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Combining Ability and Gene Action Influencing Bacterial Wilt Resistance in Brinjal

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study employed a line × tester mating design to evaluate bacterial wilt resistance in brinjal at the seedling stage. Four lines viz., L1 (SM 10), L2 (IC111010), L3 (IC89989), and L4 (IC427008) and three testers viz., T1 (IC253967), T2 (IC255756), and T3 (IC256708) were crossed to produce twelve F_1 hybrids, which, along with parents and checks, were screened at the seedling stage using the artificial root-dip inoculation method. Percent Disease Incidence (PDI) served as the criterion for resistance classification. Among all genotypes, the hybrid L3 × T1, tester T2 and the

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resistant check Arka Anand exhibited complete resistance, showing no visible wilt symptoms. Four hybrids namely, L1 × T2, L2 × T3, L3 × T2 and L4 × T2 along with line L3, testers T1 and T3 and the check Neelima were moderately resistant. Hybrids L1 × T1 and L4 × T1, line L4 and Haritha were moderately susceptible, while the remaining hybrids and lines showed moderate to high susceptibility. Combining ability analysis identified L2 × T3 (IC111010 × IC256708) as the most promising hybrid due to its desirable specific combining ability effect for resistance. Additive variance was low and negative, whereas dominance variance was high, confirming the predominance of non-additive gene action. The major contribution of line × tester interaction (64.12%), compared with lines (12.04%) and testers (23.83%), also emphasizes non-additive, dominance gene action and the significance of hybrid breeding as a better strategy to develop bacterial wilt resistant hybrids here.

Keywords: Brinjal; bacterial wilt; line x tester; combining ability; gene action; percent disease incidence.

1. INTRODUCTION

Brinjal (*Solanum melongena* L.), belonging to the family solanaceae is an important vegetable crop extensively cultivated across India and other tropical regions. Brinjal is particularly appreciated worldwide today for its rich nutritional profile, medicinal benefits and low-calorie content. (Naeem and Ugur, 2019; Quamruzzaman et al., 2020; Hazra, 2023). Despite the inherent value and importance of the crop, its cultivation and production are delimited by several hurdles, the major one being the bacterial wilt caused by *Ralstonia solanacearum.* Bacterial wilt is a highly destructive soil borne disease, which can cause yield loss ranging from 11.67 to 96.67% in India (Bainsla et al., 2016). High temperatures, humid conditions and soil acidification support the growth and survival of this pathogen (Li et al., 2017). In Kerala, the disease is particularly severe due to the humid climate and naturally acidic soils, often causing major yield losses in solanaceous crops (Ajayasree et al., 2022).

As the pathogen can grow endophytically, survive in deep soil for years, spread through water and has a wide host range, management methods such as soil fumigation, adjustment of planting dates and chemical applications have proven largely ineffective (Namisy et al., 2019). Hence, the development and cultivation of resistant varieties remain the most effective and sustainable means of controlling the disease (Barik et al., 2020; Pandiyaraj et al., 2024). However, it is often observed that open-pollinated cultivars gradually lose their resistance and desirable traits after a few generations (Duman et al., 2005), making the breeding of resistant hybrids a necessity (Chattopadhyay et al., 2012). As resistance may break down over time, continuous development of high-yielding, wilt-resistant hybrids are essential, especially since only one such hybrid has been released by Kerala Agricultural University (KAU) to date.

Parental line selection is a vital step in any hybrid breeding program and line × tester analysis (Kempthorne, 1957) is a well-established method for assessing general and specific combining abilities, understanding gene action and identifying superior parental combinations. Hybrids developed through this approach must be evaluated for bacterial wilt resistance to determine their true breeding potential. Conventionally, screening is performed in wilt-sick plots under field conditions, which is labour intensive, time-consuming and influenced by environmental variability. In contrast, screening at the seedling stage provides a rapid and cost-effective alternative, eliminating the need for wilt-sick plots. This method can also provide gnotobiotic-like environment, minimizing interference from other microorganisms and enabling a more precise evaluation of the host–pathogen interaction. However, only very few studies have applied line × tester analysis for bacterial wilt resistance at the seedling stage.

During 2020–2023, a series of studies were conducted at KAU to evaluate diverse brinjal germplasm collected from North Kerala and the National Bureau of Plant Genetic Resources (NBPGR) for yield potential and bacterial wilt resistance. In a field evaluation, Chakravaram (2023) assessed thirty genotypes and identified four superior high-yielding accessions during the summer season. In the same year, Muslepally (2023) screened the same set of genotypes for bacterial wilt resistance at the seedling stage and subsequently evaluated the resistant selections in the field, identifying two highly resistant

genotypes with comparatively lower early yield. Building upon these findings, the present investigation was carried out at KAU to perform a line × tester analysis for bacterial wilt resistance at the seedling stage, utilizing high-yielding accessions identified by Chakravaram (2023) as lines and highly resistant accessions identified by Muslepally (2023) as testers, along with additional germplasm sourced from NBPGR, to identify promising parental combinations for the development of high-yielding, bacterial wilt-resistant brinjal hybrids.

2. MATERIALS AND METHODS

2.1 Production of F_1 Hybrids

2.1.1 Experimental materials

The study involved the use of four lines and three testers. The lines, testers and their notation used in the study are given in the Table 1 and 2.

Table 1. Genotypes used as lines

Lines		Source of collection
SM 10	(L1)	Kasaragod
IC111010	(L2)	NBPGR
IC89989	(L3)	NBPGR
IC427008	(L4)	NBPGR

Table 2. Genotypes used as testers

Testers		Source of collection
IC253967	(T1)	NBPGR
IC255756	(T2)	NBPGR
IC256708	(T3)	NBPGR

Among these, two lines, SM 10 and IC111010 were high yielding genotypes identified by Chakravaram (2023) based on their studies on the performance assessment of brinjal genotypes in summer. Also, the tester IC253967 was found to be highly resistant to bacterial wilt in the studies conducted by Muslepally (2023) based on their evaluation of brinjal genotypes for resistance to bacterial wilt.

2.1.2 Hybridisation

Hybridisation was conducted in the experimental field of the Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, during January to April 2025. Seeds were sown in portrays and thirty-day-old seedlings, with ten plants per genotype, were transplanted into grow bags in the crossing block. Flowering commenced two months after transplanting. Emasculation was done on the evening prior to hybridization, between 16.00 - 17.30 h. Plump buds of the lines that were expected to open the following day were identified; stamens were carefully removed using forceps and the flowers were covered with butter paper bags. Maximum pollen dehiscence of testers was observed between 9:00 - 9:45 h. Pollen was either collected on butter paper by gently shaking the stamens with a brush or obtained by piercing the anthers along the line of dehiscence with a needle. The collected pollen was then placed on the stigma of the emasculated flower, which was subsequently bagged and tagged. Fruits that developed after successful pollination were harvested at the fully ripened stage and seeds were extracted.

2.2 Isolation and Identification of Bacteria

Tomato plants exhibiting bacterial wilt symptoms were uprooted from the experimental field of the Department of Vegetable Science, College of Agriculture, Vellanikkara. Preliminary confirmation of the disease was done using a bacterial ooze test, wherein thoroughly washed plants were cut at the collar region and immersed in clean, undisturbed water to observe bacterial streaming.

For bacterial isolation, the stem portions near the collar region of infected plants were cut into small transverse sections containing vascular tissues. Surface sterilization was carried out by immersing the samples in 1% sodium hypochlorite solution for one minute, followed by three successive rinses in sterile distilled water, each lasting one minute. The sterilized plant segments were dried using sterile tissue paper and then crushed. The bacterial ooze obtained was streaked using the quadrant streak method on Triphenyl Tetrazolium Chloride (TZC) medium.

The TZC medium was prepared following Kelman (1954). The composition included 5 g glucose, 10 g peptone and 1 g casein hydrolysate dissolved in distilled water, with the final volume adjusted to 1000 mL and the pH set to 7. Subsequently, 15 g agar was added and the medium was sterilized by autoclaving at 15 psi pressure for 15–20 minutes. After autoclaving, the medium was allowed to cool and 5 mL of a 1% (w/v) stock solution of 2,3,5-triphenyl tetrazolium chloride was added.

Table 3. Scoring of bacterial wilt resistance

Score	Description	Category
0	No wilt symptoms	Highly Resistant (HR)
1	1–10% wilted plants	Resistant (R)
2	11–20% wilted plants	Moderately Resistant (MR)
3	21–30% wilted plants	Moderately Susceptible (MS)
4	31–40% wilted plants	Susceptible (S)
5	>40% wilted plants	Highly Susceptible (HS)

For molecular identification, the isolated pure cultures were sent to the Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, for PCR amplification of the 16S rRNA gene. The obtained sequence data were analyzed using the NCBI BLAST tool to identify the organism.

2.3 Artificial Inoculation of Bacterial Wilt and Screening of Hybrids

Four-week-old seedlings, grown in protrays without any management measures against bacterial wilt, were used for artificial screening. Bacterial suspension with an OD value of 0.3 at 600 nm was prepared using the pure culture. Inoculation was done by root dip method (Kumbar et al., 2021). The seedlings were carefully uprooted from protrays and thoroughly washed. Root tips were trimmed with sterile scissors to make a wound. The wounded seedlings were immediately dipped in in bacterial suspension for 30 minutes. These were then replanted in portrays containing sterile media. A small amount of bacterial suspension was drenched into the media. The protrays were screened for bacterial wilt symptoms for 14 days.

2.4 Percent Disease Incidence and Disease Scoring

The number of wilted plants was recorded and the per cent disease incidence was calculated as per Bi-hao et al. (2009).

Percent Disease Incidence = (Number of symptomatic wilted plants / Total number of plants) x 100

Scoring of bacterial wilt incidence was done according to Hussain et al. (2005) and is given in the Table 3.

2.5 Statistical Analysis

Line × tester analysis was performed using Agricolae package in RStudio version 4.2.2.to estimate the variances and effects due to general and specific combining ability, as well as to determine the nature of gene action, following the method of Kempthorne (1957).

3. RESULTS AND DISCUSSION

3.1 Production of F_1 Hybrids

Twelve hybrid combinations were successfully produced and seeds were harvested for further analysis.

3.2 Isolation and Identification of Bacteria

Cream coloured colonies with pinkish centers appeared 48 hours after inoculation. BLASTN analysis of the 16sr RNA gene sequence gave top hits with *R. solanacearum* strains with 98.88% identity and 99% query cover.

3.3 Percent Disease Incidence (PDI) and Scoring of Hybrids

The percent disease incidence and the scoring of hybrids, parents and checks are given in Table 4.

Analysis of variance revealed significant differences among treatments for percent disease incidence (PDI). The hybrid L3 × T1 (IC89989 x IC253967), tester T2 (IC255756) and the resistant check Arka Anand were highly resistant, showing no visible symptoms. Four hybrids L1 × T2 (SM 10 x IC255756), L2 × T3 (IC111010 x IC256708), L3 × T2 (IC89989 x IC255756), L4 × T2 (IC427008 x IC255756), line L3 (IC89989), testers T1 (IC253967) and T3 (IC256708) and the check Neelima were moderately resistant, whereas L1 × T1 (SM 10 x IC253967), L4 × T1 (IC427008 x IC253967), line L4 (IC427008) and Haritha were moderately susceptible. All other hybrids and lines L1 (SM 10) and L2 (IC111010) were highly susceptible to bacterial wilt.

Table 4. PDI of hybrids, parents and checks

Genotype	PDI %	Score	Category
Lines			
L1	46.7(0.752[1])[ab]	5	HS
L2	73.7(1.033)[a]	5	HS
L3	15.7(0.407)[b]	2	MR
L4	26.1(0.537)[ab]	3	MS
Testers			
TI	15.7(0.407)[b]	2	MR
T2	0[b]	1	HR
T3	11.75(0.33)[b]	2	MR
Hybrids			
L1 X T1	27.1(0.548)[ab]	3	MS
LI X T2	15.7(0.407)[b]	2	MR
L1 X T3	46.7(0.752)[ab]	5	HS
L2 X T1	46.7(0.752)[ab]	5	HS
L2 X T2	46.7(0.752)[ab]	5	HS
L2 X T3	15.7 (0.407)[b]	2	MR
L3 X T1	0[b]	1	HR
L3 X T2	11.7 (0.35)[b]	2	MR
L3 X T3	46.7 (0.752)[ab]	5	HS
L4 X T1	27.7 (0.554)[ab]	3	MS
L4 X T2	15.7 (0.554)[b]	2	MR
L4 X T3	59.9 (0.554)[ab]	5	HS
Checks			
Haritha	21.3(0.48)[b]	3	MS
Neelima	11.7(0.35)[b]	2	MR
Arka Anand	0 [ab]	1	HR
C.D. (0.05)[2]	0.47		
SE (m)[3]	0.093		
SE (d)	0.132		
CV (%)[4]	29.92		

Table 5. ANOVA for line X tester analysis including parents for bacterial wilt resistance

Source of variation	df	Sum of Squares	Mean square	F Value	P = .05
Replications	2	0.0454	0.0227	0.857	0.4329
Treatments	18	2.5904	0.1439	5.436	0.0004**
Parents	6	1.2631	0.2105	7.952	0.0001**
Parents vs. Crosses	1	0.0133	0.0133	0.502	0.4832
Crosses	11	1.3140	0.1195	4.512	0.0003**
Lines	3	0.1583	0.0528	0.376	0.7739
Testers	2	0.3131	0.1566	1.115	0.3875
Lines × Testers	6	0.8426	0.1404	5.304	0.0005**
Error	36	0.9531	0.0265	—	—
Total	56	3.5889	—	—	—

Arcsine transformed values are given in the parenthesis
[2]C.D – Critical Difference
[3]SE - Standard Error
[4]CV – Coefficient of Variation

The high susceptibility observed in L1 and L2 corroborated the findings of Muslepally (2023), who also reported these genotypes as highly susceptible. Conversely, the highly resistant genotype T1, identified by Muslepally (2023), exhibited only moderate resistance in the present study, while the resistant check Haritha, earlier reported as highly resistant in many studies (Santhosha et al., 2015; Kumbar et al., 2021; Sivasankarreddy et al.,2025), showed moderate susceptibility under the current conditions. These discrepancies may be attributed to variations in inoculum concentration or the pathogenic diversity of *R. solanacearum* isolates. As noted by Winstead and Kelman (1952), bacterial wilt incidence is influenced by factors such as plant age, inoculum load, inoculation method and temperature. Supporting studies have similarly emphasized that the stability of bacterial wilt resistance is affected by pathogen density, strain variability, temperature and soil moisture (Santhosha et al., 2015; Kumar et al., 2017; Kumar et al., 2018; Bittner et al., 2016).

Table 6. GCA effects of parents

Line/ Tester	GCA Effect
L1	-0.003
L2	0.065
L3	-0.107
L4	0.044
T1	-0.035
T2	-0.093
T3	0.128*

Table 7. SCA effects of crosses

Line \ Tester	T1	T2	T3
L1	0.014	-0.069	0.056
L2	0.150	0.208*	-0.358*
L3	-0.137	-0.022	0.159
L4	-0.027	-0.116	0.143

** Significance at 5% level, ** significance at 1% level*

3.4 Combining Ability

From the analysis of variance for the line × tester design including parents (Table 5), it was evident that the treatments differed significantly, indicating substantial genetic variation and justifying further analysis. The variance due to Line × Tester interactions, representing specific combining ability (SCA), was also highly significant, suggesting considerable genetic variability among the parents in their respective hybrid combinations.

For percent disease incidence (PDI), a negative combining ability is desirable (Ajjappalavara et al., 2008; Chattopadhyay et al*.,* 2012; Mishra et al., 2020). None of the lines in this study exhibited a significant GCA effect, while among testers, T3 showed a significant GCA effect (Table 6). However, since this effect was positive, T3 cannot be considered a good general combiner for bacterial wilt resistance. A highly significant and negative SCA effect was observed for the cross L2 × T3 i.e., IC111010 x IC256708 (–0.358), indicating that this hybrid has the potential to transmit resistance in this specific cross combination (Table 7).

The additive variance was low and negative, whereas the dominance variance was comparatively higher for PDI (Table 8). The contribution of lines and testers to the total variance was 12.04% and 23.83%, respectively, while the line × tester interaction accounted for the highest proportion (64.12%) (Table 9). These results suggest that non-additive gene action predominates and that specific cross combinations, rather than individual parental performance, are important for bacterial wilt resistance in brinjal.

Several studies have investigated the gene action and inheritance of bacterial wilt (BW) resistance in brinjal and other solanaceous crops using different breeding approaches such as line × tester, half-diallel, full diallel, and generation mean analyses. In brinjal, Chattopadhyay et al. (2012) reported non-additive gene action from line × tester analysis, while Singh et al. (2011) observed similar patterns in tomato, indicating the scope for heterosis breeding. Half-diallel analyses in brinjal revealed contrasting results i.e., Tripathy et al. (2025) identified non-additive gene action, whereas Mishra et al. (2023) reported additive gene effects. In tomato, a full diallel analysis by Lopes et al. (2022) revealed additive control, while Mendes et al. (2018) found non-additive effects. Studies using segregating generations in brinjal also showed variation; Bainsla et al. (2016) reported additive gene control, whereas Ajjappalavara et al. (2008) noted non-additive, single-gene inheritance using line × tester followed by segregating generation analysis. Barik et al. (2021), through generation mean analysis, further revealed that BW resistance in two brinjal crosses was governed by epistatic interactions.

Table 8. Value of genetic components

Genetic Component	Value
[5]Cov H.S. (line)	-0.009741849
Cov H.S. (tester)	0.001344861
Cov H.S. (average)	-0.0009049147
[6]Cov F.S. (average)	0.02943206
[7]F = 0, Additive genetic variance	-0.003619659
F = 1, Additive genetic variance	-0.001809829
F = 0, Variance due to Dominance	0.07476152
F = 1, Variance due to Dominance	0.03738076

Table 9. Proportional contribution of lines, testers and their interactions to total variance

Source of Variation	Proportional Contribution (%)
Lines	12.04496
Testers	23.83099
Line × Tester Interaction	64.12404

Although the present study detected non-additive gene action, BW resistance is known to vary with genotype and may involve recessive genes, incomplete dominance, gene inhibition, or epistatic interactions. Environmental factors, pathogen strain variation and location-specific disease pressure further influence the expression of resistance. Because BW inheritance is strongly genotype, environment and strain-dependent, evaluating gene action under hotspot conditions is essential for identifying stable resistance sources and developing effective breeding strategies (Barik et al., 2021; Pitchai et al., 2024).

This study represents an initial effort to accelerate hybrid screening for bacterial wilt resistance at the seedling stage, an area with limited existing research on line × tester analysis. The durability and reliability of these findings can be further validated through field-level evaluations.

[5] *Covariance of Half Sib*
[6] *Covariance of Full Sib*
[7] *F denotes the inbreeding coefficient (F = 0 for random mating and F = 1 for complete inbreeding). In often cross-pollinated populations like brinjal, where both selfing and crossing occur, the actual inbreeding level lies between these two extremes.*

4. CONCLUSION

This investigation represents a novel application of line × tester analysis in brinjal aimed at the early identification of bacterial wilt–resistant hybrids during the seedling stage, thereby expediting the hybrid development process. Among the evaluated crosses, one hybrid exhibited a high level of resistance, while four showed moderate resistance. Combining ability analysis revealed that the hybrid L2 × T3 recorded a highly significant negative SCA for PDI, underscoring its potential as a promising source of bacterial wilt resistance. The predominance of positive dominance variance over low or negative additive variance, coupled with a higher proportion of line × tester interaction, further substantiates that hybrid breeding is the most effective approach for enhancing bacterial wilt resistance in brinjal.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technology ChatGPT based on GPT - 5.1 have been used during the writing or editing of manuscripts. The AI tool was only used for minimal paraphrasing and not for any other purposes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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