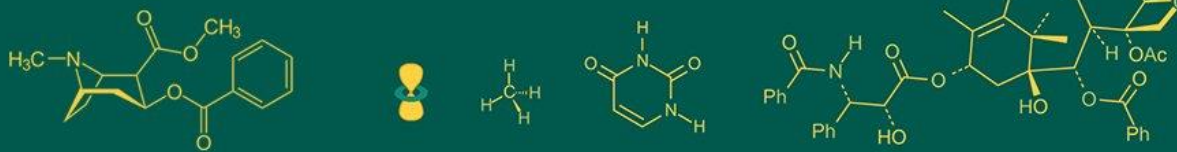


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Assessment of tomato genotypes for resistance to tomato leaf curl virus and bacterial wilt in the Eastern Plateau Region of India

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Abstract

Tomato (*Solanum lycopersicum* L.) is a crucial crop globally, revered for its nutritional value and adaptability. However, it faces significant challenges posed by the Tomato Leaf Curl Virus (ToLCV) and bacterial wilt induced by *Ralstonia solanacearum*. This research aimed to meticulously evaluate various tomato genotypes for their resistance to these debilitating diseases while identifying hybrid combinations with promising traits. Key findings include the identification of highly resistant hybrids such as DT1504 x DT911 against ToLCV and *Ralstonia solanacearum*. Assessment based on disease severity indices revealed, DT1504 and Pusa Ruby consistently exhibiting heightened vulnerability. However, genotypes like DT1504, DT911 and DT806 demonstrated commendable levels of resistance in contrast to the susceptibility observed in Pusa Ruby, Pusa Rohini, DT816 and DT806. Using line x tester analysis, the GCA and SCA hybrid combinations such as DT1504 x DT911 and Pusa Rohini x DT806 demonstrated notably negative SCA values indicating strong genetic interactions contributing to disease resistance. This study highlights the vital role of genetic diversity in breeding, emphasizing hybrids dual benefits of high yield and disease resistance. Through identifying and utilizing genetic resistance it aims to mitigate tomato diseases promoting sustainable agriculture worldwide.

Keywords: Tomato, ToLCV, *Ralstonia solanacearum*, resistance

Introduction

Tomato (*Solanum lycopersicum* L.) is a significant crop in the Solanaceae family originating from the Peru-Ecuador region of Latin America. Globally tomatoes are cultivated on a large scale ranking second only to potatoes in popularity due to their adaptability and versatility in both fresh and processed food industries. Tomato cultivation spans 5.16 million hectares worldwide yielding approximately 189.133 million metric tonnes annually as reported by the FAOSTAT in 2021. India ranks second in tomato production generating an annual yield of 20.69 million tonnes representing nearly 11.12% of the global production. Tomato is not only rich in essential nutrients like vitamins A, B, and C but also contain lycopene an antioxidant associated with various health benefits including protection against certain cancers (Chadha, 2006) [5].

Despite its significance tomato cultivation faces biotic challenges notably from the Tomato Leaf Curl Virus (ToLCV) and bacterial wilt caused by *Ralstonia solanacearum*. ToLCV belonging to the Begomovirus genus of the Geminiviridae family (Varma and Malathi, 2003) [24] exists in several variants in India including tomato leaf curl New Delhi virus (ToLCNDV), tomato leaf curl Palampur virus (ToLCPMV), tomato leaf curl Bangalore virus (ToLCBV), tomato leaf curl Karnataka virus (ToLCKV) and tomato leaf curl Gujarat virus (ToLCGV) posing severe threats to tomato production worldwide. ToLCV leads to significant yield losses ranging from 90% to 100% (Butter and Rataul, 1981, Gaikwad *et al.*, 2011, Moriones *et al.*, 2017, Kaushik and Dhaliwal, 2018) [4, 12, 19, 14] particularly in regions like North India where the prevalence of the whitefly vector is high manifesting through symptoms such as leaf curling, shrinking, cupping and stunted development. On the other hand *Ralstonia solanacearum* induces sudden plant death and stem rot causing yield losses ranging from 4.24% to 86.14% reaching up to 100% in hot and humid climates (Mishra *et al.*, 1995) [17].

It is considered to be the most important limiting factor in the successful cultivation of tomato during rainy and summer seasons when price advantage in the local and distant market is significant. The persistence of *Ralstonia solanacearum* in soil and its ability to endure various stages within water, plant residues and host plants themselves pose significant challenges for disease management.

Efforts to manage these diseases include crop rotation, chemical control of vectors and the development of resistant cultivars. However, widespread pesticide use has led to resistance, highlighting the importance of breeding for disease resistance (Horowitz *et al.*, 2005) [13]. So, breeding for disease resistance emerges as a sustainable solution given the limitations of chemical management. Several resistance genes offer promising avenues for resistant hybrids. Combining ability studies helps select diverse parents for resistant hybrids and understand genetic mechanisms. However, the lack of superior combiners hinders resistant hybrid development. Genetic analysis aids in identifying top combiners guiding hybrid development. This research aims to identify effective combiners for ToLCV and bacterial wilt resistance in superior inbred lines and hybrids enhancing sustainable tomato production and disease management.

Materials and Methods

Plant Materials (Season, strain)

The experiments were conducted at the Vegetable Research Farm, IARI Jharkhand, involving a comprehensive evaluation of tomato genotypes. Eight parental lines and fifteen hybrids were included in the study. All eight parents were crossed in a line X tester mating pattern during late

Rabi 2020-21 to create 15 hybrids. The F₁'s and their parents were studied for disease resistance. The trials were organized in a Randomized Block Design with three replications. To ensure optimal growth conditions seedlings were initially nurtured in plastic containers within a controlled environment in a greenhouse. At twenty-five days of age, they were transplanted into the field maintaining a spacing of 60 x 45 cm. All aspects of tomato cultivation practices were followed except for plant protection measures.

Maintenance and Screening for ToLCV Resistance

B. tabaci culture was established from brinjal specimens and maintained on fresh brinjal plants. ToLCV culture was obtained from infected plants and maintained in a glasshouse using tomato cv. Punjab Chuhhara. For screening, 15 hybrids and 8 parental tomato seedlings were exposed to ToLCV under greenhouse conditions *via* viruliferous whiteflies. To increase whitefly population and inoculum pressure one row of the ToLCV-susceptible cultivar Punjab Chuhhara was interspersed every 10 rows in experimental plots. Tomato seedlings aged 10-12 days were introduced with ten viruliferous whiteflies per plant. Symptom monitoring occurred at intervals up to 60 days post-planting to assess performance and resistance. For the evaluation of ToLCV, Punjab Chuhhara and Kashi Aman were employed as susceptible and resistant checks respectively. Disease severity was determined using the following formula:

$$\text{Tomato Leaf curl infected plants (\%)} = \frac{\text{Leaf curled (infected) plants/plot}}{\text{Total number of plants /plot}} \times 100$$

Table 1: The evaluation of disease reaction across different genotypes was classified according to the following scale to Tomato Leaf Curl Virus

Symptom	Symptom Severity Rating	Response value	Coefficient of infection*	Reaction
Symptomless	0	0	0-4	Immune/Highly resistant (HR)
Mild Curling up to 25%	1	0.25	5-9	Resistant (R)
Curling, wrinkling 26-50%	2	0.5	10-19	Moderately resistant (MR)
Curling, wrinkling 51-75%	3	0.75	20-39	Moderately susceptible
Severe Curling, wrinkling >75%	4	1	40-69 70-100	Susceptible (S) Highly susceptible (HS)

Banerjee and Kalloo (1987) [3].

* Coefficient of infection combines the disease percentage with the severity assigned to each grade

Bacterial wilt assessment

For the evaluation of bacterial wilt, Pusa Ruby and Arka Rakshak were employed as susceptible and resistant checks respectively. The genotypes underwent screening in a dedicated sick plot post-inoculation with a bacterial culture of 1×10^8 cfu /ml. Before transplanting, the population of *R.*

solanacearum in the soil was determined using the soil dilution plating technique. Observations on growth, yield and quality traits were carefully recorded for five randomly selected plants per replication. Bacterial wilt incidence was documented upon observation of wilt symptoms and the wilt index (WDI) was subsequently calculated.

Table 2: Bacterial wilt disease rating scale for tomato caused by *R. solanacearum*

Rating	Reaction observed	Disease index	Reaction
0	Healthy Plant (No wilting)	0.00-0.2	Highly resistant
1	< 10% of Plants showing wilt	0.21-0.3	Resistant
2	11-25% plants wilted	0.31-0.4	Moderately resistant
3	26-50% plants wilted	0.41-0.5	Moderately susceptible
4	51-75% plants wilted	0.51-0.6	Susceptible
5	>75% plants wilted	0.61-0.9 0.9-1.0	Highly susceptible

(Winstead and Kelman, 1952) [56].

Upon scoring the genotypes Disease Index (PDI) is calculated following

$$\text{Tomato Bacterial wilt Disease Index} = \frac{\text{Total sum of numerical ratings}}{\text{No. of observations} \times \text{Maximum disease rating}}$$

Statistical analysis

In this study, the statistical analysis involved One-way ANOVA to assess tomato leaf curl incidence and bacterial wilt resistance, while line × tester analysis was used for combining ability studies across three replications. Employing a randomized block design ensured robustness and minimized variability. These analyses enabled the comparison of mean differences in disease incidence and resistance among treatments or genotypes and provided insights into genetic mechanisms underlying trait inheritance.

Results

Evaluation of tomato genotypes for resistance to ToLCV: The study investigated the responses of various genotypes and hybrids to Tomato Leaf Curl Virus (TLCV) under both natural (field) and artificial (greenhouse) conditions during the late rabi season. Among parental lines and hybrids, the coefficient of infection by ToLCV ranged from 0 to 51.88 under field conditions and 0 to 80.58 under

greenhouse conditions as shown in Table 4. Notably, the hybrid DT1504 x DT911 exhibited significant resistance to TLCV remaining symptom-free until maturity across all environments. In addition, under natural conditions this hybrid displayed a reduced frequency of whitefly (*Bemisia tabaci*), suggesting a potential non-preference mechanism contributing to its TLCV resistance. Conversely, under artificial conditions, all inoculations by viruliferous whiteflies were more consistent.

Several hybrids, including Pusa Ruby x DT911, Pusa Ruby x DT802, Pusa Rohini x DT816, Pusa Rohini x DT802, Pusa Rohini x DT804, DT1504 x DT816, DT1504 x DT802 and DT1504 x DT804 exhibited no TLCV symptoms for up to 60 days under natural field conditions. After this period mild symptoms were observed only on the terminal growing shoots of the plants. This level of resistance is valuable for resistance breeding programs as symptoms after 60 days of planting do not significantly impact yield in field-grown tomatoes. Most of the accessions showed moderate disease reactions.

In greenhouse conditions, the same cross DT1504 X DT911 demonstrated a highly resistant response to ToLCV. Additionally, seven crosses exhibited resistance while eight genotypes displayed moderate resistance. Four genotypes exhibited moderate susceptibility and only two genotypes were found to be susceptible to ToLCV.

Table 3: Variability in *Lycopersicon* Accessions' Reactions to Tomato Leaf Curl Virus and bacterial wilt under field screening

Reaction	No. of genotypes (ToLCV)	Parents, Hybrids, checks	No. of genotypes (B.W)	Parents, Hybrids, checks
Highly resistant	2	DT1504 x DT911, Kashi Aman	2	DT1504 x DT911, Arka Rakshak
Resistant	8	Pusa Ruby x DT802, Pusa Ruby x DT804, Pusa Rohini x DT816, Pusa Rohini x DT802, Pusa Rohini x DT804, DT1504 x DT816, DT1504 x DT802, DT1504 x DT804	6	Pusa Ruby x DT911, Pusa Ruby x DT802, Pusa Ruby x DT804, Pusa Rohini x DT802, DT1504 x DT816, DT1504 x DT802,
Moderately resistant	9	Pusa Ruby, DT802, DT804, DT806, Pusa Ruby x DT816, Pusa Ruby x DT806, Pusa Rohini x DT911, Pusa Rohini x DT911, Pusa Rohini x DT806, DT1504 x DT806	9	DT911, DT804, DT806, Pusa Ruby x DT816, Pusa Ruby x DT806, Pusa Rohini x DT816, Pusa Rohini x DT804, Pusa Rohini x DT806, DT1504 x DT804
Moderately susceptible	4	Pusa Rohini, DT1504, DT816, Pusa Ruby x DT911	6	Pusa Rohini, DT816, DT802, Pusa Rohini x DT911, Pusa Rohini x DT911, DT1504 x DT806
Susceptible	1	DT911	1	DT1504
Highly susceptible	1	Punjab Chhuhara	1	Pusa Ruby

Table 4: Assessing tomato genotypes for resistance to tomato leaf curl virus and bacterial wilt

Character	Field			Greenhouse			B.W Disease index	B.W Disease reaction
	PDI	C.I	Reaction	PDI	C.I	Reaction		
Lines								
Pusa Ruby	37.89	18.99	MR	37.95	18.97	MR	0.53	S
Pusa Rohini	47.32	35.49	MS	50.97	38.23	MS	0.45	MS
DT1504	47.94	35.96	MS	51.39	38.54	MS	0.56	S
Testers								
DT911	48.54	48.54	S	88.17	88.17	HS	0.4	MR
DT816	48.31	36.24	MS	51.7	38.77	MS	0.48	MS
DT802	37.63	18.82	MR	33.95	25.46	MS	0.44	MS
DT804	35.75	17.88	MR	37.96	18.98	MR	0.39	MR
DT806	35.98	17.99	MR	37.98	18.99	MR	0.37	MR
Crosses								
Pusa Ruby x DT911	46.3	34.73	MS	30.18	7.54	R	0.26	R
Pusa Ruby x DT816	32.85	16.43	MR	38.13	19.06	MR	0.32	MR
Pusa Ruby x DT802	26.28	6.57	R	36	9	R	0.26	R
Pusa Ruby x DT804	32.07	8.02	R	35.25	17.62	MR	0.286	R
Pusa Ruby x DT806	36.5	18.25	MR	49.08	36.81	MS	0.41	MR
Pusa Rohini x DT911	38.88	19.44	MR	41.69	41.69	S	0.414	MS

Pusa Rohini x DT816	25.97	6.49	R	31.79	7.95	R	0.34	MR
Pusa Rohini x DT802	30.71	7.68	R	35.24	8.81	R	0.29	R
Pusa Rohini x DT804	35.7	8.93	R	37.23	18.61	MR	0.29	MR
Pusa Rohini x DT806	36.33	18.17	MR	38.15	19.07	MR	0.409	MR
DT1504 x DT911	0.21	0	HR	22.09	0	HR	0.2	HR
DT1504 x DT816	35.88	8.97	R	36.37	9.09	R	0.26	R
DT1504 x DT802	30.48	7.62	R	33.14	8.28	R	0.3	R
DT1504 x DT804	29.12	7.28	R	36.34	9.08	R	0.32	MR
DT1504 x DT806	34.19	17.1	MR	37.62	18.81	MR	0.48	MS
Checks								
Punjab Chhuhara	93.56	70.17	HS	93.73	93.73	HS	-	-
Kashi Aman	0.23	0	HR	2.76	0	HR	-	-
Pusa Ruby	-	-	-	-	-	-	0.63	HS
Arka Rakshak	-	-	-	-	-	-	0.14	HR
C.D	7.931	8.46	-	5.26	8.3	-	7.98	-
SE(m)	2.8	2.9	-	1.86	2.9	-	2.85	-
SE(d)	3.97	4.2	-	2.6	4.1	-	3.99	-
C.V	15.514	23.93	-	9	24.11	-	15.29	-

PDI= Percent Disease Index, C.I = Coefficient of infection, HR= Highly Resistant, R= Resistant, MR= Moderately resistant, MS= Moderately Susceptible, S= Susceptible, HS= Highly Susceptible

Table 5: Assessment of combining ability variance and genetic action for tomato leaf curl disease in field and greenhouse, and bacterial wilt

Genotype	PDI field GCA/SCA (ToLCV)	PDI greenhouse GCA/SCA (ToLCV)	B.W field GCA/SCA
Pusa Ruby	3.369	1.841	-1.667
Pusa Rohini	2.087	0.933	2.733
DT1504	-5.456	-2.775	-1.067
DT911	-2.97	-4.567	-3.383
DT816	0.136	-0.457	-1.383
DT802	-2.274	-1.093	-3.967
DT804	0.866	0.387	-2.217
DT806	4.242	5.73	10.95
Pusa Ruby x DT911	14.47	-2.981	-1.417
Pusa Ruby x DT816	-2.086	0.859	2.833
Pusa Ruby x DT802	-6.246	-0.635	-0.833
Pusa Ruby x DT804	-3.596	-2.865	0.167
Pusa Ruby x DT806	-2.542	5.622	-0.75
Pusa Rohini x DT911	8.332	9.437	9.683
Pusa Rohini x DT816	-7.684	-4.573	0.683
Pusa Rohini x DT802	-0.534	-0.487	-1.733
Pusa Rohini x DT804	1.316	0.023	-3.483
Pusa Rohini x DT806	-1.43	-4.4	-5.15
DT1504 x DT911	-22.801	-6.455	-8.267
DT1504 x DT816	9.769	3.715	-3.517
DT1504 x DT802	6.779	1.121	2.567
DT1504 x DT804	2.279	2.841	3.317
DT1504 x DT806	3.973	-1.222	5.9

Response of tomato germplasm resistance against *Ralstonia solanacearum*

The response of tomato germplasm to *Ralstonia solanacearum* is summarized in Table 4. Assessment of genotypes and crosses based on the wilt disease index (WDI) in Table 2 revealed that disease incidence ranged from 0.22 to 0.51 against *Ralstonia solanacearum* infection. Notably several crosses including Pusa Ruby x DT911, Pusa Ruby x DT802 and Pusa Rohini x DT802 demonstrate high levels of resistance with disease indices ranging from 0.22 to 0.32. These crosses exhibit promising potential for breeding programs aimed at developing resistant tomato cultivars. Additionally, the genotype Arka Rakshak serves as a notable reference for resistance consistently displaying a low disease index of 0.3 across the dataset. Conversely genotypes such as Pusa Ruby and DT1504 along with their respective crosses consistently exhibit higher susceptibility to the disease as reflected by disease indices ranging from 0.25 to 0.55. The genotypes DT911, DT804 and DT806

performed as moderate resistance with disease indices ranging from 0.36 to 0.4 while Pusa Rohini, DT816 and DT802 are categorized as moderately susceptible, with disease indices ranging from 0.36 to 0.47. These findings provide valuable insights for breeding efforts aimed at enhancing tomato germplasm for improved resistance against *Ralstonia solanacearum*.

Assessment of combining ability variance and genetic Action

In Table 5 the estimates of general combining ability (GCA) and specific combining ability (SCA) variances and corresponding gene actions are presented. General combining ability typically reflects additive gene action, while specific combining ability primarily arises from dominance and epistasis. Our study findings indicate both GCA and SCA for resistance against tomato leaf curl virus disease in field and greenhouse conditions as well as bacterial wilt disease in field conditions. While parental

genotypes like DT1504 (-5.456), DT911 (-2.97) and DT802 (-2.27) demonstrated negative and significant GCA effects for leaf curl virus disease, some, such as Pusa Ruby (3.369), DT806 (4.24) and Pusa Rohini (2.087) exhibit positive GCA values suggesting susceptibility. However, certain hybrid combinations including DT1504 x DT911 (-22.80) and Pusa Rohini x DT816 (-7.68) exhibited significant negative SCA effects in the desirable direction indicating enhanced resistance to the virus. Similarly, DT911 (-3.38) displays negative GCA for bacterial wilt disease hybrids like DT1504 x DT911 (-8.26) and Pusa Rohini x DT806 (-5.15) exhibit significant negative SCA in the desirable direction for bacterial wilt resistance. These findings underscore the importance of hybridization strategies in breeding programs aimed at developing disease-resistant tomato varieties. Notably, hybrid combinations with high negative SCA values such as DT1504 x DT911 and Pusa Rohini x DT806 show promise for further utilization and refinement in disease resistance breeding efforts.

Discussion

Over the years, research efforts have been dedicated to understanding the resistance of tomato varieties to Tomato Leaf Curl Virus (ToLCV) and bacterial wilt. Singh (2014)^[21] observed resistance in the wild accession H 88-78-1 to ToLCV while other genotypes exhibited varying degrees of resistance. The detrimental effects of ToLCV infection such as severe mosaic symptoms and reduced leaf size have been well-documented leading to compromised photosynthetic activities and decreased biomass compared to healthy plants (Farooq & Akanda, 2007)^[11]. Cheema and Dhaliwal (2010)^[6] reported significant heterosis in promising F1 hybrids tested under ToLCV-infested conditions. Additionally, Ullah *et al.*, 2017^[23] evaluated 21 tomato genotypes for resistance against Tomato mosaic virus (ToMV) with only one genotype showing high resistance one showing moderate resistance, four showing moderate susceptibility and the remaining 15 genotypes being susceptible. Similarly, Denoyes and Aviers (1989)^[9] found that 15 out of 25 tomato varieties exhibited resistance to bacterial wilt. Dutta and Rehman (2012)^[7] corroborated these findings in their varietal evaluation of tomatoes in the Northeast region. Notably, UK Local-2 emerged as highly resistant in a study conducted by Kumar *et al.* 2018 underlining the importance of genetic diversity in breeding programs aimed at developing disease-resistant tomato cultivars. Dheemanth *et al.*, 2020^[8] identified Abhinav as the top-performing hybrid, showcasing high yield, large fruit size and notable resistance to both bacterial wilt (1.56%) and ToLCV (5.02%). The observed higher specific combining ability (SCA) in hybrid combinations like DT1504 x DT911, Pusa Rohini x DT816, and Pusa Rohini x DT806 suggests a predominance of non-additive gene action in the inheritance of traits related to tomato leaf curl virus disease and bacterial wilt resistance. This highlights the potential effectiveness of heterosis breeding and recombination strategies with delayed selection in subsequent generations for trait improvement. These findings resonate with previous studies reporting similar non-additive gene action for yield and yield-attributing characters in tomato Mondal *et al.* (2009)^[18], Alex Sandro *et al.* (2015)^[2], Vipesh *et al.* (2015)^[25]. Therefore, understanding these genetic mechanisms is crucial for future breeding programs aimed at enhancing disease resistance in tomato varieties.

Conclusion

In summary, this research underscores the critical role of genetic diversity in addressing the persistent challenges faced by tomato cultivation notably from Tomato Leaf Curl Virus (ToLCV) and bacterial wilt. Through comprehensive evaluation of genotypes and hybrids, significant strides have been made in identifying sources of disease resistance. The findings highlight the potential of harnessing genetic reservoirs to develop resilient tomato varieties capable of withstanding disease pressures. By emphasizing sustainable breeding practices, this study paves the way for the continued advancement of agricultural resilience and the preservation of tomato cultivation worldwide.

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