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RESEARCH ARTICLE

Genotype × environment interaction and molecular marker analyses reveal promising tomato lines for yield components, TSS and tomato leaf curl disease resistance

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Abstract

The objective of this study was to analyze genotype-by-environment interactions (GEI) related to yield as well as its component traits, and resistance against tomato leaf curl disease (ToLCD) using GGE biplot analysis. Based on prior research, selected tomato genotypes were evaluated for horticultural traits such as average fruit weight, yield, total soluble solids (TSS) and ToLCD resistance (natural hot-spot conditions) over five consecutive years (2020-2025). Additionally, molecular markers were employed to identify the presence of ToLCD resistance genes (*Ty-2* and *Ty-3*) in these genotypes. Field screenings categorized the genotypes into highly resistant, resistant, and moderately resistant groups. GEI analysis was conducted using GGE biplot techniques, with the R 'metan' package utilized for various visualizations, including mean vs. stability view and MGIDI analysis. Overall, 15 superior genotypes were identified based on average fruit weight, fruit yield, TSS and ToLCD resistance such as VRT-19, VRT-34, VRT-50, VRT-51, ToDVAR-5, ToLCV-32, H-88-78-1, H-88-78-2, jointless tomatoes VRJT-24, VRJT-32, VRJT-41-2, VRJT-37, VRJT-94; orange color tomato KB-17 and KB-3-1. These genotypes can be used in breeding to introgress yield-related traits and ToLCD resistance.

Keywords: Genotype-by-environment interaction, Tomato, ToLCD resistance, Molecular markers, MGIDI.

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Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable that plays a vital role in our daily diet. It is rich in vitamins A and C, fibers, potassium, phenolic compounds and antioxidant lycopene (Nasir et al., 2015). The world's total tomato production reached to 186.10 million tons from a 4.91 million hectare area with a productivity rate of 37.84 t/ha in 2022 (FAOSTAT, 2024). To feed an increasing world population of 9.8 billion by 2050 (https://www.un.org/), there is a need to sustain tomato production in the world. Although tomato crop suffers from various biotic/abiotic stresses and climate change factors such as insect pests (whitefly and fruit borer), diseases (tomato leaf curl virus, late blight, early blight, bacterial spot and bacterial wilt), abiotic stresses such as high temperature, drought, salinity and cold stress) and fruit quality for processing parameters (Causse et al., 2016). Tomato leaf curl disease (ToLCD) is a serious disease of tomato caused by tomato leaf curl virus (ToLCV) belonging to the genus begomovirus and transmitted by whitefly (*Bemisia tabaci*) that can cause entire crop damage. Hence, breeding new cultivars requires resistance sources to tackle this notorious disease. Conventional cum molecular

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breeding approaches have been deployed to introgress resistance genes (*Ty-2* and *Ty-3*) into cultivated backgrounds. Earlier, MAS has been applied in gene pyramiding of ToLCD-resistant genes (*Ty-2/Ty-3*) and as a result, superior genotypes/cultivars have been developed in tomatoes (Foolad and Panthee, 2012; Prasanna et al., 2015a, 2015b; Kumar et al., 2019; Tiwari et al., 2022, 2023, 2024).

The availability of diverse genetic resources for ToLCD resistance is essential for effective breeding (Zhou et al., 2015). Moreover, dissecting variability across environments allows breeders to identify suitable parents for various environments (Rasheed et al., 2023). Hence, to address this issue, several multivariate methods have been applied to analyze genotype-by-environment interaction (GEO) in many crops such as tomato (Ene et al., 2022), radish (Dhand and Garg, 2023), sweet potato (Gemechu et al., 2022) and others. Of which, the Genotype plus Genotype by Environment Interaction (GGE) statistical model includes the genotype (G) main effect and the genotype-by-environment interaction (GEI) effect to elucidate the suitable genotypes in different environments (Singamsetti et al., 2023; Singh et al., 2024). The GGE model uses a biplot to identify stable genotypes across different environments (Yan et al., 2000; Yan and Kang, 2003; Yan and Tinker, 2006; Yan et al., 2007). This study aimed to evaluate tomato genotypes against ToLCD resistance under natural field conditions in different environments for five years. Moreover, molecular markers were also used to identify resistance genes *Ty-2* and *Ty-3* in these genotypes. The GEI effect was assessed for ToLCD based on the GGE and AMMI biplot analysis. Thus, our study suggests potential tomato genotypes with stable resistance to ToLCD having *Ty-2/Ty-3* genes that can be used in gene pyramiding and breeding new cultivars.

Materials and Methods

Plant materials and field experiment

Twenty-five tomato genotypes were used in the study. Field experiments were conducted in a randomized complete block design (RCBD) in three replications in five years or environments during 2020-25 during rabi seasons at the vegetable research farm of ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi (25.18 N Latitude and 82.83 E Longitude). The different years represent environments i.e. rabi season 2020-21 (Environment 1 = E1), 2021-22 (E2), 2022-23 (E3), 2023-24 (E4), and 2024-25 (E5). Genotype-by-environment interaction analysis was conducted for horticultural traits in five environments (E1-E5). Tomato seeds were sown in the first week of September and then transplanted the first week of October every year. A total of 20 plants per genotype per replication were grown in paired rows on raised beds at 60×45 cm^2 spacing following our standard cultural practices (Tiwari et al., 2024).

Observations for horticultural traits

A total of four important horticultural traits were recorded in the genotypes average fruit weight (g), fruit yield (kg/plant), total soluble solids (TSS, °Brix), and tomato leaf curl disease (ToLCD, %) were recorded. Observations were recorded on five randomly selected plants in each replication. The average per fruit eight was determined from the average of 10 fruit weights. TSS was measured by a hand refractometer, as described earlier (Tiwari et al., 2024).

Tomato leaf curl disease (ToLCD) resistance assay under natural field conditions

Thirty genotypes were tested for ToLCD resistance under natural field conditions in rabi season for five years during 2020-24 (E1-E5) using two controls: Punjab Chhuhara (highly susceptible) and Kashi Aman (highly resistant). ToLCD symptoms were recorded at 45 and 90 days after transplanting as a score: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop; 4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stop (Friedmann et al. 1998). The average score was calculated from the total scores of all plants divided by the total number of plants. Resistance/susceptible category was classified based on percent disease incidence as highly resistant (score = 0; HR: 0–5%), resistant (score = 1; R: 5·1–12·0%), moderately resistant (score = 2; MR: 12·1–25%), moderately susceptible (score = 3; MS: 25·1–50·0%), susceptible (score = 4; S: 50·1–75%), and highly susceptible (score = 5; HS: 75·1–100%) (Banerjee and Kalloo, 1987).

Molecular marker analysis for host resistance genes (Ty-2 and Ty-3)

All genotypes were tested for the presence/absence of host resistance genes (*Ty-2* and *Ty-3*) against ToLCD using gene-specific or linked molecular markers following our previous protocols (Tiwari et al., 2024). Briefly, DNA was isolated from the young leaf tissues using the CTAB method and DNA quality and quantity were checked on 1% agarose gel and NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), respectively. The Polymerase Chain Reaction (PCR) mix constitutes a total volume of 15 µL containing 7.5 µL of 2X PCR buffer, 1-µL forward and 1-µL reverse primers, 3 µL DNA of each sample, and 2.5 µL PCR grade water (Genei Laboratories Pvt Ltd, Bangalore, India). PCR cycle was set up at 94°C/4 min, 36 cycles (94°C/1 min, 55°C/1 min, and 72°C/1 min), and 72°C for 10 min in a thermal cycler (BIO-RAD, CA, USA). PCR products were resolved on 1.5% agarose gels and visualized using the Alpha Innotech Gel-Doc system (Alpha Innotech, CA, USA).

Statistical analysis

Data was analyzed through Analysis of Variance (ANOVA) for genotypes (*n* = 25) and replications (3) followed by a post-hoc analysis using the Least significant difference (LSD) test ($p < 0.05$) with the GRAPES (Version 1.1.0) software (Gopinath et al. 2021). The GEI analysis was performed through GGE biolot using the "metan" package of R software. Phylogenetic analysis among the tomato genotypes was analyzed using the DARWin software.

Results and Discussion

Performance for yield components and processing traits

A total of 25 genotypes were evaluated in five environments/ years for important horticultural traits like average fruit weight, fruit yield and processing traits like TSS (Table 1). Based on the mean data of five years, the average fruit weight was recorded the highest in H-88-78-2 (184.3 g) followed by VRT-06 (126.64 g), VRT-51 (114.27 g), VRT-19 (109.81 g), VRJT-37 (91.12 g), KB-20 (90.81 g), VRJT-94 (88.41 g) and VRT-50 (87.26 g), whereas the lowest fruit weight was noticed on cherry tomato lines VRCRT-14 (8.17 g) and VRCYT-3 (8.90 g).

Similarly, the highest TSS was recorded in cherry tomatoes VRCYT-3 (8.36 °Brix) and VRCRT-14 (6.37 °Brix); common tomato VRT-67 (5.43 °Brix), jointless tomatoes like VRJT-32 (5.33 °Brix), VRJT-37 (5.22 °Brix) and VRJT-94 (5.22 °Brix), whereas lowest TSS was observed in KB-20 (3.98 °Brix) and ToLCV-16 (4.09 °Brix). Fruit yield per plant was recorded as maximum in jointless tomato VRJT-37 (2.79 kg), VRJT-24 (2.77 kg) and, VRJT-94 (2.70 kg) and common tomato ToDVAR (2.56 kg), whereas minimum yielding lines were H-88-78-2 (0.56 kg) and cherry tomato VRCRT-14 (0.88 kg). Many studies illustrated genetic parameters analysis and inheritance studies in tomatoes (Singh et al., 2015; Hussain et al., 2024). Hence, the findings of this study would be useful for increasing yield and component traits, processing, and ToLCD resistance in tomatoes.

Tomato leaf curl disease (ToLCD) resistance assays under field conditions and through molecular markers

Twenty-five tomato genotypes, including two controls (highly resistant: Kashi Aman, and highly susceptible: Punjab Chhuhara) were evaluated against ToLCD under natural field conditions in rabi seasons for five consecutive years (2020-2025) representing five environments (E1-E5). Year-wise results are summarised in Table 2. Fifteen genotypes were highly resistant (HR: 0–5%) such as VRT-19, VRT-34, VRT-50, VRT-51, ToDVAR-5, ToLCV-32, H-88-78-1, H-88-78-2, VRJT-24, VRJT-32, VRJT-41-2, VRJT-37, VRJT-94, KB-17 and KB-3-1; nine were resistant (R: 5·1–12·0%) such as VRT-06, VRT-30, VRT-67, ToLCV-16, ToLCV-28, VRCRT-14, VRCYT-3, KB-2 and KB-20; and one (VRT-02) was moderately susceptible (25·1–50%) compared to controls like Kashi Aman (HR: 0-5%) and Punjab Chhuhara (HS: 75·1–100%) (Table 2). Thus, we identified 15 potential highly resistant tomato genotypes, which can be used as parental lines in tomato breeding.

Molecular markers-based study showed the *Ty-3* resistance gene-specific band (519 bp) in 16 genotypes *viz*., VRT-06, VRT-19, VRT-30, VRT-34, VRT-50, VRT-51, ToDVAR-5, ToLCV-16, ToLCV-32, H-88-78-2, VRJT-32, VRJT-37, VRJT-94, KB-17, KB-20 and control (Kashi Aman) (resistant: 519 bp, susceptible: 269 bp) (Fig. 1). However, heterozygous condition of *Ty-3* gene was observed in VRT-67, ToLCV-28 and KB-2. *Ty-2* resistance gene-specific band (523 bp) was not amplified in any genotypes except positive control cv.

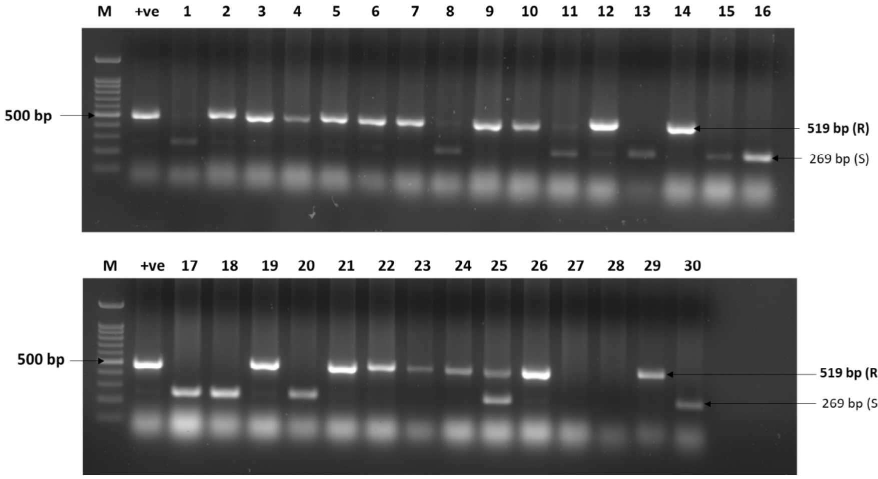

Fig. 1: Identification of ToLCD resistance gene *Ty-3* using SCAR molecular marker in tomato genotypes. Samples on the gel images: #1. VRT-02, #2. VRT-06, #3. VRT-19, #4. VRT-30, #5. VRT-34, #6. VRT-50, #7. VRT-51, #8. VRT-67, #9. ToDVAR-5, #10. ToLCV-16, #11. ToLCV-28, #12. ToLCV-32, #13. H-88-78-1, #14. H-88-78-2, #15. VRCRT-14, #16. VRCRT-15, #17. VRCYT-3, #18. VRJT-24, #19. VRJT-32, #20. VRJT-41-2, #21. VRJT-37, #22. VRJT-94, #23. KB-17, #24. KB-10, #25. KB-2, #26. KB-20, #27. KB-3-1, #28. KB-3-2, #29. Kashi Aman (+ve control), #30. Punjab Chhuhara. Note: Sample #16, #24, and #28 were additional samples here.

Table 1: Evaluation of tomato genotypes for yield related horticultural traits during 2020-2025 under natural field conditions at research farm of ICAR-IIVR, Varanasi

Sr. No.	*Genotype*	*Code*	*Average fruit wt. (g)*						*TSS (°Brix)*						*Yield/plant (kg)*					
			2020-21	*2021-22*	*2022-23*	*2023-24*	*2024-25*	*Mean*	*2020-21*	*2021-22*	*2022-23*	*2023-24*	*2024-25*	*Mean*	*2020-21*	*2021-22*	*2022-23*	*2023-24*	*2024-25*	*Mean*
1	VRT-02	G1	18.34	17.30	22.80	23.80	19.19	20.28	4.72	5.04	5.03	4.61	5.31	4.94	1.13	1.10	1.74	2.06	1.95	1.60
2	VRT-06	G2	120.71	123.22	143.00	126.40	119.87	126.64	4.31	4.33	5.02	4.61	5.35	4.73	1.33	1.07	2.24	1.02	1.45	1.42
3	VRT-19	G3	113.44	114.93	109.67	110.47	100.53	109.81	4.14	4.27	4.85	4.35	5.12	4.55	2.07	1.74	1.52	1.16	1.62	1.62
4	VRT-30	G4	52.39	53.65	54.87	45.27	50.40	51.31	4.37	4.21	4.71	4.04	5.05	4.48	1.03	0.97	0.98	1.01	1.08	1.02
5	VRT-34	G5	64.59	69.70	59.80	53.07	59.93	61.42	4.50	4.85	5.09	4.25	4.72	4.68	1.94	1.60	1.93	1.66	1.54	1.74
6	VRT-50	G6	82.56	86.41	89.87	97.33	80.13	87.26	4.40	4.64	4.63	4.30	4.81	4.56	2.08	1.69	1.64	1.52	1.72	1.73
7	VRT-51	G7	100.31	100.00	123.20	112.73	135.08	114.27	4.41	4.27	4.07	4.00	4.69	4.29	1.96	1.46	1.89	1.20	1.44	1.59
8	VRT-67	G8	32.39	31.42	33.80	32.77	35.07	33.09	5.20	5.42	5.80	4.99	5.73	5.43	1.12	1.02	2.27	0.99	1.23	1.33
9	ToDVAR-5	G9	72.33	67.00	74.83	70.53	84.73	73.89	4.60	4.62	4.77	4.61	4.20	4.56	2.47	2.27	2.60	2.71	2.74	2.56
10	ToLCV-16	G10	46.50	50.25	74.33	69.73	48.93	57.95	3.71	3.51	4.00	4.35	4.85	4.09	1.06	1.06	1.75	0.93	1.07	1.18
11	ToLCV-28	G11	70.00	72.33	70.00	89.80	63.07	73.04	4.23	4.27	4.14	4.61	5.12	4.47	1.97	2.10	1.88	1.11	1.44	1.70
12	ToLCV-32	G12	78.75	77.94	75.47	71.20	77.73	76.22	4.60	4.36	4.03	3.77	4.50	4.25	2.02	1.93	1.80	2.28	2.07	2.02
13	H-88-78-1	G13	28.67	37.67	34.00	35.67	32.40	33.68	4.80	5.00	5.17	4.94	4.20	4.82	1.69	1.85	1.54	1.61	1.53	1.64
14	H-88-78-2	G14	160.00	189.33	195.00	189.67	187.50	184.30	4.40	4.37	4.60	4.48	4.10	4.39	0.54	0.64	0.47	0.51	0.64	0.56
15	VRCRT-14	G15	6.83	7.50	10.63	8.30	7.58	8.17	5.28	5.57	7.37	6.43	7.22	6.37	0.52	0.66	1.21	1.00	0.99	0.88
16	VRCYT-3	G16	8.00	7.55	10.10	7.70	11.13	8.90	8.34	8.43	8.09	8.93	8.01	8.36	1.04	1.05	1.44	1.06	1.04	1.13
17	VRJT-24	G17	64.33	58.00	61.70	62.60	64.83	62.29	4.93	4.82	4.98	4.61	4.80	4.83	2.87	2.77	2.85	2.63	2.72	2.77
18	VRJT-32	G18	59.67	61.00	62.67	65.67	81.67	66.13	5.23	5.27	5.33	5.04	5.76	5.33	1.10	1.07	1.20	1.05	1.10	1.10
19	VRJT-41-2	G19	79.57	81.00	83.00	82.73	80.20	81.30	4.08	4.10	4.03	4.23	4.43	4.17	1.32	1.40	1.41	1.41	1.33	1.37
20	VRJT-37	G20	97.67	100.67	101.00	77.53	78.73	91.12	5.51	5.57	5.49	4.82	4.71	5.22	3.01	2.93	2.93	2.40	2.65	2.79
21	VRJT-94	G21	85.70	87.67	90.00	87.80	90.87	88.41	5.28	5.33	5.37	5.35	4.75	5.22	2.85	2.83	2.97	2.16	2.70	2.70
22	KB-17	G22	40.93	42.74	74.93	67.07	58.07	56.75	4.79	4.63	4.25	4.50	4.67	4.57	0.96	1.09	1.69	1.21	1.22	1.23
23	KB-2	G23	35.33	36.24	29.93	30.87	30.47	32.57	4.83	4.77	5.00	4.40	4.47	4.69	1.07	1.20	0.73	0.83	1.07	0.98
24	KB-20	G24	99.67	101.00	99.00	82.00	72.40	90.81	4.03	3.73	3.64	4.23	4.28	3.98	2.08	2.13	2.11	1.75	2.03	2.02
25	KB-3-1	G25	50.05	50.44	49.33	50.13	45.60	49.11	4.79	4.77	4.93	5.09	6.22	5.16	1.97	1.73	1.03	0.99	1.03	1.35
Mean			66.75	69.00	73.32	70.03	68.64	69.55	4.78	4.81	4.98	4.78	5.08	4.89	1.65	1.57	1.75	1.45	1.57	1.60
CD (P < 0.05)			2.62	1.94	2.26	2.46	1.82		0.23	0.14	0.1	0.15	0.10		0.15	0.21	0.16	0.15	0.11	
SE(m)			0.92	0.68	0.79	0.86	0.63		0.08	0.05	0.03	0.05	0.03		0.05	0.07	0.05	0.05	0.04	
CV (%)			2.38	1.7	1.87	2.13	1.61		2.99	1.78	1.3	1.93	1.21		5.67	8.2	5.84	6.42	4.45	

Table 2: Evaluation of tomato genotypes against Tomato leaf curl disease (ToLCD) resistance under natural field (hot-spot) conditions during 2020-2025 at research farm of ICAR-IIVR, Varanasi

Sr. No.	Genotype	Code	ToLCD (Per cent disease infection) reaction							*Ty-3/ Ty-2* gene
			2020-21	2021-22	2022-23	2023-24	2024-25	Mean	Class	
1	VRT-02	G1	25.00	27.50	32.83	24.53	50.00	31.97	MS	-
2	VRT-06	G2	5.00	6.83	9.16	0.00	5.00	5.20	R	*Ty-3*
3	VRT-19	G3	0.00	0.00	0.00	0.00	0.00	0.00	HR	*Ty-3*
4	VRT-30	G4	10.00	1.66	3.00	0.00	25.00	7.93	R	*Ty-3*
5	VRT-34	G5	2.66	0.66	3.00	0.00	2.33	1.73	HR	*Ty-3*
6	VRT-50	G6	0.00	3.33	2.00	0.00	0.00	1.07	HR	*Ty-3*
7	VRT-51	G7	10.00	4.33	5.06	0.00	0.00	3.88	HR	*Ty-3*
8	VRT-67	G8	5.00	10.50	10.66	0.00	5.00	6.23	R	*Ty-3 (H)*
9	ToDVAR-5	G9	0.00	0.00	0.00	0.00	0.00	0.00	HR	*Ty-3*
10	ToLCV-16	G10	20.0	11.33	12.16	0.00	10.00	10.70	R	*Ty-3*
11	ToLCV-28	G11	3.66	5.66	8.33	0.00	15.00	6.53	R	*Ty-3 (H)*
12	ToLCV-32	G12	5.00	3.00	2.33	0.00	3.33	2.73	HR	*Ty-3*
13	H-88-78-1	G13	0.00	0.00	0.00	10.00	5.00	3.00	HR	-
14	H-88-78-2	G14	0.00	0.00	0.00	0.00	0.00	0.00	HR	*Ty-3*
15	VRCRT-14	G15	10.00	9.00	10.00	10.00	5.00	8.80	R	-
16	VRCYT-3	G16	2.50	4.00	5.33	10.00	7.50	5.87	R	-
17	VRJT-24	G17	0.00	0.00	0.00	5.00	10.00	3.00	HR	-
18	VRJT-32	G18	0.00	0.00	0.00	0.00	0.00	0.00	HR	*Ty-3*
19	VRJT-41-2	G19	4.00	0.66	5.66	3.33	5.00	3.73	HR	-
20	VRJT-37	G20	0.00	0.66	0.00	0.00	0.00	0.13	HR	*Ty-3*
21	VRJT-94	G21	0.00	1.00	0.00	5.00	5.00	2.20	HR	*Ty-3*
22	KB-17	G22	0.00	0.00	0.00	0.00	2.50	0.50	HR	*Ty-3*
23	KB-2	G23	5.00	1.66	5.00	0.00	15.00	5.33	R	*Ty-3 (H)*
24	KB-20	G24	0.00	2.00	5.00	10.00	30.00	9.40	R	*Ty-3*
25	KB-3-1	G25	2.50	3.00	1.33	0.00	0.00	1.37	HR	-
Kashi Aman (Resistant control)			2.50	0.00	0.00	0.00	2.50	1.00	HR	*Ty-3*
Punjab Chhuhara (Susceptible control)			95.33	94.33	98.33	98.00	97.00	96.60	HS	-
SE(m)			0.62	0.83	1.13	1.32	2.44			
CD ($P < 0.05$)			1.77	2.36	3.21	3.76	6.94			
CV (%)			14.68	22.06	30.71	21.90	24.75			

ToLCD symptoms were recorded at 45 and 90 days after transplanting as score: 0 = no visible symptoms; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop; 4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stops (Friedmann et al. 1998). Average score was calculated from total scores of all plants divided by total number of plants. Resistance/susceptible category was classified based on the Per cent disease incidence as highly resistant (score = 0; HR: 0-5%), resistant (score = 1; R: 5·1–12·0%), moderately resistant (score = 2; MR: 12·1–25%), moderately susceptible (score = 3; MS: 25·1–50·0%), susceptible (score = 4; S: 50·1–75%), and highly susceptible (score = 5; HS: 75·1–100%) (Banerjee and Kalloo, 1987). Note: Kashi Aman (Resistant Control) and Punjab Chhuhara (Susceptible Control)

Sankranti (Table 2). Our findings are in line with an earlier report on the validation of *Ty-2* and *Ty-3* genes in tomato resistance breeding. Resistant genotypes were reported earlier in tomatoes against ToLCD resistance by field and artificial screening and molecular markers (Prasanna et al., 2015a, 2015b). Recently, Zannat et al. (2023) identified the best-performing 25 genotypes and ToLCD resistance based on genetic parameters. They suggest that such genotypes

Table 3: Joint ANOVA (GGE biplot) for various horticultural traits in tomato genotypes

Source	*Df*	*Average fruit weight (g)*		*TSS (°Brix)*		*Fruit yield (kg/ plant)*		*ToLCD (%)*	
		Sum Sq	*Mean Sq*	*Sum Sq*	*Mean Sq*	*Sum Sq*	*Mean Sq*	*Sum Sq*	*Mean Sq*
ENV	4	1716	428.98**	5.67	1.41**	3.69	0.92**	301	75.2**
REP	10	944	94.36**	4.34	0.43**	1.88	0.18**	577	57.7**
GEN	24	552750	23031.25**	283.70	11.82**	129.55	129.55**	10017	417.4**
GEN:ENV	96	17763	185.04**	38.58	0.40**	20.90	20.90**	2131	22.2**
Residuals	240	485	2.02	2.13	0.008	2.40	2.40	1391	5.8

**Statistical significance at $P < 0.01$ for genotype-by-environment interaction analysis

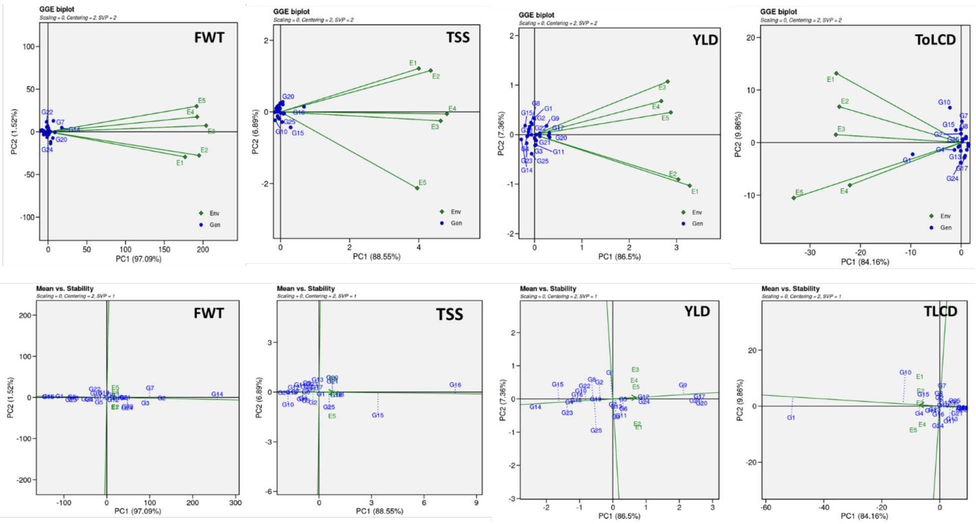

Fig. 2: The basic «GGE biplot» and «Mean vs. Stability» view of 25 tomato genotypes across the testing environments for traits such Average fruit weight (FWT) (g), Total soluble solids (TSS) (°Brix), Yield (YLD) (kg/plant), and ToLCD (tomato leaf curl disease) resistance using default parameters of the R software (Scaling = 0, Centering = 2, SVP i.e. singular value partition = 2). Genotypes with Codes are given in Table 1

could be considered in future hybridization programs in tomato breeding based on traits like fruit diameter, number of fruits per plant, fruit weight and yield. Overall, our study identifies ToLCD-resistant sources for exploitation in breeding through conventional or molecular approaches.

Stability analysis using GGE biplot

Twenty-five genotypes were evaluated for five consecutive years for economically important traits such as average per fruit weight, TSS, fruit yield per plant and ToLCD resistance. GGE biplot ANOVA is given in Table 3. In the basic GGE biplot, the first two components accounted for > 98.6% (average fruit weight), 95.3% (TSS), 93.8% (yield per plant), and 94.0% (ToLCD) of the total variance, thus affirming the comprehensive representation of total variability for these traits (Fig. 2). The identification of ideal genotypes was done based on the mean vs. stability view of the GGE biplot. The stability of genotypes is determined by their projection from the average environment coordinate (AEC) abscissa (horizontal axis) towards the AEC ordinate (vertical axis). The genotype that falls on the AEC abscissa with almost zero or the shortest projection (i.e., vertical dotted lines) towards the AEC ordinate is considered to be the most stable, while the genotype with the longest projection in either direction with the AEC abscissa is considered to be unstable (Khan et al. 2021). The AEC abscissa indicates the mean values, while the AEC ordinate reflects the stability of genotypes. The arrow sign on the AEC abscissa indicates the higher mean values. In this study, most genotypes were stable, with minor projections in G7 (VRT-51) for fruit weight and G15 (VRCRT-14) for TSS. Similarly, for fruit yield, the study identified all stable genotypes except G15 (VRCRT-14), G25 (KB-3-1) and G8 (VRT-67). For ToLCD also, most genotypes were stable except, G1 (VRT-02) and G10 (ToLCV-16). Our study was supported by previous work on stability analysis in crops (Mullualem et al., 2024; León et al., 2021). Overall, the stable genotypes exhibited the highest mean performance values (towards the arrow side on the horizontal axis) and the lowest vector (i.e., shortest projection on the vertical axis).

Breeders have used the multi-trait genotype-ideotype distance index (MGIDI) tool to select genotypes based on multiple traits. It is a multivariate selection tool that incorporates various trait information into a single value and ranks genotypes based on their distance from an ideal

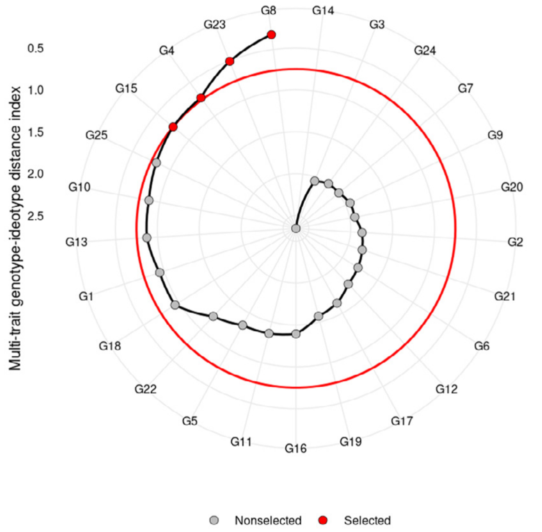

Fig. 3: Multi-trait genotype-ideotype distance index (MGIDI) view of the GGE biplot, where red color indicates selected genotypes). Genotypes with Codes are given in Table 1

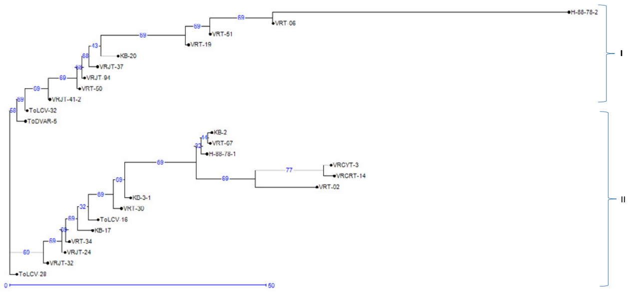

Fig. 4: Phylogeny analysis based on the Weighted Neighbor-Joining method showing genetic distances in tomato genotypes.

genotype. It allows to deployment of versatile selection criteria and also uses principal components to select ideal genotypes based on the Euclidean distance between an ideal genotype and other genotypes for multiple traits. Thus, in this study, red circle indicates selected genotypes such as G8 (VRT-67), G23 (KB-2), G4 (VRT-30) and G15 (VRCRT-14) based on four traits data across five environments (Fig. 3). The MGIDI is a novel approach and proven to be highly effective in selection of genotypes and designing efficient breeding strategies in various crops (Olivoto and Nardino 2021). ToLCD high values of percent disease infection indicate a susceptible reaction. On the contrary, a lower value represents resistance. Hence, the genotypes having desirable traits pave the way for efficient and effective tomato breeding for improvement of horticultural, processing and ToLCD resistance traits.

Phylogenetic Analysis

Phylogenetic analysis of tomato genotypes, based on four traits, was conducted using the Weighted Neighbor-Joining method in DARWin software. The analysis identified two distinct clusters: Cluster I, comprising 11 genotypes, and Cluster II, containing 14 genotypes (Fig. 4). Notably, genotype H-88-78-2 was positioned farthest within Cluster I, whereas ToLCV-28 occupied the most distant position at the opposite end of Cluster II.

Conclusion

In summary, tomato genotypes were characterized for yield average fruit weight, fruit yield, TSS and ToLCD resistance under field conditions for five years. Molecular markers were also used to identify *Ty-2* and *Ty-3* resistance genes through gene-specific molecular markers. Collectively, 15 superior genotypes based on average fruit weight, fruit yield, TSS and ToLCD resistance were identified such as VRT-19, VRT-34, VRT-50, VRT-51, ToDVAR-5, ToLCV-32, H-88-78-1, H-88-78-2, jointless tomatoes VRJT-24, VRJT-32, VRJT-41-2, VRJT-37, VRJT-94; orange color tomato KB-17 and KB-3-1. Further, GGE biplot and MGIDI stability analysis over the years identified these potential lines, which can be used in breeding.

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सारांश

इस अध्ययन का उद्देश्य उपज से संबंधित जीनोटाइप x पर्यावरण इंटरैक्शन (जीईआई) के साथ-साथ इसके घटक लक्षणों और जीजीई बाइप्लॉट विश्लेषण का उपयोग करके टमाटर लीफ कर्ल रोग (टीओएलसीडी) के प्रति प्रतिरोध का विश्लेषण करना था। पूर्व शोध के आधार पर, चयनित टमाटर जीनोटाइप का मूल्यांकन बागवानी लक्षणों जैसे औसत फल वजन, उपज, कुल घुलनशील ठोस (टीएसएस) और टीओएलसीडी प्रतिरोध (प्राकृतिक हॉट-स्पॉट स्थितियां) के लिए लगातार पांच वर्षों में किया गया था। इसके अतिरिक्त, इन जीनोटाइप में टीओएलसीडी प्रतिरोध जीन (Ty-2 और Ty-3) की उपस्थिति की पहचान करने के लिए आणविक मार्करों को नियोजित किया गया था। फील्ड स्क्रीनिंग ने जीनोटाइप को अत्यधिक प्रतिरोधी, प्रतिरोधी और मध्यम प्रतिरोधी समूहों में वर्गीकृत किया। जीईआई विश्लेषण जीजीई बाइप्लॉट तकनीकों का उपयोग करके किया गया था कुल मिलाकर, औसत फल भार, फल उपज, टीएसएस और टीओएलसीडी प्रतिरोध के आधार पर 15 बेहतर जीनोटाइप की पहचान की गई जैसे कि वीआरटी-19, वीआरटी-34, वीआरटी-50, वीआरटी-51, टीओडीवीएआर-5, टीओएलसीवी-32, एच-88-78-1, एच-88-78-2, जोड़ रहित टमाटर वीआरजेटी-24, वीआरजेटी-32, वीआरजेटी-41-2, वीआरजेटी-37, वीआरजेटी-94; नारंगी रंग के टमाटर केबी-17 और केबी-3-1। इन जीनोटाइप का उपयोग प्रजनन में उपज-संबंधित विशेषताओं और टीओएलसीडी प्रतिरोध को शामिल करने के लिए किया जा सकता है।