Screening of parents and hybrids for resistance to ToLCV under field and artificial condition

The field screening took place during the early summer season of 2022 and 2023, with data collection occurring at 30, 60, and 90 days after transplanting.

Table 1 : The specifics details of SCAR markers, including gene-specific primers and the annealing temperature used for detecting tomato leaf curl virus (ToLCV)

Gene	Marker	Sequences	Marker type	Annealing temperature (°C)	PCR Product size (bp)		Reference
					Resistant (R)	Susceptible (S)	
Ty3	P6-25	F-5'GGTAGTGGAAATGATGCTGCTC-3' R-5' GCTCTGCCTATTGTCCCATATATAACC-3'	Co dominant	53°C-1min	450	320	Ji et al. (2007)
	SCAR 1	F-5'GCTCAGCATCACCTGAGACA-3' R-5'TGCAGGAACAGAATGATAGAAAA-3'	Co-dominant	58°C-20 sec	519	269	Dong et al. (2016)
	FLUW 25F	F-5'CAAGTGTGCATATACTTCATA(T/G) TCACC-3' R- 5'CCA TAT ATA ACC TCT GTT TCT ATT TCG AC-3'	Co dominant	53°C-1min	640	475	Salus et al. (2007)

Table 2 : Performance of the parents, hybrids and double hybrids for tomato leaf curl virus (ToLCV) disease reaction

Parent and hybrid	PDI of tomato leaf curl						PDS	CI	Disease group
	30DAT	Arc sine transformed	60 DAT	Arc sine transformed	90 DAT	Arc sine transformed			
Parents									
CBESL129	13.33	21.42	40.00	39.23	86.67	68.58	82.67	71.64	HS
CBESL133	6.67	14.96	33.33	35.26	66.67	54.74	37.33	24.89	MS
CBESL142	20.00	26.57	53.33	46.91	86.67	68.58	89.33	77.42	HS
CBESL143	6.67	14.96	40.00	39.23	53.33	46.91	45.33	24.18	MS
CBESL146	20.00	26.57	33.33	35.26	46.67	43.09	57.33	26.76	MS
CBESL154	0.00	0.00	20.00	26.57	53.33	46.91	45.33	24.18	MS
CBESL159	0.00	0.00	6.67	14.96	26.67	31.09	20.00	5.33	HR
CBESL160	0.00	0.00	13.33	21.42	33.33	35.26	34.67	11.56	MR
CBESL162	0.00	0.00	6.67	14.96	13.33	21.42	14.67	1.96	HR
CBESL164	0.00	0.00	6.67	14.96	13.33	21.42	28.00	3.73	R
CBESL168	0.00	0.00	6.67	14.96	13.33	21.42	22.67	3.02	R
CBESL169	0.00	0.00	6.67	14.96	6.67	14.96	25.33	1.69	HR
F_1 hybrids									
H1(CBESL142× CBESL160)	6.67	14.96	20.00	26.57	86.67	68.58	26.67	23.11	MS
H2(CBESL146× CBESL160)	0.00	0.00	13.33	21.42	40.00	39.23	34.67	13.87	MR
H3(CBESL154× CBESL168)	13.33	21.42	26.67	31.09	73.33	58.91	34.67	25.42	MS
H4(CBESL142× CBESL168)	0.00	0.00	13.33	21.42	40.00	39.23	32.00	12.80	MR
H5(CBESL133× CBESL169)	0.00	0.00	6.67	14.96	6.67	14.96	17.33	1.16	HR
H6(CBESL143× CBESL159)	0.00	0.00	6.67	14.96	13.33	21.42	24.00	3.20	R
H7(CBESL146× CBESL162)	0.00	0.00	6.67	14.96	13.33	21.42	25.33	3.38	R
H8(CBESL129× CBESL164)	0.00	0.00	13.33	21.42	40.00	39.23	33.33	13.33	MR
Double hybrids									
H1xH7	0.00	0.00	13.33	21.42	46.67	43.09	32.00	14.93	MR
H5xH7	0.00	0.00	6.67	14.96	13.33	21.42	12.00	1.60	HR
H8xH7	0.00	0.00	13.33	21.42	40.00	39.23	30.67	12.27	MR
H7xH5	6.67	14.96	13.33	21.42	53.33	46.91	32.00	17.07	MR
H1xH5	0.00	0.00	13.33	21.42	33.33	35.26	36.00	12.00	MR
H8xH5	0.00	0.00	13.33	21.42	33.33	35.26	36.00	12.00	MR
H4xH5	0.00	0.00	6.67	14.96	13.33	21.42	18.67	2.49	HR
Check hybrids									
Arka Rakesh	13.33	21.42	26.67	31.09	46.67	43.09	34.67	16.18	MR
Arka Vishesh	0.00	0.00	20.00	26.57	20.00	26.57	20.00	4.00	HR
Mean	4.56	6.11	18.67	23.45	40.78	38.01	34.21	16.35	-
S.D.	6.21	9.47	12.19	8.77	23.86	15.43	17.27	17.93	-
S.Ed	1.15	1.76	2.26	1.63	4.43	2.87	3.21	3.33	-
CD	2.38	3.62	4.66	3.36	9.13	5.90	6.61	6.86	-

Table 3 : Reaction of parents and hybrids against ToLCV at glass house condition

Parental line/ hybrid	PDI	Arc sine PDI	PDS	Arc sine PDS	CI	Disease reaction
Parental lines						
CBESL129	96.67	79.48	44	41.55	72.50	HS
CBESL133	73.33	58.91	28	31.95	36.67	MS
CBESL142	73.33	58.91	52	46.15	73.33	HS
CBESL143	96.67	79.48	44	41.55	72.50	HS
CBESL146	96.67	79.48	48	43.85	72.50	HS
CBESL154	46.67	43.09	28	31.95	23.33	MS
CBESL159	6.67	14.96	12	20.27	3.33	HR
CBESL160	16.67	24.09	32	34.45	8.33	R
CBESL162	6.67	14.96	12	20.27	3.33	HR
CBESL164	23.33	28.88	4	11.54	5.83	R
CBESL168	13.33	21.42	16	23.58	6.67	R
CBESL169	13.33	21.42	12	20.27	3.33	HR
F_1 hybrids						
H1 (CBESL142×CBESL160)	50.00	45.00	32	34.45	37.50	MS
H2 (CBESL146×CBESL160)	33.33	35.26	20	26.57	16.67	MR
H3 (CBESL154×CBESL168)	46.67	43.09	20	26.57	23.33	MS
H4 (CBESL142×CBESL168)	20.00	26.57	20	26.57	15.00	MR
H5 (CBESL133×CBESL169)	13.33	21.42	8	16.43	3.33	HR
H6 (CBESL143×CBESL159)	16.67	24.09	12	20.27	8.33	R
H7 (CBESL146×CBESL162)	13.33	21.42	12	20.27	3.33	HR
H8 (CBESL129×CBESL164)	16.67	24.09	32	34.45	8.33	R
Double cross hybrids						
H1xH7	33.33	35.26	20	26.57	16.67	MR
H5xH7	10.00	18.43	16	23.58	2.50	HR
H8xH7	20.00	26.57	12	20.27	10.00	MR
H7xH5	20.00	26.57	8	16.43	15.00	MR
H1xH5	60.00	50.77	16	23.58	15.00	MR
H8xH5	46.67	43.09	8	16.43	11.67	MR
H4xH5	13.33	21.42	8	16.43	3.33	HR
Check hybrids						
Arka Rakshak	60	50.76852	28	31.94	16.8	MR
Arka Vishesh	40	39.23155	24	29.33	9.6	R
Mean	38.56	38.18	20.67	26.15	21.54	-
S.Ed	27.16	18.29	12.40	8.62	21.85	-
CD	4.96	3.34	2.26	1.57	3.99	-

PDI- Percentage of disease incidence, PDS- Percentage of disease severity, CI- Coefficient of the infection, Arcsine- Arcsine data transformation value, HR- Highly resistant, R- Resistant, MR- Moderately resistant, MS- Moderately susceptible, S- Susceptible, S. Ed- Standard error difference, C.D- Critical difference at 5 per cent level of significance

Based on the per cent disease incidence (PDI), the parents and hybrids were categorized into highly resistant (0-10%), moderately resistant (10-30%), moderately susceptible (30-70%), and highly susceptible (70-90%) (Sharma et al., 1984).

Observation on field screening at 30th, 60th, 90th days after transplanting indicated that the three parents (CBESL159, CBESL162 and CBESL169), two hybrids (H5 and H7) and two double cross hybrids (H5XH7 and H4XH5) exhibited a highly resistant disease reaction with a Coefficient of infection (CI) value less than 2, indicating a high level of resistance throughout the period. Whereas, CBE SL 164, CBE SL 168, H6 and H7 exhibited a resistant (R) reaction to ToLCV with CI value less than 4.5, while, the check hybrid Arka Vishesh displayed highly resistant and Arka Rakshak exhibited a moderately resistant response with CI value of 4.5 and 16.18, respectively (Table 2). These lines are highly suitable for ToLCV-resistant breeding. The present results are in conformity with the findings of Purushothaman et al. (2023).

Screening of tomato parents and hybrids for resistance to ToLCV under artificial screening at glass house condition at 45 days after transplantation indicated that the coefficient of infection (CI) ranged from 2.5 to 73.33 across all parent and hybrids (Table 3), including the two check hybrids. Three parents (CBESL159, CBESL162 and CBESL169), two hybrids (H5 and H7) and two double hybrids (H5XH7 and H4XH5), displayed a highly resistant reaction with CI value below 4, indicating a robust resistance level. On the other hand, three parental lines (CBESL160, CBESL164 and CBESL168), two hybrids (H8 and H6) and check hybrid Arka Vishesh were deemed resistant (Table 3 & 4).

Three parental lines, CBESL159, CBESL162, and CBESL169, exhibited a high degree of resistance to tomato leaf curl virus (ToLCV). This resistance trait was successfully transferred to two hybrid lines, H5 and H7. Additionally, the double cross hybrids H5xH7 and H4xH5, which were derived from these hybrids, also demonstrate enhanced resistance to ToLCV under both field conditions and artificial screening methods. These findings align with the results reported by Babu et al. (2018) who revealed that AVRDC tomato lines containing resistance-conferring crosses displayed greater resistance to ToLCV, while, maintaining high yield potential.

Observations revealed that lines carrying *Ty* genes demonstrated resistance to ToLCV disease, especially those containing *Ty-3* genes exhibiting a heightened level of resistance. Similar results were reported by Vijeth et al. (2018) and Dhital et al. (2023) in tomato lines harbouring *Ty* genes. It was also observed that many parents and hybrids carrying resistant genes displayed mild symptoms of the disease to the presence of other viruses, mixed infections are often encountered due to the transmission vector, as whiteflies are capable of transmitting more than one type of virus (Diaz Pendon et al., 2010). In conclusion, the successful transfer of ToLCV resistance from the CBESL lines to the hybrid and double cross lines, along with their consistent performance under various screening conditions, demonstrates the potential of these materials for developing ToLCV-resistant tomato varieties with high yield potential.

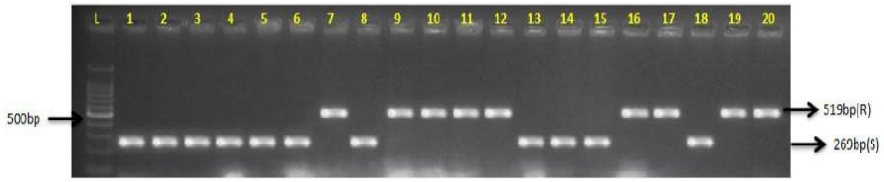
To uncover novel resistance loci, it is essential to investigate molecularly whether the resistance reported is controlled by allelic variants of the known *Ty* genes.

Table 4 : A scale of whitefly mediated screening for categorizing the parental lines, hydrids and double hybrids for disease reaction to tomato leaf curl virus (TLCV) accordance with the reaction by Banerjee and Kalloo (1987)

Scale of CI value	Disease reaction	No. of. parents and hybrids	Parent and hybrid
0-4	HR	7	CBESL159, CBESL162, CBESL169, H5, H7, H5XH7, H4XH5
5-9	R	6	CBESL160, CBESL164, CBESL168, H6,H8,Arka Vishesh
10-19	MR	8	H2, H4, H1XH7, H8XH7, H7XH5,H1XH5, H8XH5, Arka Rakshak
20-39	MS	4	CBESL133, CBESL154, H1, H3
40-69	S	0	-
70-100	HS	4	CBESL129, CBESL142, CBESL143, CBESL146

Comprehensive approach that combines molecular analysis with inoculation is imperative. The integration of molecular and phenotypic screening methods will provide a more accurate and reliable assessment of resistance, ultimately leading to the development of more effective and durable resistance in tomato cultivars. By adopting this approach, breeders can accelerate the development of ToLCV-resistant varieties, enhancing the sustainability and productivity of tomato production worldwide.

Molecular validation of *Ty-3* genes in parents and hybrids using SCAR markers



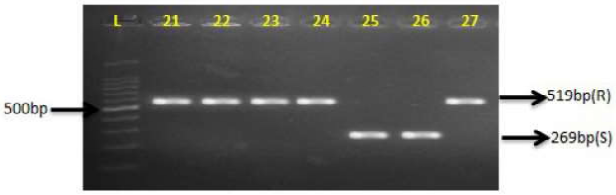
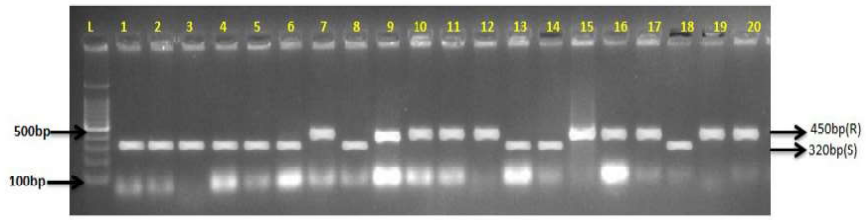


Fig. 1 : SCAR 1 marker



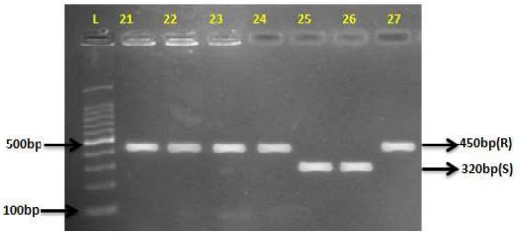


Fig. 2 : P625 marker

L: indicates lane- 100 bp ladder ,1. CBESL 129 2. CBE SL 133 3. CBESL 142 4. CBESL 143 5. CBESL 146 6. CBESL 154 7. CBESL 159 8. CBE SL 160 9. CBESL 162 10. CBESL 164 11. CBESL 168 12. CBESL 169 13. H1 14. H2 15. H3 16. H4 17. H5 18. H6 19. H7 20. H8 21.H1xH7 22.H5xH7 23.H7xH5 24.H1xH5 25.H8xH5 26.H8xH7 27.H4xH5 R indicate - Resistant lines, S - indicate Susceptible lines

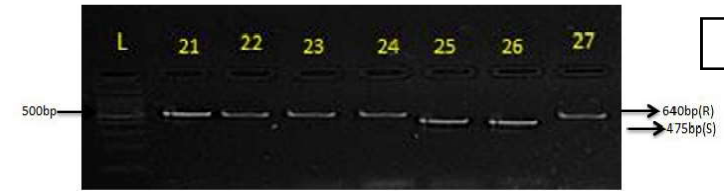


Fig. 3 : FLUW25F marker

Reproducible and reliable markers are pivotal in breeding for disease resistance. Ji et al. (2007) identified SCAR markers linked to begomovirus resistance loci on chromosome 6 of *S. chilense*. The SCAR1 co-dominant marker, upon PCR amplification, produced a single molecular band at 519 bp in five parents (CBESL 159, CBESL 162, CBESL 164, CBESL 168 and CBESL 169), four hybrids (H4, H5, H7 and H8) and five double cross hybrids (H1xH7, H5xH7, H7xH5, H8xH5 and H4xH5), which carry the *Ty-3* resistant allele in a homozygous state. Conversely, thirteen parents and hybrids such as CBESL 129, CBESL 133, CBESL 142, CBESL 143, CBESL 146, CBESL 154, CBESL 160, H1, H2, H3, H6, H1xH5, and H8xH7, observed amplicon at 269 bp signifies the absence of the Ty-3 gene (Fig. 1). This outcome aligns with Dong et al. (2016) and Nevame et al. (2018) findings, where they employed the SCAR1 marker to assess segregating populations of the cross between *S. lycopersicum*-A45 (resistant) and *S. lycopersicum*-A39 (susceptible) for the presence of Ty-3 gene.

The P6-25 marker yielded a solitary band of 630 bp in CBESL 159, CBESL 162, CBESL 164, CBESL 168, CBESL 169, H4, H5, H7, H8, H1xH7, H5xH7, H7xH5, H8xH5, and H4xH5, indicating the presence of the *Ty-3* resistant allele. Conversely, the remaining thirteen parents and hybrids, such as CBESL 129, CBESL 133, CBESL 142, CBESL 143, CBESL 146, CBESL 154, CBESL 160, H1, H2, H3, H6, H1xH5, and H8xH7, displayed a single band at 320 bp, indicating the absence of the *Ty-3* gene (Fig. 2). These results align with the findings of Prasanna et al. (2015), where they employed the P6-25 marker to analyse F_4 plants (double heterozygous) and F_5 progenies for the *Ty-2* and *Ty-3* genes. Ji et al. (2007) created PCR primers P6-25F2/P6-25R5 to detect *Ty-3* from *S. chilense* LA1969. These primers produced 450-bp fragments in TLCV resistant lines (LA2779 or Ih902), a 320-bp fragment for absence of *Ty-3* in lines (LA1932 and Gc171).

The FLUW25F marker distinguishes between resistant and susceptible, with a resistant band at 640 bp and a susceptible band at 475 bp (Chen et al., 2015). The gel electrophoresis results (Fig. 3) revealed a distinct pattern among the parents and hybrids tested for the presence of the *Ty-3* gene. The presence of a band at 640 bp signified the presence of the resistance allele in lines CBESL 159, CBESL 162, CBESL 164, CBESL 168, CBESL 169, H4, H5, H7, H8, H1xH7, H5xH7, H7xH5, H8xH5, and H4xH5, while, a band at 475 bp was observed in CBESL 129, CBESL 133, CBESL 142, CBESL 143, CBESL 146, CBESL 154, CBESL 160, H1, H2, H3, H6, H1xH5, and H8xH7, indicating the existence of susceptible alleles at the loci. The results were in accordance with the findings of Salus et al. (2007) and Martin et al. (2007) who developed PCR primers FLUW-25 for selecting *Ty-3/Ty-3* alleles of the *Ty3* locus.

Parents (CBESL 159, CBESL 162, CBESL 164, CBESL 168 and CBESL 169) hybrids (H4, H5, H7 and H8) and double hybrids (H1xH7, H5xH7, H7xH5 and H4xH5) exhibited resistance under field, artificial and molecular screening. The four hybrids (H4, H5, H7 and H8) possess the *Ty-3* resistance gene, indicating that this resistance is contributed by the parental lines (CBESL 159, CBESL 162, CBESL 164, CBESL 168 and CBESL 169), which are confirmed resistant by molecular screening. The double crosses (H1xH7, H5xH7, H7xH5, H8xH5 and H4xH5) include resistant parents and hybrids displayed high level of resistance to ToLCV.

Hybrid H6 (CBE SL 143 x CBE SL 159) exhibits resistance in field and glass house conditions, yet does not show resistance in molecular screening. This discrepancy in molecular screening results, as highlighted by Kumar et al. (2019), could be linked to the absence of amplification against specific primers/genes in certain breeding lines, possibly due to non-specificity at the primer-binding sites. Additionally, Sharma et al. (2019) suggested that this lack of amplification might be influenced by the activation of other tomato leaf curl virus (ToLCV) resistant genes from the parental lines.

Growth, yield and quality performance of *Ty-3* confirmed resistance lines with check hybrids (Table 5 & 6) resulted that double cross hybrids H5xH7 and H4xH5 were better than parental lines and check hybrids. These double crosses coupled with high yield and resistance can be used for further breeding lines.

CONCLUSION

The evaluation of parents and hybrids for resistance to tomato leaf curl virus (ToLCV) in both field and greenhouse conditions unveiled varying degrees of disease response. While, certain parents and hybrids exhibited strong resistance, others displayed moderate resistance or susceptibility. Notably, Arka Vishesh and

Table 5 : *Per se* performance for growth and yield of *Ty-3* confirmed resistance lines with check hybrids

Parent and hybrid	Plant height (cm)	No. primary branches	Days to first flowering (days)	No. of fruits cluster^{-1}	Single fruit weight (g)	No. of fruits plant^{-1}	Yield/ plant (g)
CBESL159	74.32	10.85	31.46	2.48	53.12	43.16	1960.419
CBESL162	111.26	8.56	29.46	2.75	58.46	44.56	2367.367
CBESL169	90.25	9.85	30.16	2.46	61.13	39.16	2291.545
H5	114.56	11.89	31.46	4.06	87.25	79.15	6324.451
H7	176.66	17.23	23.12	4.05	80.12	77.13	6076.446
H5xH7	199.43	9.90	20.99	4.55	88.83	86.15	6408.669
H4xH5	227.33	14.66	19.91	3.83	97.17	51.81	6866.964
Arka Rakshak	139.23	8.00	35.33	4.00	75.30	47.33	3559.26
Arka Vishesh	157.07	8.33	32.00	4.33	78.49	51.00	4000.53
Mean	143.35	11.03	28.21	3.61	75.54	57.72	4428.41
S.Ed	45.33	3.49	8.92	1.14	23.89	18.25	1400.38
C.D. at 5%	93.38	7.19	18.38	2.35	49.21	37.60	2884.79

S.Ed: standard error difference, C.D: critical difference at 5 per cent level of significance

Table 6 : *Per se* performance for quality of *Ty-3* confirmed resistance lines with check hybrids

Parent and hybrid	Pericarp thickness (cm)	No. of locules	TSS (^{o}brix)	TA	Ascorbic acid (mg/100 g)	Lycopene content (mg/100 g)	β-carotene content (mg/100 g)
CBESL159	0.67	3.00	6.34	0.1	24.75	8.55	11.85
CBESL162	0.54	3.00	6.95	0.27	34.32	9.61	15.46
CBESL169	0.72	4.00	5.11	0.41	35.17	8.48	9.11
H5 (CBESL133×CBESL169)	0.74	4.00	5.32	0.29	55.71	11.75	14.56
H7 (CBESL146×CBESL162)	0.66	2.00	7	0.42	54.32	10.81	15.43
H5xH7	0.79	4.00	6.98	0.4	42.34	10.81	13.54
H4xH5	0.71	5.00	7.96	0.59	57.98	12.38	23.4
Arka Rakshak	0.50	3.00	4.80	0.38	31.72	7.63	5.58
Arka Vishesh	0.53	3.67	5.70	0.33	33.83	9.77	6.00
Mean	0.65	3.52	6.24	0.35	41.13	9.98	12.77
S.Ed	0.21	1.11	1.97	0.11	13.01	3.15	4.04
C.D. at 5%	0.42	2.29	4.06	0.23	26.79	6.50	8.32

S.Ed: standard error difference, C.D: critical difference at 5 per cent level of significance

CBESL159, CBESL162, CBESL169 and H5 (CBESL133×CBESL169), H7 (CBESL146× CBESL162), H5xH7, H4xH5 demonstrated high resistance, while additional genotypes like CBESL160, CBESL164, CBESL168, H6 and H8 showed varying degrees of resistance. These underwent further molecular screening using SCAR markers, including SCAR-1, P625, and FLUW25F, to validate the presence of *Ty-3* gene. The parents CBESL159, CBESL162, CBESL169, hybrids H5 (CBESL133× CBESL169) and H7 (CBESL146×CBESL162), and double cross hybrids H5xH7 and H4xH5 have the potential to act as valuable breeding materials for the creation of resistance against ToLCV diseases. Particularly, the double cross hybrids provide important insights for future breeding endeavors by

developing lines with high yield, ToLCV resistance coupled with market quality aimed at producing resilient tomato hybrids or varieties, crucial for ensuring sustainable tomato production despite the challenges posed by ToLCV.

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