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Article

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QTL-seq-Based Mapping and Validation of Molecular Markers Associated with Bacterial Wilt Resistance in Eggplant (*Solanum melongena* L.)

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Abstract

Bacterial wilt caused by *Ralstonia solanacearum* is a major constraint to eggplant (*Solanum melongena* L.) production, and the genetic basis of resistance remains insufficiently characterized. In this study, we investigated the inheritance and genomic basis of bacterial wilt resistance using an F_2 population of 260 individuals derived from a cross between the resistant cultivar Utkal Anushree and the susceptible line BCB 40. Phenotypic evaluation following artificial inoculation indicated that resistance was predominantly controlled by a major recessive locus. QTL-seq analysis of contrasting resistant and susceptible DNA bulks identified three candidate genomic regions on chromosome 3 (91.0-91.8 Mb) and chromosome 11 (6.7-7.0 Mb and 7.4-8.4 Mb). Screening of 110 SSR markers identified emb01D10 and em135a as polymorphic between the parents and contrasting DNA bulks. Genotyping of the F_2 population further confirmed their close association with the resistance locus, demonstrating their potential as molecular markers for bacterial wilt resistance. Comparative sequence analysis of the candidate regions identified several SNPs and InDel polymorphisms, providing additional sequence variation for fine mapping and candidate gene identification. Collectively, these results define genomic regions associated with bacterial wilt resistance and identify closely linked molecular markers in eggplant. The genomic resources generated in this study provide a basis for further molecular dissection of resistance and facilitate marker-assisted selection for bacterial wilt resistance breeding.

Keywords:
Solanum melongena; bacterial wilt; *Ralstonia solanacearum*; QTL-seq; bulked segregant analysis; SSR markers; marker-assisted selection.

Introduction

Eggplant (*Solanum melongena* L.) is one of the most important vegetable crops of the family Solanaceae, cultivated extensively in tropical and subtropical regions because of its high economic value, adaptability to diverse agro-climatic conditions and nutritional significance (Gramazio *et al.*, 2018). The fruits are rich in dietary fibre, vitamins, minerals, chlorogenic acid, phenolic compounds, flavonoids and anthocyanins, which contribute to their antioxidant and health-promoting properties (Plazas *et al.*, 2013). Asia accounts for more than 90% of global eggplant production, with China and India being the leading producers, highlighting the crop's importance in food and nutritional security (FAO, 2023; Xiao *et al.*, 2024). In India, eggplant is cultivated across diverse agro-climatic regions and serves as an important source of income for small and marginal farmers because it can be cultivated year-round and has continuous market demand (NHB, 2024). However, the productivity of eggplant is severely constrained by several biotic stresses, among which bacterial wilt caused by *Ralstonia solanacearum* is one of the most destructive diseases, particularly in warm and humid environments where disease incidence may lead to substantial yield losses (Hayward, 1991; Elphinstone, 2005; Sivasankarreddy *et al.*, 2024)

Ralstonia solanacearum, the causal agent of bacterial wilt, is a soil-borne, Gram-negative, vascular pathogen belonging to the *R. solanacearum* species complex (RSSC), one of the most genetically diverse groups of phytopathogenic bacteria (Genin and Denny, 2012; Sivasankarreddy *et al.*, 2024). The pathogen infects more than 200 plant species representing over 50 botanical families, with solanaceous crops such as eggplant, tomato, potato, pepper and tobacco being particularly susceptible (Hayward, 1991; Elphinstone, 2005). Infection is initiated through the roots, followed by rapid colonisation of the xylem vessels, where the bacterium proliferates and produces extracellular polysaccharides, cell wall-degrading enzymes and Type III secretion system effectors that disrupt water transport, resulting in irreversible wilting and eventual plant death (Genin and Denny, 2012; Mansfield *et al.*, 2012). The pathogen can persist in soil, irrigation water, weeds and asymptomatic host plants for extended periods, making eradication extremely difficult and contributing to frequent disease outbreaks under favorable environmental conditions (Sivasankarreddy *et al.*, 2024). Although several management practices, including crop rotation, soil amendments, biological control agents, grafting and chemical treatments, have been evaluated, their effectiveness remains inconsistent due to the pathogen's extensive genetic variability, broad host range and long-term survival in diverse ecological niches (Elphinstone, 2005; Barik *et al.*, 2020). Therefore, the development of genetically resistant cultivars remains the most effective, economical and environmentally sustainable approach for managing bacterial wilt and ensuring stable eggplant production (Barik *et al.*, 2020; Sivasankarreddy *et al.*, 2024).

Despite the availability of resistant germplasm, the genetic basis of bacterial wilt resistance in eggplant remains complex, with resistance being governed by one or more quantitative trait loci (QTLs) and influenced by the genetic background of both the host and the pathogen (Barik *et al.*, 2020; Wang *et al.*, 2024). Over the past decade, conventional linkage mapping has identified several genomic regions associated with bacterial wilt resistance however, these approaches are often constrained by the requirement for large mapping populations, high-density genetic maps, multiple generations and extensive phenotypic evaluations, making them time-consuming and labour-intensive (Wang *et al.*, 2024). The advent of next-generation sequencing (NGS) technologies has led to the development of QTL-seq, an approach that integrates bulked segregant analysis (BSA) with whole-genome resequencing to rapidly identify genomic regions associated with target traits through genome-wide allele frequency comparisons between phenotypically contrasting bulks (Takagi *et al.*, 2013). Compared with conventional QTL mapping, QTL-seq offers a rapid, cost-effective and high-resolution strategy for detecting major-effect loci while substantially reducing the time and resources required for genetic analysis (Takagi *et al.*, 2013;

Xiao *et al.*, 2024). Furthermore, genomic regions identified through QTL-seq can be translated into breeder-friendly molecular markers, including simple sequence repeats (SSRs), which remain widely used in plant breeding because of their co-dominant inheritance, high reproducibility, ease of detection and suitability for marker-assisted selection (MAS) (Barik *et al.*, 2020). Therefore, integrating QTL-seq with SSR marker validation provides an efficient framework for identifying reliable markers that can accelerate the development of bacterial wilt-resistant eggplant cultivars.

Although recent studies have successfully identified genomic regions associated with bacterial wilt resistance using conventional QTL mapping and sequencing-based approaches, the translation of these discoveries into reliable molecular tools for routine breeding applications remains limited (Barik *et al.*, 2020; Wang *et al.*, 2024; Xiao *et al.*, 2024). In particular, the validation of closely linked SSR markers in segregating populations, especially in Indian eggplant germplasm, has received relatively little attention, thereby limiting their practical deployment in marker-assisted breeding programmes. Furthermore, the considerable genetic diversity of the *Ralstonia solanacearum* species complex and the quantitative nature of bacterial wilt resistance necessitate the development of robust and reproducible molecular markers that are effective across diverse genetic backgrounds (Genin and Denny, 2012; Sivasankarreddy *et al.*, 2024). Therefore, the present study integrated phenotypic evaluation of an F_2 mapping population with QTL-seq and subsequent SSR marker validation to identify genomic regions associated with bacterial wilt resistance and develop reliable molecular markers. The findings of this study are expected to facilitate marker-assisted selection and accelerate the development of bacterial wilt-resistant eggplant cultivars, thereby contributing to sustainable disease management and genetic improvement of this economically important crop.

Materials and methods

2.1 Experimental site and plant materials

The study was conducted during 2021–2024 under the All India Coordinated Research Project (AICRP) on Vegetable Crops, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Kalyani, Nadia, West Bengal, India. Field experiments were carried out at the AICRP experimental farm, while laboratory experiments were conducted in the Plant Pathology laboratories of BCKV.

Two contrasting brinjal (*Solanum melongena* L.) genotypes, Utkal Anushree, a bacterial wilt-resistant genotype, and BCB 40, a highly susceptible genotype, were used as parental lines. The genotypes, Utkal Anushree and BCB-40 are the brinjal genotypes that were collected from the BCKV University Research Farm. Utkal Anushree is a released variety from OUAT, Bhubaneswar, Odisha, India, and BCB-40 is a breeding line developed and mentioned at BCKV, Kalyani, India. An F_2 mapping population comprising 260 individuals was developed from the cross Utkal Anushree × BCB 40. The F_2 population was phenotypically evaluated for bacterial wilt resistance following artificial inoculation with *Ralstonia solanacearum*. Based on disease reaction, resistant and susceptible F_2 individuals were selected for bulked segregant analysis (BSA), QTL-seq and subsequent marker validation.

2.2 Isolation and maintenance of *Ralstonia solanacearum*

Naturally infected brinjal plants exhibiting characteristic bacterial wilt symptoms were collected from diseased experimental fields. The presence of *Ralstonia solanacearum* was confirmed using the bacterial ooze test, in which longitudinal sections of infected vascular tissue were immersed in sterile distilled water and examined for the characteristic milky bacterial streaming. The bacterial ooze was streaked onto Triphenyl Tetrazolium Chloride (TTC) medium and incubated at 30°C for 48–72 h following the method described by Kelman (1954). Typical virulent colonies, characterised by a fluidal white to cream appearance with a pink centre, were purified and maintained on TTC medium for subsequent experiments.

2.3 Artificial inoculation and phenotyping of the F_2 population

Eggplant seedlings were raised under protected nursery conditions and transplanted to the experimental field at approximately 35 days after sowing. A virulent isolate of *R. solanacearum* was cultured and adjusted to a concentration of approximately 1×10^8 CFU mL^{-1}. Prior to transplanting, seedling roots were gently dipped in the bacterial suspension up to the root–shoot junction for approximately 10–15 min. To ensure successful pathogen establishment, a second inoculation was performed 30–35 days after transplanting by injecting the bacterial suspension into the root zone using a sterile syringe.

The F_2 mapping population consisting of 260 individuals was phenotyped following artificial inoculation. Resistant and susceptible plants were identified based on their phenotypic response to bacterial wilt and used for subsequent molecular analyses, including bulked segregant analysis and QTL-seq.

2.4 Genomic DNA extraction and PCR amplification

Genomic DNA was isolated from young leaf tissues of the parental lines and F_2 individuals using the cetyltrimethylammonium bromide (CTAB) method. DNA quality and concentration were assessed using a Bio-Spectrometer and by electrophoresis on 1% agarose gels. Polymerase chain reaction (PCR) amplification was performed in a final reaction volume of 25 µL containing 12.5 µL PCR Master Mix (Xcelris Labs, India), 2.0 µL genomic DNA, 1.0 µL each of forward and reverse primers, and 8.5 µL nuclease-free water. PCR amplification was carried out with an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 45 s and extension at 72°C for 1.5 min, with a final extension at 72°C for 8 min.

2.5 Construction of DNA bulks and whole-genome sequencing

Genomic DNA was extracted from young leaves of the resistant parent (Utkal Anushree), susceptible parent (BCB 40) and selected F_2 individuals. Based on phenotypic evaluation for bacterial wilt resistance, equal amounts of DNA from 20 resistant and 20 susceptible F_2 plants were pooled separately to construct the Resistant Bulk (RB) and Susceptible Bulk (SB). DNA concentrations were normalized prior to pooling to ensure equal representation of each individual in the respective bulk. Whole-genome sequencing libraries were prepared from the two parental lines and the two DNA bulks following the Illumina TruSeq DNA library preparation protocol. Paired-end libraries with an average insert size of approximately 350 bp were sequenced on the Illumina HiSeq platform to generate 150 bp paired-end reads for QTL-seq analysis.

2.6 QTL-seq analysis

Raw sequencing reads were quality-checked using FastQC v0.11.8 (Andrews, 2010) and filtered with Trimmomatic v0.39 (Bolger et al., 2014). High-quality reads were analysed using QTL-seq v2.2.3. Reads were aligned to the eggplant reference genome using BWA (Li and Durbin, 2009) and variants were identified using SAMtools v1.14 (Li et al., 2009). SNP-index and Δ(SNP-index) values were calculated for the resistant and susceptible bulks and genomic regions exceeding the 95% and 99% confidence intervals were considered candidate QTLs for bacterial wilt resistance (Takagi et al., 2013).

2.7 SSR marker screening and validation

A total of 110 genomic SSR markers distributed across the eggplant genome were screened to identify polymorphisms between the resistant parent (Utkal Anushree) and the susceptible parent (BCB 40). Polymorphic SSR markers were subsequently used for bulked segregant analysis (BSA) to identify markers associated with bacterial wilt resistance. For marker validation, genomic DNA was isolated from all 260 F_2 individuals. Resistant and susceptible DNA bulks were screened using the polymorphic SSR markers identified during parental screening. Markers exhibiting clear polymorphism between the two parents and contrasting amplification patterns between the resistant and susceptible bulks were further

validated in the entire F_2 population to assess marker segregation and co-segregation with bacterial wilt resistance.

2.8 Statistical analysis

Phenotypic and genotypic segregation data of the F_2 population were analysed using Statistical Analysis System (SAS) version 9.3 (SAS Institute Inc., Cary, NC, USA). The goodness-of-fit of the observed segregation ratios to the expected Mendelian ratios was evaluated using the chi-square (χ^2) test according to the method described by Dabholkar (1999). Differences were considered significant at $P < 0.05$.

3. Results

3.1 Inheritance analysis of bacterial wilt resistance in the F_2 population

A total of 260 F_2 individuals derived from the cross Utkal Anushree × BCB 40 were evaluated for bacterial wilt resistance. Among them, 209 plants were susceptible and 51 were resistant, approximating a 3:1 segregation ratio. Chi-square analysis indicated that the observed segregation did not deviate significantly from the expected Mendelian ratio, suggesting that bacterial wilt resistance in this population is controlled by a single recessive gene, with susceptibility being dominant (Table 1).

Table 1: Phenotypic Chi–square values of segregating F2 populations

Cross	**Number of plants**	**Observed frequency**		**Observed ratio**	**Expected frequency**		**Expected ratio**	**Chi square value**
		Susceptible	Resistant		Susceptible	Resistant		
Utkal Anushree × BCB 40	260	209	51	3:1 (approx)	195	65	3:1	4.02

This result is significant at $p<0.05$

3.2 QTL-seq-based identification of genomic regions associated with bacterial wilt resistance

Sequencing data quality

Whole-genome resequencing generated high-quality reads for both parental lines and the resistant and susceptible bulks after quality filtering. The resistant parent (Utkal Anushree) and susceptible parent (BCB 40) yielded 73,546,810 and 47,114,708 clean reads, respectively, while the resistant and susceptible bulks produced 256,469,286 and 268,502,883 clean reads, respectively. The proportion of clean reads ranged from 79.36% to 92.32% across all samples, indicating high-quality sequencing data suitable for downstream QTL-seq analysis (Table 2).

Table 2: Summary of reads statistics of the re-sequenced samples of the parents along with the contrasting bulks

Sample	**Input Read Pairs**	**Clean reads (Both Surviving)**	**% Clean reads**
Susceptible bulk	290,743,438	268,502,883	92.32
Resistant bulk	311,543,844	256,469,286	82.32

Parent 1 (Utkal Anushree)	82,960,147	73,546,810	88.65
Parent 2 (BCB 40)	59,363,578	47,114,708	79.36

Identification of candidate QTL regions

QTL-seq analysis identified three candidate genomic regions associated with bacterial wilt resistance. One region was detected on chromosome 3 (91.0–91.8 Mb), while two regions were identified on chromosome 11 (6.7–7.0 Mb and 7.4–8.4 Mb) (Fig. 1). Genome annotation of these intervals revealed 96 predicted genes within the chromosome 3 QTL region and 84 predicted genes within the chromosome 11 QTL regions.

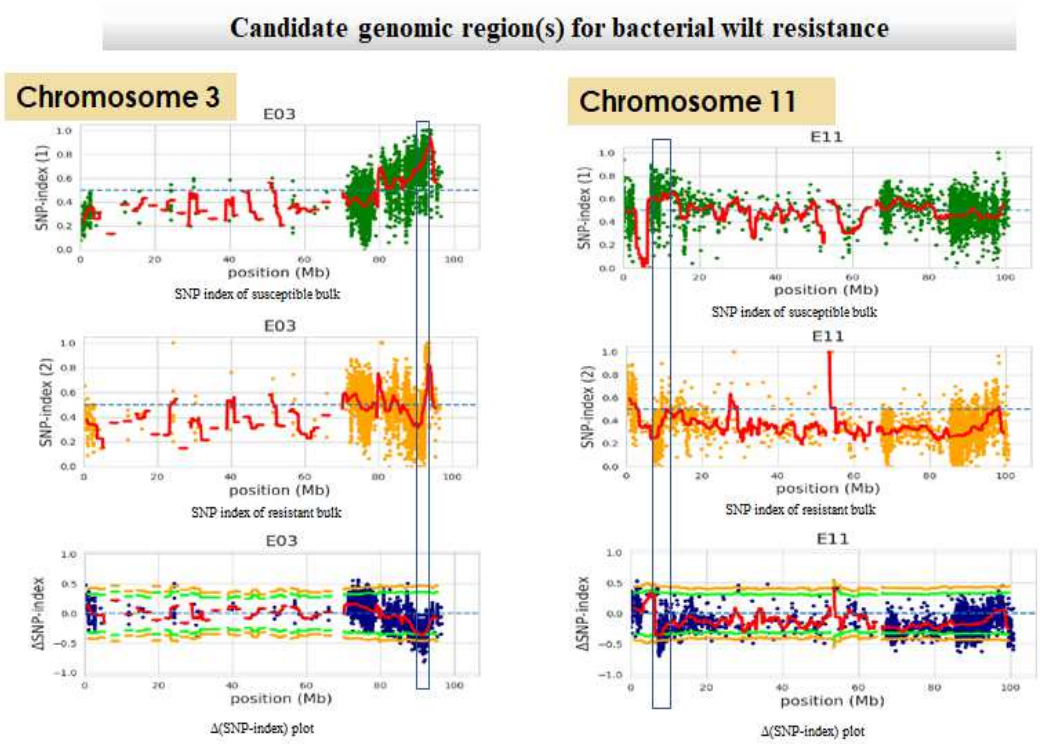


Fig.1: Identification of QTL region on chromosome 3(91–91.8 Mb) and chromosome 11(6.7 -7.0 Mb and 7.4- 8.4 MB) associated with bacterial wilt resistance using Illumina sequencing and Bulk Segregant Analysis.

3.3 SSR marker screening and polymorphism analysis:

A total of 110 genomic SSR markers were screened for polymorphism between the parental genotypes, Utkal Anushree and BCB 40, together with the resistant and susceptible DNA bulks. Of these, two SSR markers *(emb01D10 and em135a)* exhibited clear polymorphism between the parents and contrasting amplification patterns in the resistant and susceptible bulks, whereas the remaining markers were monomorphic (Table 3; Fig. 2).

Table 3: Amplification pattern of genomic SSR primers in parental population

Sl. No.	Primer Name	Remarks
1.	ctg180-1	Monomorphic
2.	ctg221163-1	Monomorphic
3.	ctg299666-1	Monomorphic
4.	ctg395359-1	Monomorphic
5.	ctg502673-1	Monomorphic
6.	ctg613726-1	Monomorphic
7.	ctg716550-1	Monomorphic
8.	ctg822477-1	Monomorphic
9.	ctg938394-1	Monomorphic
10.	ctg1052242-1	Monomorphic
11.	ctg1173161-1	Monomorphic
12.	ctg1296337-1	Monomorphic
13.	ctg1447558-1	Monomorphic
14.	ctg1648601-1	Monomorphic
15.	ctg7180000086494	Monomorphic
16.	ctg7180000226029	Monomorphic
17.	ctg7180000297805	Monomorphic
18.	ctg7180000371440	Monomorphic
19.	ctg7180000464128	Monomorphic
20.	ctg7180000558466	Monomorphic
21.	ctg7180000644963	Monomorphic
22.	ctg7180000726194	Monomorphic
23.	ctg7180000820595	Monomorphic
24.	ctg7180001157526	Monomorphic
25.	ctg7180001049083	Monomorphic
26.	ctg7180001157526	Monomorphic
27.	ctg7180001268341	Monomorphic
28.	ctg7180001374918	Monomorphic
29.	ctg7180001512531	Monomorphic
30.	ctg7180001609856	Monomorphic
31.	ctg7180001666973	Monomorphic
32.	ctg7180000051126	Monomorphic
33.	ctg7180000155189	Monomorphic
34.	ctg7180000221826	Monomorphic
35.	ctg7180000237036	Monomorphic
36.	ctg7180000284638	Monomorphic
37.	ctg7180000312265	Monomorphic
38	ctg7180000347216	Monomorphic
39.	ctg7180000390242	Monomorphic
40.	ctg7180000446964	Monomorphic
41.	ctg7180000477657	Monomorphic
42.	ctg7180000543481	Monomorphic

Sl. No.	Primer Name	Remarks
43.	ctg7180000575579	Monomorphic
44.	ctg7180000629202	Monomorphic
45.	ctg7180000663758	Monomorphic
46.	ctg7180000681652	Monomorphic
47.	ctg7180000734981	Monomorphic
48.	ctg7180000792978	Monomorphic
49.	ctg7180000851986	Monomorphic
50.	ctg7180000891625	Monomorphic
51.	ctg7180000964573	Monomorphic
52.	ctg7180001019247	Monomorphic
53.	ctg7180001072837	Monomorphic
54.	ctg7180001133218	Monomorphic
55.	ctg7180001185405	Monomorphic
56.	ctg7180001250610	Monomorphic
57.	ctg7180001291258	Monomorphic
58.	ctg7180001343208	Monomorphic
59.	ctg7180001408328	Monomorphic
60.	ctg7180001476396	Monomorphic
61.	ctg7180001538888	Monomorphic
62.	ctg7180001582954	Monomorphic
63.	ctg7180001624126	Monomorphic
64.	ctg7180001655595	Monomorphic
65.	ctg7180000573331	Monomorphic
66.	ctg7180000584752	Monomorphic
67.	ctg7180000782676	Monomorphic
68.	ctg7180001055785	Monomorphic
69.	ctg7180001167402	Monomorphic
70.	ctg7180001198804	Monomorphic
71.	ctg7180001292517	Monomorphic
72.	ctg7180001305066	Monomorphic
73.	emb01D10	Polymorphic
74.	emh02E08	Monomorphic
75.	SSR–46	Monomorphic
76.	emh21J12	Monomorphic
77.	STM0007	Monomorphic
78.	emh01J23	Monomorphic
79.	emb01N07	Monomorphic
80.	emf21E10	Monomorphic
81.	emg11I03	Monomorphic
82.	emb01N07	Monomorphic
83.	eme36B08	Monomorphic
84.	em135 a	Polymorphic

Sl. No.	Primer Name	Remarks
85.	em119 a	Monomorphic
86.	Primer 1	Monomorphic
87.	Primer 2	Monomorphic
88.	Primer 3	Monomorphic
89.	ctg7180000312142	Monomorphic
90.	ctg7180000325902	Monomorphic
91.	ctg7180000333771	Monomorphic
92.	ctg7180000421734	Monomorphic
93.	ctg7180000431986	Monomorphic
94.	ctg7180000570722	Monomorphic
95.	ctg7180000580911	Monomorphic
96.	ctg7180000708758	Monomorphic
97.	ctg7180000735141	Monomorphic
98.	ctg7180000769871	Monomorphic
99.	ctg7180000775495	Monomorphic
100.	ctg7180000778901	Monomorphic
101.	ctg7180000791464	Monomorphic
102.	ctg7180000802052	Monomorphic
103.	ctg7180000898211	Monomorphic
104.	ctg7180000910528	Monomorphic
105.	ctg7180000945698	Monomorphic
106.	ctg7180000948211	Monomorphic
107.	ctg7180000956085	Monomorphic
108.	ctg7180000962309	Monomorphic
109.	ctg7180000978643	Monomorphic
110.	ctg7180000997480	Monomorphic

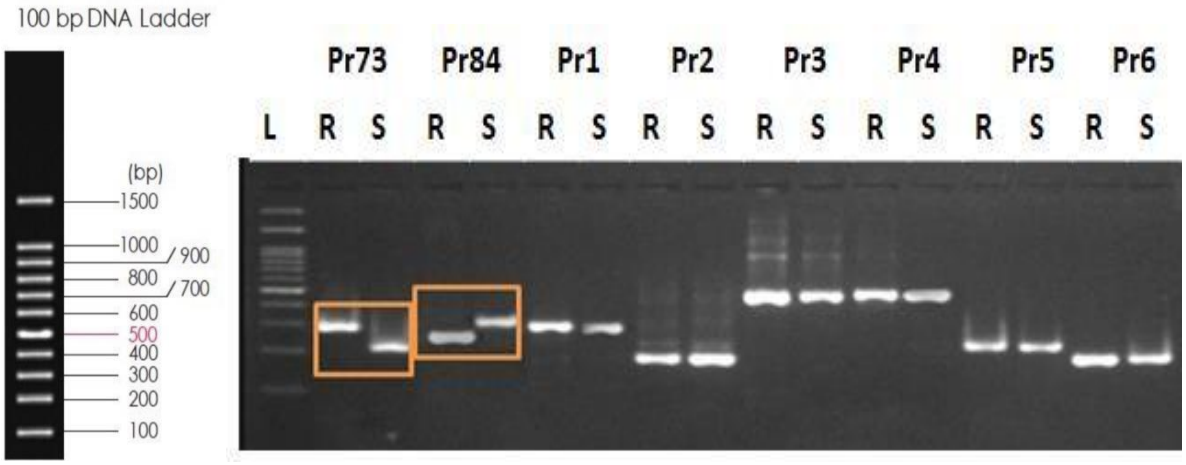


Fig.2: Amplification pattern of genomic SSR primers in parental population i.e. Utkal Anushree (Resistant, R) and BCB 40 (Susceptible, S). Highlighted boxes indicate polymorphisms between the two parents. L: ladder, Pr represents the primers used according to serial number given in the Table 3 (73, 84, 1-6). The uncropped gel image has been provided in the supplementary file.

3.4 Bulk segregant analysis using SSR markers

Among the 110 SSR markers screened, only *emb01D10* and *em135a* differentiated the resistant parent (Utkal Anushree) from the susceptible parent (BCB 40) and produced contrasting amplification patterns between the resistant and susceptible DNA bulks (Table 4; Fig. 3). These polymorphic markers were subsequently used to genotype the entire F_2 population (260 individuals) for marker validation.

Table 4: Primer sequence of SSR marker against to bacterial wilt in brinjal

SSR Marker	Primer sequence (5'-3')	
	Forward	Reverse
emb01D10	AAGAATCGGTCCTCTTTGCATTGT	TGCTTTTCACCTCTCCGCTATCTC
em135 a	ATCCTGTTGCTGCTCATTTTCCTC	AGGAGGATCCAAGAGGTTTGTTGA

Table 5: Amplified product size of two (emb01D10 and em135a) polymorphic marker

Population	SSR Marker	Amplified product size (bp)	
		Resistant	Susceptible
Utkal Anushree × BCB 40	emb01D10	300	210
	em135 a	250	300

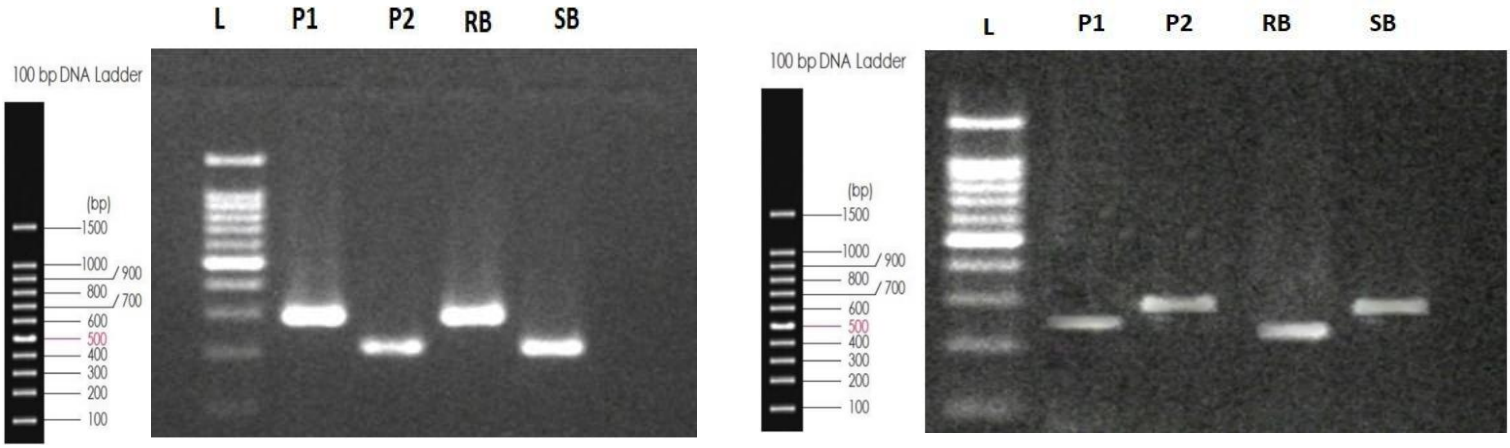


Fig. 3. Bulk segregant analysis of bacterial wilt resistance using polymorphic SSR markers emb01D10 and em135a. The polymorphic markers differentiated the resistant parent (P1, Utkal Anushree), susceptible parent (P2, BCB 40), resistant bulk (RB) and susceptible bulk (SB) of the F_2 population. L, 100 bp DNA ladder. The uncropped gel image has been provided in the supplementary file.

3.5 Validation of SSR markers in the F_2 population

The polymorphic SSR markers emb01D10 and em135a were validated in the 260 F_2 individuals derived from the cross Utkal Anushree × BCB 40. Both markers clearly distinguished the resistant and susceptible genotypes and exhibited co-dominant segregation in the F_2 population (Figs. 4 and 5). Genotypic segregation of both markers conformed to the expected 1:2:1 Mendelian ratio, confirming their stable inheritance and suitability for marker-assisted selection (Table 6).

Table 6: Segregation pattern of SSR markers (emb01D10 and em135 a) in segregating F_2 populations for bacterial wilt resistance in eggplant.

Cross	SSR marker	Observed F2 genotype			Total number of recombinants	Recombination frequency (%)	Expected ratio	Chi square value	P value at 5%
		Resistance (A)	Heterozygous Susceptible (AB)	Susceptible (B)					
Utkal Anushree × BCB 40	emb01D 10	67	132	61	33	12.69	1:2:1	0.34	0.84
	em135a	71	125	64	36	13.85	1:2:1	0.76	0.68

Marker emb01D10 exhibited a recombination frequency of 12.69% with a χ^2 value of 0.34 (P = 0.84), whereas em135a showed a recombination frequency of 13.85%, a χ^2 value of 0.76, and P = 0.68. Both markers followed the expected 1:2:1 Mendelian segregation ratio, indicating no significant deviation from the expected inheritance pattern (Table 6).

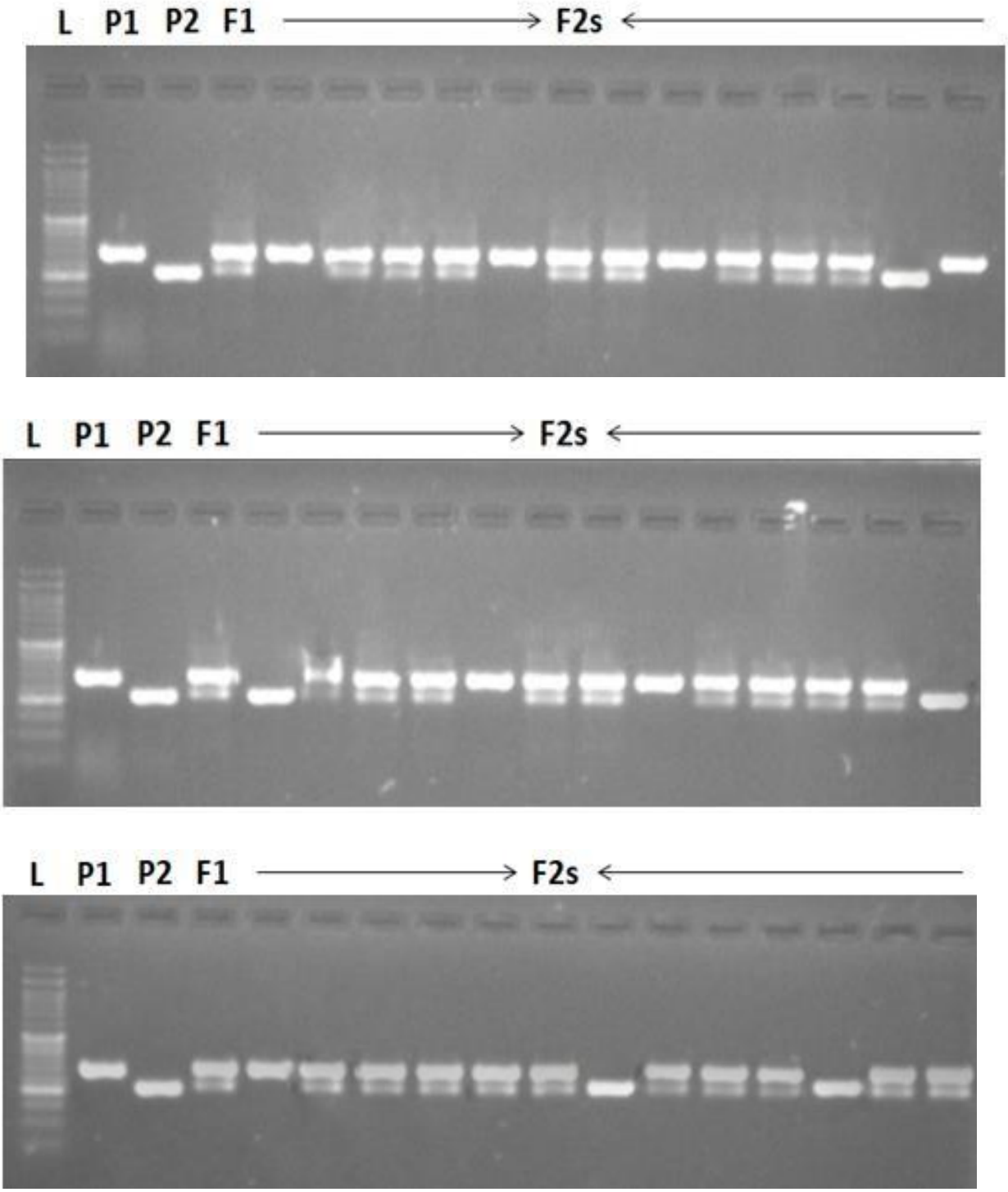


Fig. 4. Validation of the emb01D10 SSR marker in the parental lines, F_1 hybrid, and F_2 population derived from the cross Utkal Anushree × BCB 40. P1, Utkal Anushree (resistant); P2, BCB 40 (susceptible); F_1, hybrid; F_2, segregating population; L, 100 bp DNA ladder.

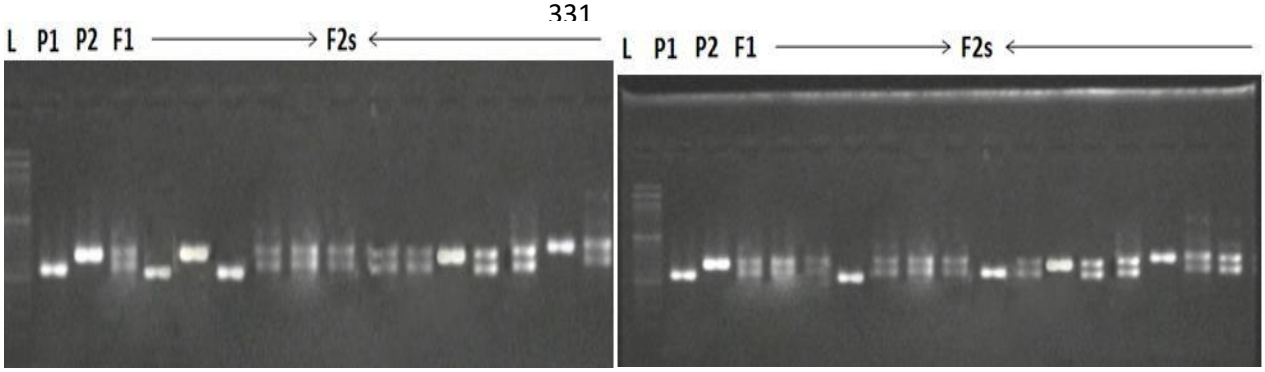


Fig. 5: Validation of the em135a SSR marker in the parental lines, F_1 hybrid and F_2 population derived from the cross Utkal Anushree × BCB 40. P1, Utkal Anushree (resistant); P2, BCB 40 (susceptible); F_1, hybrid; F_2, segregating population; L, 100 bp DNA ladder. The uncropped gel image has been provided in the supplementary file.

3.6 Nucleotide sequence comparison between resistant and susceptible brinjal genotypes

Pairwise alignment of the nucleotide sequences from the resistant genotype Utkal Anushree (Contig 1) and the susceptible genotype BCB 40 (Contig 2) revealed multiple single nucleotide polymorphisms (SNPs) and insertion–deletion (In-Del) polymorphisms across the aligned region (Fig. 6). These sequence variations distinguish the resistant and susceptible parental genotypes within the candidate genomic region associated with bacterial wilt resistance.



Fig. 6. Pairwise nucleotide sequence alignment of the candidate genomic region between the resistant genotype Utkal Anushree (Contig 1) and the susceptible genotype BCB 40 (Contig 2). Asterisks (*) indicate conserved nucleotides, while mismatched bases represent single nucleotide polymorphisms (SNPs) and gaps (–) represent insertion–deletion (InDel) polymorphisms

4. Discussion

Bacterial wilt remains one of the most challenging diseases in eggplant because of the extensive genetic diversity, broad host range and long-term persistence of *Ralstonia solanacearum* in soil. Consequently, host plant resistance represents the most practical and environmentally sustainable strategy for disease management. Understanding the genetic architecture of resistance and identifying tightly linked molecular markers are therefore essential prerequisites for accelerating resistance breeding through marker-assisted selection.

The inheritance of bacterial wilt resistance has been reported to vary considerably among different eggplant genetic backgrounds. Monogenic recessive inheritance has been described by Arunkumar et al. (2016), whereas dominant and quantitative inheritance have also been reported in other mapping populations (Gopalakrishnan et al., 2005; Bainsla et al., 2016; Salgon et al., 2018). Such variation is not unexpected because resistance is influenced by the resistance source, pathogen race and genetic background of the mapping population. The inheritance pattern observed in the present study therefore suggests that the resistance present in Utkal Anushree is conditioned primarily by a major recessive locus, providing a relatively simple genetic system for marker development and breeding.

Accurate phenotyping is fundamental for successful genetic mapping because the reliability of molecular analyses depends directly on correct classification of resistant and susceptible individuals. Artificial inoculation under controlled conditions provided uniform disease pressure, thereby reducing environmental variation during disease assessment. High-quality phenotyping is particularly important in QTL-seq studies because incorrect classification of individuals into resistant and susceptible bulks can substantially reduce mapping resolution and the accuracy of QTL detection.

The integration of QTL-seq with bulked segregant analysis enabled rapid identification of genomic regions associated with bacterial wilt resistance. Compared with conventional linkage mapping, QTL-seq substantially reduces the time required for marker discovery while maintaining high mapping resolution through whole-genome resequencing. Similar applications of QTL-seq have successfully identified bacterial wilt resistance loci in eggplant and other solanaceous crops (Salgon et al., 2018; Xiao et al., 2024). Although the genomic intervals detected in different studies are not always identical, variation in QTL position is expected because of differences in parental genotypes, pathogen isolates and population structure. Nevertheless, the genomic regions identified in this study represent promising targets for fine mapping and candidate gene identification.

Among the SSR markers evaluated, only emb01D10 and em135a exhibited polymorphism and remained consistently associated with the resistance locus following validation in the segregating population. Marker validation is an essential step because polymorphism between parental lines alone does not guarantee successful application in breeding populations. The successful validation of these markers indicates their close linkage with the bacterial wilt resistance locus and highlights their potential utility for marker-assisted selection. Similar approaches integrating bulked segregant analysis and molecular marker validation have been successfully applied to identify bacterial wilt resistance loci in eggplant (Gopalakrishnan et al., 2005; Lebeau et al., 2013; Salgon et al., 2018; Xiao et al., 2024). Compared with dominant marker systems such as RAPD and AFLP, SSR markers remain advantageous because they are co-dominant, highly reproducible, cost-effective and readily applicable in routine breeding programmes.

The identification of SNPs and In-Del polymorphisms between the resistant and susceptible parental genotypes further supports the presence of genetic variation within the candidate resistance region. Such sequence polymorphisms provide useful resources for developing high-resolution molecular markers and facilitate future fine mapping and functional characterisation of candidate genes associated with bacterial wilt resistance.

Overall, the combined use of precise phenotyping, QTL-seq, bulked segregant analysis, and SSR marker validation provides a robust framework for identifying genomic regions associated with bacterial wilt resistance in eggplant. The validated SSR markers identified in this study constitute useful tools for marker-assisted breeding, while the candidate genomic regions provide a foundation for future gene discovery and functional validation aimed at developing durable bacterial wilt-resistant cultivars.

5. Conclusion

This study elucidated the genetic basis of bacterial wilt resistance in eggplant by integrating phenotypic evaluation, QTL-seq, bulked segregant analysis, and SSR marker validation. The results indicate that resistance in the Utkal Anushree × BCB 40 population is primarily governed by a major recessive locus. QTL-seq identified candidate genomic regions associated with resistance, while the SSR markers emb01D10 and em135a were successfully validated as closely linked markers suitable for marker-assisted selection. Furthermore, sequence polymorphism analysis revealed SNPs and InDels within the candidate regions, providing valuable resources for fine mapping and candidate gene identification. Collectively, these findings advance our understanding of the genetic architecture of bacterial wilt resistance and provide practical molecular tools for accelerating resistance breeding in eggplant. Future research should focus on validating the identified QTLs across diverse genetic backgrounds and functionally characterizing the underlying resistance genes to facilitate the development of durable bacterial wilt-resistant cultivars.

Author contribution

SO: Conceptualization, methodology, investigation, data curation, formal analysis, and writing original draft. SS: Investigation, phenotypic evaluation, data collection, and formal analysis. UB: Investigation, molecular analysis, and data curation. MI: Investigation, molecular marker analysis, and data curation. BM: Investigation, phenotyping, and data collection. KP: Methodology, molecular analysis, and validation. AC: Resources, supervision, and validation. AD: Investigation, data curation, and validation. RB: Methodology, supervision, and interpretation of results. SSD: Conceptualization, methodology, supervision, interpretation of results, and writing, review, and editing. AKM: Conceptualization, supervision, project administration, funding acquisition, and writing, review and editing. All authors contributed to interpreting the results, critically reviewed the manuscript, and approved the final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The raw whole-genome sequencing (WGS) data generated and analyzed in this study for Solanum melongena (including the parents Utkal Anushree and BCB 40, as well as the bacterial wilt disease-resistant and susceptible F_2 bulks) have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the BioProject accession number PRJNA1544810 (Study: SRP746294). The corresponding BioSample accession from SAMN64241093 to SAMN64241100, and the individual Sequence Read Archive (SRA) accession from SRR41216414 to SRR41216421. Weblink for the datasets: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1544810?reviewer=hmgq255b763mcjk7ui0hth12l5

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