Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Genome-Wide Association Study Identifies Resistance Loci to Bacterial Wilt in Capsicum species Using a Cotyledon-Based Rapid Screening Method

Jaw-rong Chen
World Vegetable Center
Hung-chia Chang
World Vegetable Center
Jutatape Watcharachaiyakup
Kasetsart University
Derek Barchenger
World Vegetable Center
Ricardo Oliva
Alliance of Bioversity International and CIAT
Ya-ping Lin
ya-ping.lin@worldveg.org

World Vegetable Center

Research Article

Keywords: Genome-Wide Association Study (GWAS), Bacterial Wilt, Ralstonia solanacearum species complex (RSSC), Capsicum species, Cotyledon-Based Rapid Screening

Posted Date: September 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10967581/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Genome-Wide Association Study Identifies Resistance Loci to Bacterial Wilt in *Capsicum* species Using a Cotyledon-Based Rapid Screening Method

Jaw-rong Chen[1], Hung-chia Chang[1], Jutatape Watcharachaiyakup[2], Derek Barchenger[1], Ricardo Oliva[1,3], Ya-ping Lin[1]

Affiliation

[1]World Vegetable Center, Shanhua, Tainan, Taiwan, 74151

[2]Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand, 73140

[3]Alliance of Bioversity International and CIAT, Palmira, Colombia, 763537

Contact Email

Jaw-rong Chen: jaw-rong.chen@worldveg.org

Hung-chia Chang: hung-chia.chang@worldveg.org

Jutatape Watcharachaiyakup: jutatape.w@ku.th

Derek Barchenger: derek.barchenger@worldveg.org

Ricardo Oliva: r.oliva@cgiar.org

Ya-ping Lin: ya-ping.lin@worldveg.org

Corresponding author: Ya-ping Lin, ya-ping.lin@worldveg.org (ORCID: 0000-0002-9575-2007)

Abstract

Bacterial wilt, caused by the *Ralstonia solanacearum* species complex (RSSC), remains a major constraint to pepper (*Capsicum* spp.) production. To facilitate large-scale resistance screening and genetic analysis, we developed a rapid assay at the two-cotyledon stage and applied it to 540 accessions representing four domesticated *Capsicum* species. Comparison with conventional greenhouse screening demonstrated the reliability of the assay, with the strongest correlation observed at 10 days post-inoculation ($r = 0.73$) and a 50% wilting threshold providing the best discrimination between resistant and susceptible responses. Among the germplasm evaluated, 21.3% of accessions exhibited moderate to strong resistance, with resistant sources identified across *C. annuum*, *C. frutescens*, *C. chinense*, and *C. baccatum*. Genome-wide association studies (GWAS) conducted separately in the multi-species panel and a *C. annuum* subset identified 62 and 22 significant SNPs, respectively, distributed across the pepper genome, supporting a polygenic architecture of bacterial wilt resistance. Independent validation of 149 accessions confirmed nine resistance-associated SNPs. Notably, two validated SNPs on chromosome 8, S8_125376969 and S8_139497915, identified independently from the multi-species and *C. annuum* analyses, respectively, were in strong linkage disequilibrium and defined a resistance-associated interval consistent with previously reported bacterial wilt resistance QTLs. Resistance-associated alleles at validated loci reduced the disease index by up to 30.5%. Together, these results establish cotyledon-based screening as an efficient platform for large-scale resistance evaluation and identify validated genomic regions that provide useful targets for marker-assisted breeding and pre-breeding for bacterial wilt resistance in pepper.

Keywords

Genome-Wide Association Study (GWAS), Bacterial Wilt, *Ralstonia solanacearum* species complex (RSSC), *Capsicum* species, Cotyledon-Based Rapid Screening

Introduction

Peppers (*Capsicum* spp.) are globally important horticultural crops with economic, nutritional, agricultural, and cultural value. They are cultivated worldwide for fresh consumption, processed products, and spice production, contributing significantly to the livelihoods of millions of smallholders and farmers (Bosland and Votava 2012). Their culinary versatility has made them integral to regional cuisines, from the spicy curries of South Asia to the smoky stews of Latin America, while their adaptability to diverse agroclimatic zones makes them suitable for sustainable agricultural systems (Pickersgill 2018). The domestication of *Capsicum* species began over 6,000 years ago in Mesoamerica, particularly in present-day Mexico, where early farmers selected wild progenitors for traits such as fruit size, pungency, color, and morphology (Perry et al. 2007; Kraft et al. 2014). This long history of selection and cultivation gave rise to five primary domesticated species: *C. annuum* (e.g., bell pepper, jalapeño), *C. frutescens* (e.g., tabasco), *C. chinense* (e.g., habanero, ghost pepper), *C. baccatum* (widely cultivated in South America), and *C. pubescens* (distinguished by its thick fruit walls and black seeds) (García et al. 2016). These species were subsequently disseminated worldwide via trade and cultural exchange, becoming vital to both traditional food systems and modern agriculture.

Bacterial wilt, caused by the *Ralstonia solanacearum* species complex (RSSC), is a major constraint to pepper (*Capsicum* spp.) production, particularly in tropical and subtropical regions where warm and humid conditions favor disease development (Hayward 1991; Schaad et al. 2001). As a soil-borne pathogen with a broad host range spanning over 200 plant species in 53 botanical families (Elphinstone 2005; Peeters 2013), RSSC is notoriously persistent in the soil and difficult to manage through conventional approaches. Its prevalence and strain diversity vary geographically and are heavily influenced by environmental factors (Huet 2014; Prior et al. 2016), making localized resistance breeding a critical component of disease control strategies.

In bacterial wilt, early root infection and initial vascular colonization are critical determinants of disease outcome, as successful establishment of the pathogen in the xylem often precedes rapid systemic spread and irreversible wilting symptoms (Huet 2014; Lowe-Power et al. 2018). Resistance mechanisms acting at early infection stages can therefore play a decisive role in limiting disease progression. While early-stage phenotyping platforms have been successfully applied to evaluate bacterial wilt resistance in tomato (Singh et al. 2018), comparable high-throughput screening systems for pepper remain limited. Given the extensive diversity of both RSSC strains and domesticated *Capsicum* species, evaluating resistance at early developmental stages across diverse genetic backgrounds may facilitate the identification of both conserved and lineage-specific resistance loci.

Among current management strategies for bacterial wilt, the use of resistant cultivars or grafting onto resistant rootstocks remains the most sustainable and economically viable approach (Lebeau et al. 2011). However, the genetic architecture underlying bacterial wilt resistance in pepper is complex and polygenic (Kang et al. 2016; Naveena et al. 2020), presenting significant challenges for conventional breeding. Quantitative trait loci (QTL) mapping has identified several genomic regions associated with bacterial wilt resistance in pepper, underscoring the multigenic nature of this trait. One of the earliest major resistance loci, *Bw1*, was identified by Mimura et al. (2009) in a doubled haploid population derived from ‘California Wonder’ × ‘LS2341’. This locus accounted for approximately one-third of the observed phenotypic variation and provided the foundation for subsequent studies of bacterial wilt resistance (Mimura et al. 2009). However, progress in marker-assisted breeding was initially limited by the large pepper genome (~3.5 Gb) and the availability of genomic resources (Kim et al. 2014). The advent of next-generation sequencing technologies has greatly accelerated the identification of resistance loci and associated molecular markers.

Recent studies have revealed the complex genetic architecture underlying bacterial wilt resistance in pepper. Du et al. (2019) identified the major QTL *qRRs-10.1* on chromosome 10 and proposed several defense-related genes, including NBS-LRR resistance genes, as candidates. Chae et al. (2022) subsequently mapped *pBWR-1* on chromosome 1 and reported multiple resistance-associated genes, including RPM1-like, NB-ARC, and thaumatin-like proteins. In addition to bi-parental QTL mapping, genome-wide association studies (GWAS) have emerged as a powerful approach for dissecting complex traits in crops (Demirjian et al. 2023; Qi et al. 2024). Unlike conventional QTL mapping, which is limited to alleles segregating within a biparental population, GWAS exploits historical recombination events in diverse germplasm, enabling higher mapping resolution and broader allele discovery. Additionally, given the polygenic nature of bacterial wilt resistance in pepper, the evaluation of genetically diverse germplasm is essential to enhance breeding strategies. Using a combined QTL

mapping and GWAS approach, Lee et al. (2024) identified *QTL.Bw5* on chromosome 5, together with additional loci on chromosomes 4 and 8. These findings further demonstrate that bacterial wilt resistance in pepper is controlled by multiple genomic regions and diverse genetic mechanisms.

Despite this advantage, GWAS requires large, genetically diverse populations to achieve sufficient statistical power, and phenotyping for disease resistance at this scale is often labor-intensive, time-consuming, and resource-demanding. Recent advances in tomato bacterial wilt research have demonstrated the utility of early-stage phenotyping using seedling assays. Specifically, screening at the two-cotyledon stage combined with root-dip inoculation has proven effective for evaluating resistance while dramatically reducing the labor, time, and space needed (Singh et al. 2018). This high-throughput approach enables the efficient screening of large germplasm collections with sufficient accuracy for downstream genetic analyses and breeding applications.

In this context, the present study aimed to (i) develop a rapid and reliable seedling-based screening platform for evaluating bacterial wilt resistance in pepper, and (ii) identify genomic regions associated with resistance through GWAS. To capture the broadest possible genetic and phenotypic diversity, we screened 540 accessions spanning four domesticated *Capsicum* species, including *C. annuum*, *C. chinense*, *C. frutescens*, and *C. baccatum*. These species not only represent the core of cultivated *Capsicum* diversity but also offer distinct genetic backgrounds that align with species-specific resistance loci. Including multiple domesticated species enabled us to identify both shared and lineage-specific SNPs, enhancing the relevance of our findings for molecular breeding across diverse genetic pools.

Materials and Methods

Development of rapid screening for bacterial wilt resistance in pepper

To develop and evaluate whether the rapid screening method using cotyledons can serve as an alternative of conventional disease resistance screening of pepper bacterial wilt in greenhouses, twenty-two pepper (*Capsicum* spp.) accessions (Table 1) were evaluated with both methods to examine the correlation between two screening methods.

The RSSC strain Pss71, originally isolated from infected sweet pepper (*C. annuum*) in Yunlin County, Taiwan, in 1991, was previously employed for routine screening of bacterial wilt resistance. This strain has been taxonomically classified as Phylotype I, Biovar 3, Sequevar 34, based on both biochemical assays and molecular characterization performed by the Bacteriology Unit at the World Vegetable Center. For inoculation, the strain was revived from cryopreserved stocks and cultured on 2,3,5-triphenyl tetrazolium chloride (TTC)-amended medium at 30°C for 48 hours. Subsequently, the bacterial colonies were transferred to 523 medium and incubated for an additional 24 hours to obtain an active culture. The bacterial mass was harvested and suspended in sterile distilled water, with the concentration adjusted to an optical density of 0.3 at 600 nm (OD_{600}), corresponding to approximately 1×10^8 CFU/mL based on a previously established OD600–CFU calibration for the strain.

For the rapid screening method, a screening protocol in growth chambers was modified (Singh et al. 2018),

incorporating root-tip excision with sterile scissors to create a wound and facilitate bacterial entry into the internal tissues. In brief, seeds were surface-sterilized with 1% sodium hypochlorite for 5 minutes, followed by three rinses with sterile water (Carisse et al., 2000). Susceptible (PBC1367) and resistant (PBC066) lines were included as controls. Sterilized seeds were placed on moist, sterile cotton layered with tissue paper in tissue culture containers and incubated in the dark at 28°C for 7 days. Post-germination, seedlings were maintained under a 12-hour photoperiod (4891 Lux; 90 µmol/s·m²; 30 cm lamp distance) until the cotyledon stage. Fourteen-day-old seedlings (two-cotyledon stage) were used for inoculation. Following root-tip excision, seedlings were inoculated by immersing the excised root region in the bacterial suspension for 1 min. Inoculated seedlings were air-dried in petri dishes for 5 minutes and transferred into 2 mL microcentrifuge tubes containing sterile water. The seedlings were incubated at 28°C under a 12-hour light/dark cycle. The experiment followed a randomized complete block design (RCBD) with three replicates and ten seedlings per accession per replicate. Six non-inoculated seedlings per accession, with root tips excised, served as negative controls. Wilting percentage (W%) was recorded at 7-, 10-, and 14-days post-inoculation (DPI). Symptom scoring criteria are illustrated in Figure 1A.

The conventional screening method was followed the established protocol of the World Vegetable Center (AVRDC 1994). In brief, seeds were surface-sterilized with 1.6% HCl for 15 minutes, rinsed under running water for 10 minutes, immersed in 10% trisodium phosphate for 1 hour, rinsed again for 10 minutes, and air-dried for 1 hour. Seeds were sown into 9-cm pots filled with a soil mixture (soil: rice hulls: sand: compost = 3:1:1:1) and grown under natural sunlight at the World Vegetable Center in Tainan, Taiwan. Seedlings with 4-6 true leaves (3-4 weeks old) were inoculated by wounding roots 1-2 cm from the stem base with a scalpel, followed by the application of 30 mL of bacterial suspension (1 x 10^8 CFU/mL) to each pot. The average temperature and relative humidity were 30.2°C/22°C and 100%/74.1% (max/min), respectively.

Disease severity was assessed weekly for four weeks using a disease index (DI) based on a 0–5 scale (Winstead and Kelman 1952):

- 0 = No symptoms
- 1 = One leaf partially wilted
- 2 = Two to three leaves wilted
- 3 = All leaves wilted except the top two or three
- 4 = All leaves wilted
- 5 = Plant dead

The DI (%) was calculated as follows:

$$\mathrm{DI} = \left(\frac{N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3 + N_4 \times 4 + N_5 \times 5}{N_t \times V}\right) \times 100\%$$

Where N_0 - N_5 indicate the number of plants per disease severity class, N_t is the total number of plants, and V is the highest scale.

Resistance reaction was categorized based on DI at 4 weeks after inoculation (WAI) as:

- Resistant (R): DI ≤ 20%
- Moderately resistant (MR): 20% < DI ≤ 40%
- Moderately susceptible (MS): 40% < DI ≤ 60%
- Susceptible (S): DI > 60%

Phenotypic evaluation of GWAS

Another strain, Pss2074, was used for the GWAS because it was the latest representative strain of the predominant bacterial wilt population in Taiwan. It was isolated from infected sweet pepper in Pingtung County (2019) and identified as Phylotype I, Biovar 3, Sequevar 34, same as Pss71. This strain was used to evaluate 540 Capsicum accessions from the World Vegetable Center GenBank, including *C. annuum* (439), *C. baccatum* (21), *C. chinense* (38), and *C. frutescens* (42). The experiment was conducted between February 7 and July 6, 2023 (Table S1).

All accessions were screened using the rapid seedling assay as previously described. Disease assessment was based on both wilting percentage (W%) and disease index (DI) using a 0–3 scale:

- 0 = No symptoms
- 1 = Small necrotic lesion or discoloration at the stem base
- 2 = Necrotic lesion or discoloration extending to 75% of the stem
- 3 = Lesion or discoloration extending beyond 75% of the stem

Wilting percentage (W%) was calculated as:

$$W\% = \left(\frac{N_w}{N_t}\right) \times 100\%$$

Where N_w is the number of wilted plants, and N_t is the total number of plants.

Disease index was computed as:

$$DI = \left(\frac{N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3}{N_t \times V}\right) \times 100\%$$

Where N_0 - N_3 refer to the number of plants in each severity class, N_t is the total number of plants, and V is the highest scale.

GWAS

Two GWAS analyses were performed: one included 439 *C. annuum* accessions and the other contained a broader panel of 540 diverse pepper germplasms (*C. annuum*, C. *baccatum*, *C. chinense*, *C. frutescens*). SNPs were obtained from Tripodi et al. (2021) and filtered for a minor allele frequency (MAF) < 0.05. Principal component analysis (PCA) was assessed performed using the GAPIT package (Lipka et al., 2012). Genome-

wide association mapping was conducted using the FarmCPU and BLINK models implemented in the GAPIT package, with the first three principal components included as covariates to account for population structure (Huang et al. 2019; Lipka et al. 2012; Liu et al. 2016). To prioritize candidate SNPs for validation, SNPs with *p* value < 0.001 were preliminarily selected and designated as significant SNPs for subsequent analysis.

Marker validation

To validate candidate SNP markers, an independent panel of 149 *Capsicum* accessions, including 92 *C. annuum* entries (Table S5), was screened using the rapid protocol. The genotypes of this panel were also retrieved from Tripodi et al., (2021). The accessions were selected based on the allelic composition of the GWAS-significant SNPs that significantly contribute to resistant and susceptible phenotypes.

Disease assessment was based on both wilting percentage and disease index using a 0–3 scale, the same procedures as described in the GWAS. Association between markers and bacterial wilt resistance (DI) was assessed using ANOVA in the R program. The significant SNPs were considered successfully validated GWAS SNPs. For these significant SNPs, the resistance reduction rate was calculated as:

$$\text{Reduction Rate (\%)} = \left(\frac{Max\ DI - Min\ DI}{Max\ DI}\right) \times 100$$

Results

Evaluation of the consistency between rapid screening and conventional greenhouse method

To evaluate the reliability of the rapid screening protocol relative to the conventional greenhouse method, a set of 22 pepper (*Capsicum* spp.) varieties with known phenotypic responses to *R. solanacearum* species complex strain Pss71 were assessed under both conditions. The results demonstrated that bacterial wilt resistance can be effectively evaluated at the two-cotyledon stage under controlled growth chamber conditions (Table 1). No disease-like symptoms were observed in the mock-inoculated controls. Pearson correlation coefficients between the disease index (DI) scores obtained from greenhouse evaluations and the wilting percentages recorded at 7, 10, and 14 DPI in the growth chamber were 0.12, 0.73, and 0.37, respectively. Notably, the highest correlation was observed at 10 DPI ($r = 0.73$, $p < 0.001$), indicating a strong concordance between the two screening methods at this time point and effectively distinguishing resistant from susceptible accessions.

To further validate the discriminatory power of the rapid assay, various wilting percentage thresholds (20%, 30%, 40%, and 50%) recorded at 10 DPI were compared with resistance classifications obtained from greenhouse screening. The chi-square test revealed a significant agreement at the 50% threshold ($p = 0.004$; Table S2), suggesting that a wilting percentage below 50% at 10 DPI provides a useful threshold for distinguishing resistant from susceptible accessions under the rapid screening conditions. Overall, these findings indicate that the rapid screening method provides a time-efficient alternative to conventional greenhouse screening, particularly when disease symptoms are assessed at 10 DPI using a 50% wilting threshold as the classification criterion.

Identification of novel resistance resources in diverse *Capsicum* germplasm

To uncover novel resistance sources, the rapid screening was conducted on 540 Capsicum accessions representing five cultivated species (Table S1). Disease severity was assessed using the DI at 10 DPI, based on its established correlation with conventional greenhouse-based evaluations. The analysis revealed that bacterial wilt resistance is not restricted to a single species but across all these four domesticated *Capsicum* species (Figure 2). Of the accessions evaluated, 21.3% displayed resistant or moderately resistant phenotypes (DI ≤ 40%). Eighteen accessions were classified as resistant, with DI values ranging from 5.6% to 20.0%. Notably, three *C. annuum* accessions, VI028661, VI046792, and VI012092, demonstrated particularly high resistance, with DI values of 5.6%, 6.7%, and 8.9%, respectively (Table S1). In addition, highly resistant or moderately resistant accessions were identified in non-annuum species. These included VI012273 (*C. frutescens*, DI = 11.1%), VI012269 (*C. baccatum*, DI = 17.8%), and VI029467 (*C. chinense*, DI = 23.5%), each representing the most resistant genotype within their respective species (Table S1). Resistance was more frequently observed among *C. baccatum*, *C. chinense*, and *C. frutescens* accessions compared to *C. annuum* (Table 2), suggesting possible species-specific co-evolutionary dynamics between *Capsicum* hosts and RSSC. Collectively, these results highlight the effectiveness of rapid seedling-based screening in identifying bacterial wilt resistance across taxonomically diverse *Capsicum* germplasm and demonstrate its potential to accelerate resistance gene discovery and pre-breeding efforts.

GWAS identifies candidate loci for bacterial wilt resistance in *Capsicum*

To characterize the genetic diversity within the *Capsicum* germplasm collection, a total of 24,362 high-quality SNPs were analyzed across 540 accessions. PCA revealed three to four distinct genetic clusters that were generally consistent with the four major domesticated *Capsicum* species: *C. annuum*, *C. chinense*, *C. baccatum*, and *C. frutescens* (Supplementary Figure 1A). PC1 and PC2 explained 38.33% and 7.88% of the genetic variation, respectively. *C. annuum* was clearly differentiated from the other three species, while *C. baccatum* was genetically distinct from *C. frutescens*. Within *C. annuum*, two to three genetic sub-groups were observed, with PC1 and PC2 explained 11.61% and 4.72% of the variation, respectively (Supplementary Figure 1B). Based on the observed population structure, the first three PCs were subsequently included as covariates in the GWAS models to control for potential confounding effects.

GWAS were conducted on two populations: a multi-species *Capsicum* panel and a *C. annuum*-specific panel. Quantile–quantile plots demonstrated an appropriate control of population structure, as indicated by the expected normal distribution of *p*-values (Supplementary Figure 2). DI was used as the primary phenotypic trait for GWAS, due to its role as the central selection indicator in screening assays (Figure 3). In the multi-species analysis, 15,607 high-quality SNPs were retained after filtering for MAF, resulting in the identification of 62 significant SNPs distributed across all 12 pepper chromosomes (Table S3). Similarly, in the *C. annuum* panel, 5,941 SNPs were retained, leading to the detection of 22 significant SNPs (Table S4). Of particular interest, four common SNPs, S2_149827954, S4_204959303, S5_26175517, and S11_81044687, were consistently detected in both populations. These genomic regions represent promising candidates for the development of markers applicable across multiple *Capsicum* species in breeding programs aimed at enhancing bacterial wilt resistance.

However, due to the relatively low marker density in both analyses (224 kb per SNP in the *Capsicum* spp. panel and 589 kb per SNP in the *C. annuum* panel), we prioritized SNP validation over candidate gene identification.

Validation of SNPs conferring resistance

To confirm the relevance of SNPs identified through GWAS, a phenotypic screening was conducted on an independent panel of pepper accessions. These accessions have different alleles from the selected candidate SNPs (Table 3). Disease index (DI) values ranged from 5.56% to 100% across the 149 *Capsicum* accessions evaluated (Tables S5). Of these, six SNPs derived from the multi-species GWAS were significantly associated with bacterial wilt resistance (Table S5). In a subset of 92 *C. annuum* accessions, 3 SNPs identified from the *C. annuum*-specific GWAS showed significant associations with DI (Table 3). In the multi-species panel, SNPs S8_125376969, S4_237675229, and S12_180241186, exhibited the strongest resistance-associated effects, with the maximum DI reduction reaching 20.25% between allelic classes (Table 3). In the *Capsicum annuum* dataset, SNPs S11_81044687, S8_139497915, and S3_2899880 showed the greater phenotypic effects than *Capsicum* spp. significant SNPs, with a maximum DI reduction of 30.50% (Table 3). Moreover, SNPs S8_125376969 (identified in the multi-species panel) and S8_139497915 (identified in the *C. annuum* dataset) exhibited linkage disequilibrium, with $r^2 = 0.195$ and $D' = 0.786$ (p value < 0.001), which was further supported by a chi-square test (p value < 0.001). This finding suggests that this QTL could serve as a robust, cross-species marker for bacterial wilt resistance in *Capsicum*.

Discussion

A rapid screening system for bacterial wilt based on two-cotyledon seedlings enables high-throughput evaluation, significantly reducing time, space, and labor requirements. Moreover, this method is not constrained by seasonal climatic conditions, thereby accelerating the identification of resistant accessions. Despite its advantages, the method has certain limitations, including variability in seed quality and germination rates, as well as the potential loss of resistant lines due to lignification after the seedling stage. Nevertheless, it allows for the rapid acquisition of phenotypic data on bacterial wilt resistance across a large number of accessions within a short period. By applying this method to 540 accessions, we identified putative sources of resistance across *C. annuum*, *C. chinense*, *C. frutescens*, and *C. baccatum*, underscoring that resistance is not restricted to a single species but is distributed throughout the domesticated *Capsicum* gene pool. A similar finding was reported by Ro et al (2024), where the presence of highly resistant accessions within non-annuum species suggests both lineage-specific and potentially conserved mechanisms of resistance, highlighting a complex evolutionary dynamic between *Capsicum* hosts and RSSC.

Among the loci identified in this study, the region on chromosome 8 is particularly noteworthy. Two independently identified SNPs, S8_125376969 from the multi-species panel and S8_139497915 from the *C. annuum* panel, were significantly associated with resistance in the validation population and showed strong linkage disequilibrium, spanning an approximately 14-Mb resistance-associated interval. This region is consistent with previous evidence implicating chromosome 8 in bacterial wilt resistance. The major bacterial wilt resistance locus *Bw1*, originally identified in the resistant accession ‘LS2341’, explained approximately

33% of the phenotypic variation (Mimura et al. 2009), and subsequent genome-based analysis localized the CAMS451 marker associated with *Bw1* to chromosome 8 (Naveena et al. 2020). More recently, Lee et al. (2022) identified *Bwr6w-8.1* on chromosome 8 in a population derived from the resistant cultivar 'Konesian Hot', with the closely linked marker C08_134064617 located within the interval identified in our study. Lee et al. (2024) further detected bacterial wilt resistance-associated loci on chromosome 8 through a combination of biparental QTL mapping and GWAS. Together, the repeated detection of resistance-associated variation in this region across different genetic backgrounds and mapping approaches suggests that chromosome 8 represents an important genomic region underlying bacterial wilt resistance in pepper.

The broader distribution of significant loci across the genome is also consistent with the quantitative nature of bacterial wilt resistance reported previously. In addition to *Bw1* on chromosome 8, major resistance loci have been reported on chromosome 10 (*qRRs-10.1*; Du et al. 2019) and chromosome 1 (*pBWR-1*; Chae et al. 2022), while isolate-specific QTLs have been detected on chromosomes 5, 6, 7, 8, 9, and 10 (Lee et al. 2022). Likewise, Lee et al. (2024) identified loci on chromosomes 4, 5, and 8 using complementary biparental mapping and GWAS approaches. Our detection of resistance-associated SNPs across multiple chromosomes therefore reinforces the view that bacterial wilt resistance in pepper is polygenic and likely involves multiple genetic mechanisms. At the same time, the validation of the chromosome 8 interval in an independent population provides stronger evidence for this region than for individual GWAS signals detected only in the discovery panel. Given the relatively low marker density of the present GWAS, however, further fine mapping with higher-density markers will be required to determine whether the validated chromosome 8 associations represent the same resistance locus reported in previous studies or closely linked but distinct loci.

A limitation of this study is that resistance was evaluated using a single RSSC strain, despite the considerable genetic and pathogenic diversity of the RSSC (Salgon et al. 2017). Strain-specific variation in virulence and host adaptation means that resistance effective against one strain may not confer broad-spectrum protection under field conditions (Kwon et al. 2021). However, the use of a single, well-characterized strain is appropriate at the discovery stage, as it reduces pathogen-derived variability and improves detection of host genetic effects underlying quantitative resistance, particularly in GWAS where phenotypic noise can obscure polygenic signals (Kwon et al. 2024). The identification of multiple resistance-associated loci across the genome, including the validated Chr08 interval, is consistent with bacterial wilt resistance in pepper being quantitatively inherited (Subedi et al. 2024). Nonetheless, validation using additional strains will be necessary to assess the breadth and durability of the identified loci for breeding applications.

Following inoculation, seedlings were maintained in sterilized water to ensure uniform post-inoculation conditions and minimize confounding effects from soil-borne microorganisms, thereby enabling high experimental consistency for large-scale phenotyping. While this simplified system may impose mild physiological stress due to the absence of nutrients and beneficial microbes, all accessions were subjected to identical conditions, and resistant genotypes consistently exhibited reduced wilting, indicating that observed differences primarily reflect genetic variation in host–pathogen interactions rather than differential plant vigor. As bacterial wilt is fundamentally a vascular disease characterized by pathogen proliferation in the xylem and subsequent disruption of water transport (Lowe-Power et al., 2018), early symptom development in this water-

based assay may reflect early disease responses associated with bacterial wilt development. Under these highly controlled conditions, variation arising from disease escape mechanisms, such as differences in seedling vigor or growth rate, is minimized, allowing resistance associated with pathogen restriction to be more clearly expressed. Moreover, the cotyledon-stage assay likely emphasizes resistance mechanisms acting during early vascular colonization and establishment, rather than traits involved in restricting late-stage systemic spread of the pathogen. Nevertheless, resistance expressed at the seedling stage does not necessarily equate to durable resistance under greenhouse or field conditions, where additional factors such as root architecture, lignification, and systemic defense regulation may contribute to disease suppression (Huet 2014). Consequently, some accessions may be underestimated or overestimated in the seedling assay. Despite these limitations, the strong correspondence observed between seedling wilting scores at 10 DPI and greenhouse disease indices indicates that the assay captures resistance-associated phenotypic variation relevant to disease development, supporting its use as a high-throughput screening and genetic mapping tool, with subsequent validation under more complex growth conditions required to confirm resistance durability.

Collectively, our results add to the limited but growing body of studies mapping bacterial wilt resistance in pepper and expand the repertoire of known genomic regions. While the loci identified here showed moderate phenotypic effects, they nonetheless provide valuable entry points for molecular breeding and marker-assisted selection. To achieve durable resistance, however, future studies must integrate diverse *Capsicum* germplasm, multiple pathogen isolates, and functional validation of candidate genes. By combining rapid phenotyping with genomic approaches, the foundation is now set for building a more comprehensive genetic architecture of bacterial wilt resistance and accelerating resistance breeding in pepper.

Acknowledgments

We thank for the long-term strategic donors to the World Vegetable Center: Taiwan, the United States, Australia, the United Kingdom, Germany, Thailand, South Korea, the Philippines, and Japan.

References

AVRDC (1994) Preliminary screening of resistance to bacterial wilt in eggplant. In: AVRDC 1993 progress report. Asian Vegetable Research and Development Center, Tainan, Taiwan, pp 96–98. http://worldveg.tind.io/record/20396

Bosland PW and Votava EJ (2012) Pepper: vegetable and spice Capsicums, 2nd edn. CABI, Wallingford.

Carisse O, Ouimet A, Toussaint V, Philion V (2000) Evaluation of the effect of seed treatments, bactericides, and cultivars on bacterial leaf spot of lettuce caused by Xanthomonas campestris pv. vitians. Plant Dis 84:295–299. https://doi.org/10.1094/PDIS.2000.84.3.295

Chae SY, Lee K, Do JW, Hong SC, Lee KH, Cho MC, Yang EY, Yoon JB (2022) QTL mapping of resistance to bacterial wilt in pepper plants (Capsicum annuum) using genotyping-by-sequencing (GBS). Horticulturae 8:115.

https://doi.org/10.3390/horticulturae8020115

Demirjian C, Vailleau F, Berthomé R, Roux F (2023) Genome-wide association studies in plant pathosystems: success or failure? Trends Plant Sci 28:471–485. https://doi.org/10.1016/j.tplants.2022.11.006

Du H, Wen C, Zhang X, Xu X, Yang J, Chen B, Geng S (2019) Identification of a major QTL (qRRs-10.1) that confers resistance to Ralstonia solanacearum in pepper (Capsicum annuum) using SLAF-BSA and QTL mapping. Int J Mol Sci 20:5887. https://doi.org/10.3390/ijms20235887

Elphinstone JG (2005) The current bacterial wilt situation: a global view. In: Allen C, Prior P, Hayward AC (eds) Bacterial wilt disease and the Ralstonia solanacearum species complex. APS Press, St Paul, pp 9–28.

García CC, Barfuss MHJ, Sehr EM, Barboza GE, Samuel R, Moscone EA, Ehrendorfer F (2016) Phylogenetic relationships, diversification and expansion of chili peppers (Capsicum, Solanaceae). Ann Bot 118:35–51. https://doi.org/10.1093/aob/mcw079

Hayward AC (1991) Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annu Rev Phytopathol* 29:65–87.

Huang M, Liu X, Zhou Y, Summers RM, Zhang Z (2019) BLINK: a package for the next level of genome-wide association studies with both individuals and markers in the millions. Gigascience 8: /giy154. https://doi.org/10.1093/gigascience/giy154

Huet G (2014) Breeding for resistances to Ralstonia solanacearum. Front Plant Sci 5:715. https://doi.org/10.3389/fpls.2014.00715

Kang YJ, Ahn YK, Kim KT, Kim DH, Lim JR, Lee JY, Kim HJ, Kim BD, Kang BC (2016) Resequencing of Capsicum annuum parental lines (YCM334 and Taean) for the genetic analysis of bacterial wilt resistance. BMC Plant Biol 16:235. https://doi.org/10.1186/s12870-016-0931-0

Kim S, Park M, Yeom SI, Kim YM, Lee JM, Lee HA, Seo E, Choi J, Cheong K, Kim KT et al (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat Genet 46:270–278. https://doi.org/10.1038/ng.2877

Kraft KH, Brown CH, Nabhan GP, Luedeling E, Luna Ruiz JDJ, Coppens d'Eeckenbrugge G, Hijmans RJ, Gepts P (2014) Multiple lines of evidence for the origin of domesticated chili pepper, Capsicum annuum, in Mexico. Proc Natl Acad Sci USA 111:6165–6170 https://doi.org/10.1073/pnas.1308933111

Kwon JS, Lee J, Shilpha J, Jang H, Kang WH (2024) The landscape of sequence variations between resistant and susceptible hot peppers to predict functional candidate genes against bacterial wilt disease. BMC Plant Biol 24:1036.

https://doi.org/10.1186/s12870-024-05742-w

Kwon JS, Nam JY, Yeom SI, Kang WH (2021) Leaf-to-whole plant spread bioassay for pepper and Ralstonia solanacearum interaction determines inheritance of resistance to bacterial wilt for further breeding. Int J Mol Sci 22:2279. https://doi.org/10.3390/ijms22052279

Lebeau A, Daunay MC, Frary A, Palloix A, Wang JF, Dintinger J, Chiroleu F, Wicker E, Prior P (2011) Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to diverse strains in the Ralstonia solanacearum species complex. Phytopathology 101:154–165. https://doi.org/10.1094/PHYTO-02-10-0048

Lee JH, Siddique MI, Jang S, Kim GW, Choi GJ, Kwon JK, Kang BC (2024) Identification of QTLs associated with resistance to bacterial wilt in pepper (Capsicum annuum L.) through bi-parental QTL mapping and genome-wide association analysis. Sci Hortic 329:112987. https://doi.org/10.1016/j.scienta.2024.112987

Lee S, Chakma N, Joung S, Lee JM, Lee J (2022). QTL Mapping for Resistance to Bacterial Wilt Caused by Two Isolates of Ralstonia solanacearum in Chili Pepper (Capsicum annuum L.). Plants 11:1551. DOI: 10.3390/plants11121551.

Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang Z (2012) GAPIT: genome association and prediction integrated tool. Bioinformatics 28:2397–2399. https://doi.org/10.1093/bioinformatics/bts444

Liu X, Huang M, Fan B, Buckler ES, Zhang Z (2016) Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. PLoS Genet 12. https://doi.org/10.1371/journal.pgen.1005767

Lowe-Power TM, Khokhani D, Allen C (2018) How Ralstonia solanacearum exploits and thrives in the flowing plant xylem environment. Trends Microbiol 26:929–942. https://doi.org/10.1016/j.tim.2018.06.002

Mimura Y, Kageyama T, Minamiyama Y, Hirai M (2009) QTL analysis for resistance to Ralstonia solanacearum in Capsicum accession 'LS2341'. J Jpn Soc Hortic Sci 78:307–313. https://doi.org/10.2503/jjshs1.78.307

Naveena GS, Sandeep V, Ponnam N, Kumari M, Acharya GC, Srinivas P, Sangeetha G, Sahu GS (2020) Genetic analysis of bacterial wilt resistance in hot pepper (Capsicum annuum L.). Veget Sci 47:153–156. https://isvsvegsci.in/index.php/vegetable/article/view/33/32

Peeters N, Guidot A, Vailleau F, Valls M (2013) Ralstonia solanacearum, a widespread bacterial plant pathogen in the post-genomic era. Mol Plant Pathol 14:651–662. https://doi.org/10.1111/mpp.12038.

Perry L, Dickau R, Zarrillo S, Holst I, Pearsall DM, Piperno DR, Berman MJ, Cooke RG, Rademaker K, Ranere AJ, Raymond JS, Sandweiss DH, Scaramelli F, Tarble K, Zeidler JA (2007) Starch fossils and the domestication and dispersal of chili peppers (Capsicum spp. L.) in the Americas. Science 315:986–988.

https://doi.org/10.1126/science.1136914

Pickersgill B (2018) Parallel vs. convergent evolution in domestication and diversification of crops in the Americas. Front Ecol Evol 6:56.

https://doi.org/10.3389/fevo.2018.00056

Prior P, Ailloud F, Dalsing BL, Remenant B, Sanchez B, Allen C (2016) Genomic and proteomic evidence supporting the division of the plant pathogen Ralstonia solanacearum into three species. *BMC Genomics* 17:90.
https://doi.org/10.1186/s12864-015-2232-z

Qi T, Song L, Guo Y, Chen C, Yang J (2024) From genetic associations to genes: methods, applications, and challenges. Trends Genet 40:642–667. https://doi.org/10.1016/j.tig.2024.04.008

Ro N, Lee GA, Ko HC, Oh H, Lee S, Haile M, Kim H, Lee J, Hyun DY, Lee MC (2024) Exploring disease resistance in pepper (Capsicum spp.) germplasm collection using Fluidigm SNP genotyping. Plants 13:1344.
https://doi.org/10.3390/plants13101344

Salgon S, Jourda C, Sauvage C, Daunay MC, Reynaud B, Wicker E, Dintinger J (2017) Eggplant resistance to the Ralstonia solanacearum species complex involves both broad-spectrum and strain-specific quantitative trait loci. Front Plant Sci 8:828.
https://doi.org/10.3389/fpls.2017.00828

Schaad NW, Jones JB, Chun W (2001) Laboratory guide for the identification of plant pathogenic bacteria, 3rd edn. APS Press, St Paul.

Singh N, Kumar R, Ray SK (2018) An innovative approach to study Ralstonia solanacearum pathogenicity in 6- to 7-day-old tomato seedlings by root dip inoculation. Bio Protoc 8: e3065.
https://doi.org/10.21769/BioProtoc.3065

Subedi N, Cowell T, Cope-Arguello M, Paul P, Cellier G, Bkayrat H, Bonagura N, Cadatal A, Chen R, Enriquez A, Parasar R (2024) Characterization of Ralstonia pseudosolanacearum diversity and screening tomato, pepper, and eggplant resistance to manage bacterial wilt in South Asia. PhytoFrontiers 4:393–403.
https://doi.org/10.1094/PHYTOFR-10-23-0136-R

Tripodi P, Rabanus-Wallace MT, Barchi L, Kale S, Esposito S, Acquadro A, Schafleitner R, van Zonneveld M, Prohens J, Diez MJ, Börner A, Salinier J, Caromel B, Bovy A, Boyaci F, Pasev G, Brandt R, Himmelbach A, Portis E, Finkers R, Lanteri S, Paran I, Lefebvre V, Giuliano G, Stein N (2021) Global range expansion history of pepper (Capsicum spp.) revealed by over 10,000 genebank accessions. Proc Natl Acad Sci USA 118. https://doi.org/10.1073/pnas.2104315118

Winstead NN, Kelman A (1952) Inoculation techniques for evaluating resistance to Pseudomonas solanacearum. Phytopathology 42:623–634.

Statements and Declarations

Funding

Funding for this research was provided by the Ministry of Agriculture, Taiwan (111AS-1.2.4-ST-a1, 112AS-1.2.3-ST-a4).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Jaw-rong Chen, Hung-chia Chang, and Ya-ping Lin. The first draft of the manuscript was written by Jaw-rong Chen, Jutatape Watcharachaiyakup, and Ya-ping Lin. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available in the supplementary data.

Figures

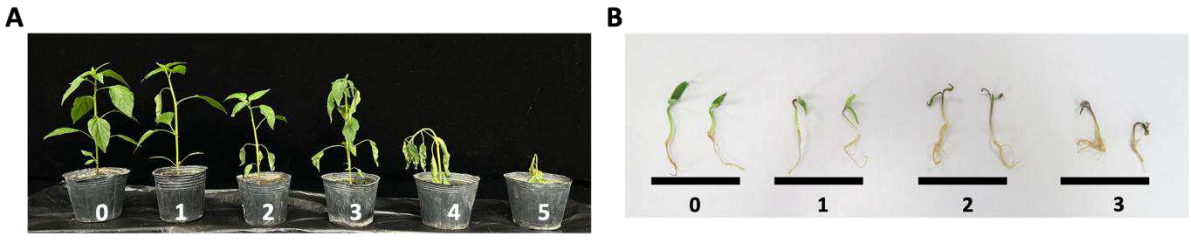


Figure 1. Disease severity scoring. (A) Conventional scoring method: Disease symptoms on each plant were assessed weekly over a four-week period using a 0–5 scale, as described by Winstead and Kelman (1952). The scoring criteria were: 0 = no symptoms; 1 = one leaf partially wilted; 2 = two to three leaves wilted; 3 = all leaves wilted except the top two to three leaves; 4 = all leaves wilted; and 5 = plant dead. (B) Cotyledon rapid scoring method: Disease severity was evaluated at 10 days post-inoculation using a modified 0–3 scale. The scoring criteria were: 0 = no symptoms; 1 = small necrotic lesion on the stem; 2 = necrotic lesion extending to 75% of the stem; and 3 = necrotic lesion extending beyond 75% of the stem.

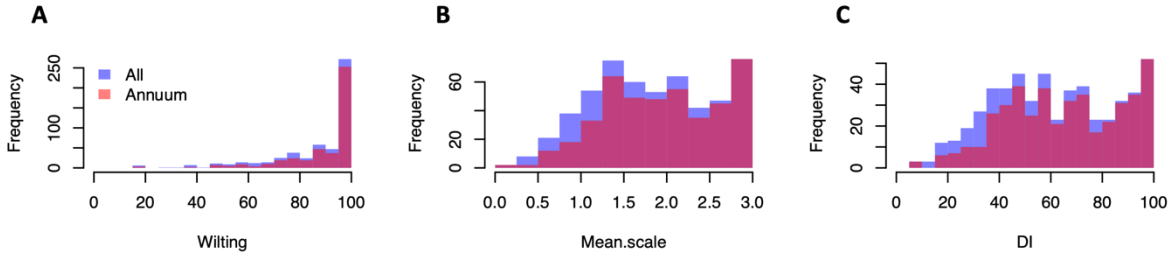


Figure 2. Phenotypic distribution of disease response in *Capsicum* spp. (blue) and *Capsicum annuum* (red) based on the cotyledon rapid screening method. (A) Wilting percentage; (B) Mean disease scale; (C) Disease index.

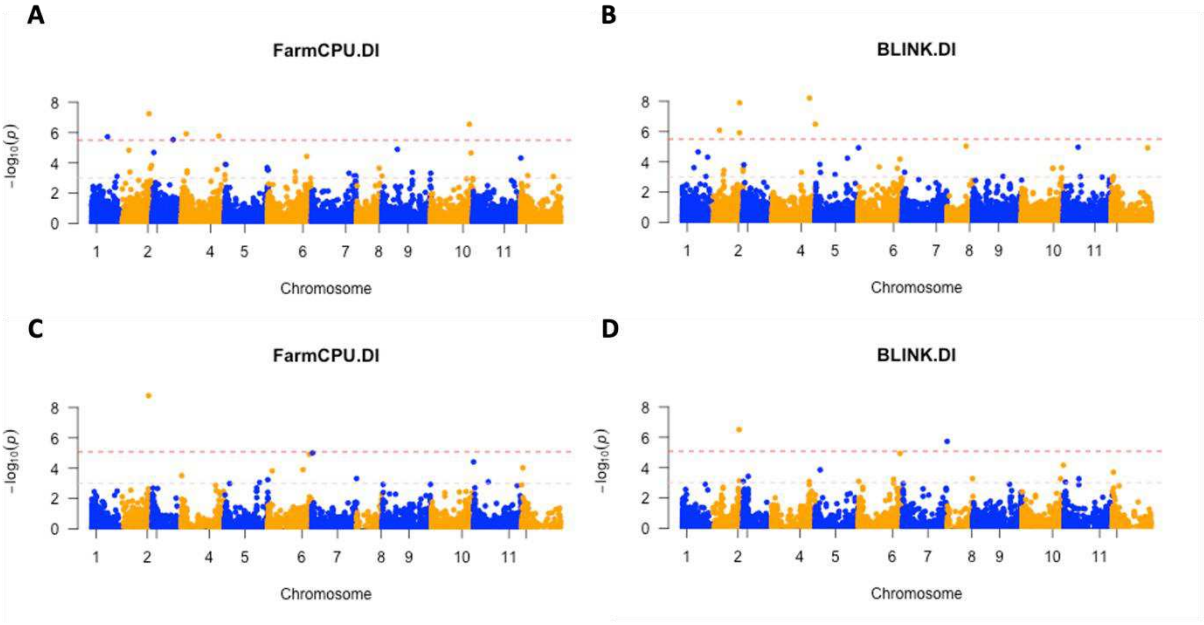


Figure 3. Manhattan plots of the disease index based on genome-wide association studies.

(A, B) FarmCPU and BLINK models, respectively, for *Capsicum* spp.; (C, D) FarmCPU and BLINK models, respectively, for *Capsicum annuum*.

516 **Tables**

517 Table 1. Evaluation of *Capsicum* resistance to bacterial wilt via the conventional scoring and the cotyledon rapid
518 scoring method.

Accession	DI via conventional greenhouse method[a]	Wilting percentage via cotyledon rapid scoring method[b]		
		7 DAI	10 DAI	14 DAI
AVPP0002	83.8	6.7	46.7	70.0
AVPP0105	15.8	10.0	33.3	86.7
AVPP0203	84.2	23.3	82.1	97.9
AVPP0205	10.0	16.7	27.8	55.6
AVPP0303	95.8	16.7	76.7	73.3
AVPP0305	36.7	0.0	40.3	67.2
AVPP0409	17.5	16.7	73.3	90.0
AVPP0705	27.5	48.5	45.2	93.0
AVPP1108	26.7	20.0	30.0	46.7
AVPP1327	31.7	31.9	45.9	79.6
AVPP1333	100.0	6.7	90.0	100.0
AVPP1337	94.2	6.7	70.0	100.0
AVPP1339	37.5	0.0	4.2	41.7
AVPP1517	13.3	40.0	50.0	93.3
AVPP9813	24.2	5.6	36.1	48.3
VI014995	25.8	24.1	31.9	100.0
VI037556	22.5	3.3	6.7	86.7
1705-5642-1	25.0	40.0	63.3	96.7
PBC142	84.2	13.9	62.5	100.0
0837-7613 (S.CK)	100.0	80.0	96.7	100.0
PBC1367 (S.Ck)	96.2	26.7	90.0	100.0
PBC066 (R. Ck)	15.8	3.7	40.7	81.5

519 [a] Disease index (DI) of the fourth week in the greenhouse.

520 [b] Wilting percentage assessed at 7, 10, and 14 days post-inoculation in the growth chamber.

521

522

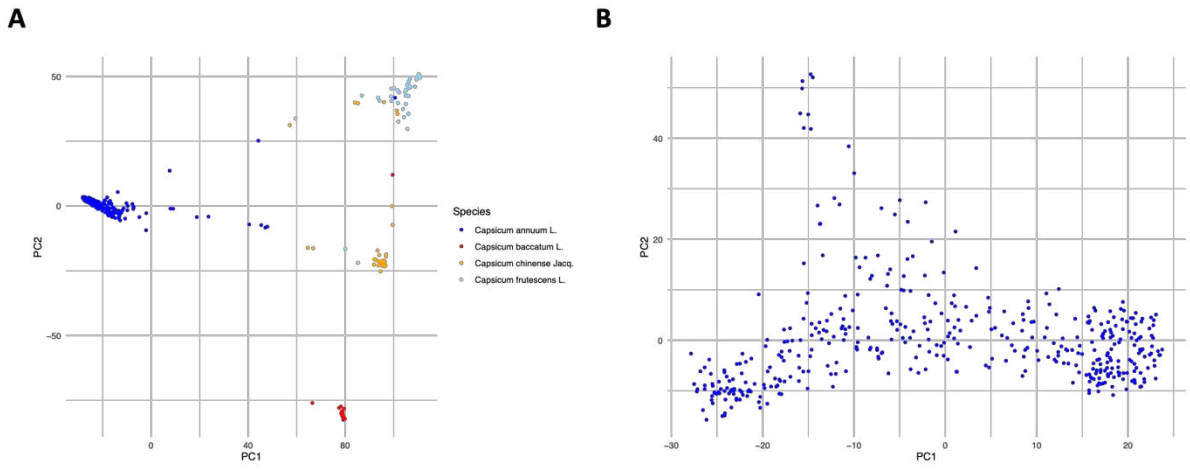
Table 2. Summary of resistance to *Ralstonia solanacearum* among *Capsicum* species based on the cotyledon rapid screening method. Resistance was determined under different threshold criteria; percentages are shown in parentheses.

Capsicum spp.	Number	W%≤50	DI≤20	DI≤30	DI≤40
C. annuum L.	439	12 (2.7)	9 (2.1)	26 (5.9)	62 (14.1)
C. baccatum L.	21	2 (9.5)	1 (4.8)	5 (23.8)	11 (52.4)
C. chinense Jacq.	38	1 (2.6)	0 (0.0)	5 (13.2)	21 (55.3)
C. frutescens L.	42	12 (28.6)	8 (19.0)	14 (33.3)	21 (50.0)
Total	540	27 (5)	18 (3.3)	50 (9.3)	115 (21.3)

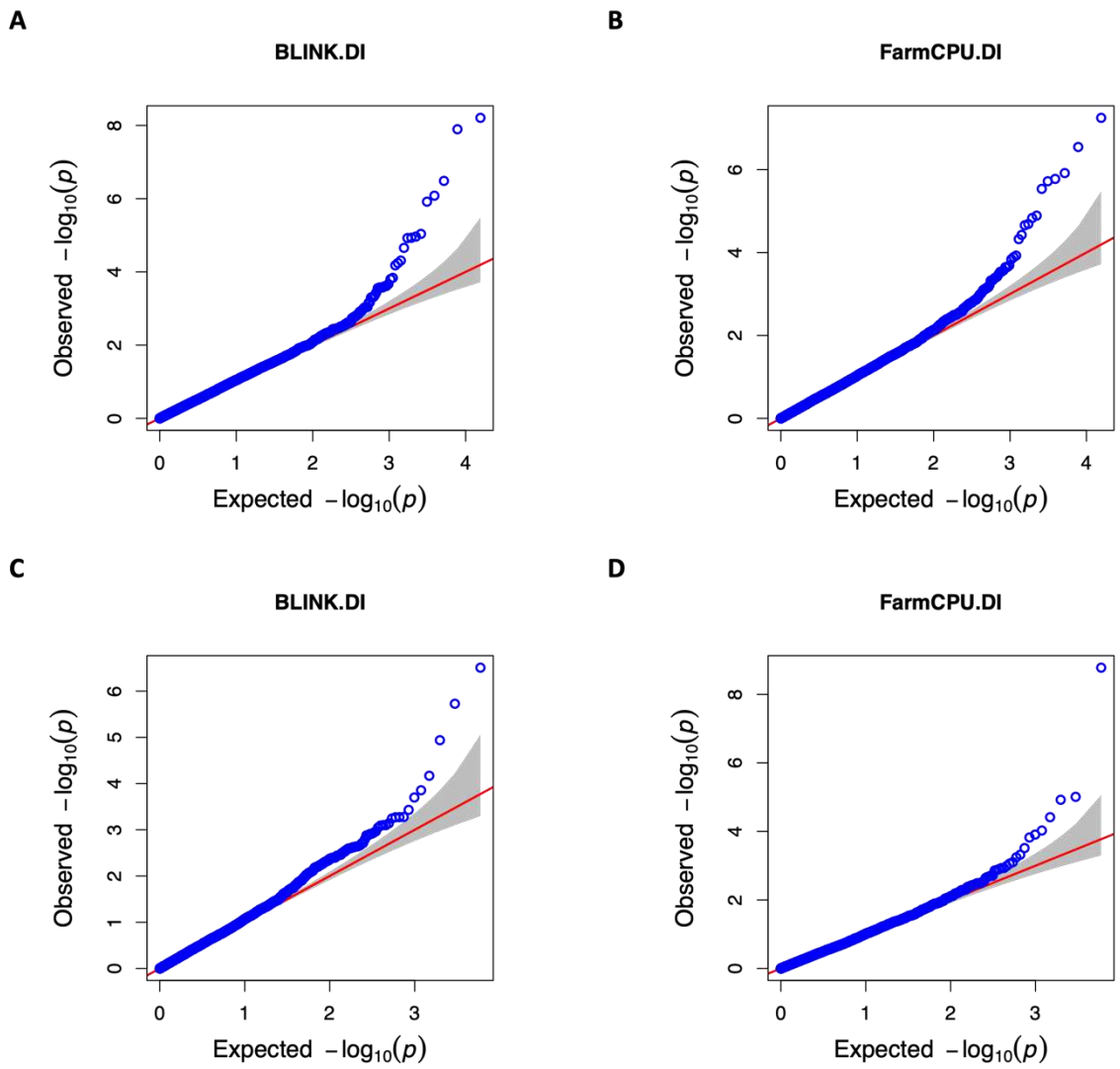
Table 3. Summary of the three most impactful candidate SNPs associated with disease index in *Capsicum* spp. and *Capsicum annuum*

Group	Candidate SNP	R/S Allele	DI %		*p*-value	Reduction Rate (%)
			R allele	S allele		
Capsicum spp.	S8_125376969	G/A	58.07	72.82	0.002	20.25
	S4_237675229	A/G	57.83	70.89	0.009	18.43
	S12_180241186	T/C	51.97	62.43	0.040	16.75
	S6_229196282	A/G	57.90	66.77	0.029	13.28
	S6_23118467	T/A	57.81	66.31	0.043	12.82
	S2_157176424	T/C	60.45	61.60	0.027	1.87
Capsicum annuum	S11_81044687	C/A	52.97	69.13	0.002	30.50
	S8_139497915	G/T	59.27	76.99	0.012	29.91
	S3_2899880	C/A	57.50	71.84	0.011	24.94

Supplementary data



Supplementary Figure 1. Principal component analysis of the (A) *Capsicum* spp.; (B) *Capsicum annuum* used in this study.



Supplementary Figure 2. Quantile–quantile (Q–Q) plots for genome-wide association analyses. (A) *Capsicum* spp.; (B) *Capsicum annuum*. Q–Q plots are shown for wilting percentage, mean disease scale, and disease index using the FarmCPU and BLINK models.

Supplementary Table 1. Phenotypes of the accessions screened in this study.

Supplementary Table 2. Phenotypes obtained from conventional greenhouse screening and cotyledon rapid screening, based on wilting percentage.

Supplementary Table 3. Candidate SNPs identified from GWAS of *Capsicum* spp.

Supplementary Table 4. Candidate SNPs identified from GWAS of *Capsicum annuum*.

Supplementary Table 5. Disease index and genotypes at candidate SNP loci for validated *Capsicum* spp. accessions.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Suptablesupdated.xlsx