

Identification and fine-mapping of a novel locus qSCL2.4 for resistance to *Sclerotinia sclerotiorum* in sunflower (*Helianthus annuus*)

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Abstract

Sclerotinia sclerotiorum (*S. sclerotiorum*) as a necrotrophic fungus severely threatens sunflower production. Despite abundant quantitative trait locus (QTL) for *S. sclerotiorum* resistance have been reported, the genetic and molecular basis remains largely unclear. Here, we performed QTL analysis for *S. sclerotiorum* resistance using a recombinant inbred line (RIL) population derived from resistant C6 and susceptible B728 parental lines. A high-density genetic linkage map of 17 linkage groups with 6,059 single nucleotide polymorphism (SNP) markers spanning 2,763 cM was constructed. Lesion length (LL) and lesion area (LA) were evaluated across field, climate chamber, and greenhouse trials using standardized inoculation protocols. Sixteen QTL for LL and twelve QTL for LA were identified with each QTL explaining 1.58 to 32.86% of the phenotypic variation. Of these, a major-effect QTL, qSCL2.4 on chromosome 2, explained up to 30.22% of phenotypic variance, which had allele conferring increased *S. sclerotiorum* resistance derived from the parent C6. Fine-mapping through a BC1F3 population derived from B728 × RIL-5 crosses narrowed the locus to a 226.7 kb interval. Functional annotation identified HaWRKY48 (LOC110907968), encoding a WRKY transcription factor, as the candidate gene. Seven nonsynonymous SNPs in HaWRKY48 distinguished resistant and susceptible alleles, with higher expression in B728 post-inoculation. A KASP marker targeting the causal SNP (Chr02:141503867) effectively discriminated haplotypes in 137 sunflower lines, with the favorable allele reducing lesion expansion by 40.06–48.92%. This study highlights qSCL2.4 as a pivotal locus for resistance and provides functional markers to accelerate breeding for *sclerotinia*-resistant sunflower cultivars.

Key message

A major QTL, *qSCL2.4*, governing resistance to *S. sclerotiorum* in sunflower was fine-mapped to a 226.7 kb interval on chromosome 2, with the candidate gene *HaWRKY48* harboring seven nonsynonymous SNPs distinguishing resistant and susceptible alleles. A developed KASP marker targeting the causal SNP effectively identifies favorable haplotypes, reducing lesion expansion by 40–49%, providing a pivotal tool for breeding *sclerotinia*-resistant sunflower varieties.

1 Introduction

Sunflower (*Helianthus annuus* L.) serves as a major oilseed crop globally, playing a crucial role in edible oil production (Liu et al. 2017). However, its productivity is seriously imperiled by *Sclerotinia sclerotiorum*, a kind of necrotrophic fungus causing *sclerotinia* stem/head rot (Vuong et al. 2004). Current disease control methods mainly rely on limited fungicide applications, but their unsatisfactory efficacy and negative environmental impacts have spurred the search for more sustainable alternatives (del Río et al. 2007). Therefore, developing resistant cultivars through advanced breeding approaches has become the most promising strategy (Zhao et al. 2004; Bastien et al. 2014). Resistance to *S. sclerotiorum* is a polygenic trait characterized by additive and epistatic interactions, moderate heritability, and significant genotype-environment interactions (Hahn, 2002). Quantitative trait locus (QTL) mapping has emerged as a key technique for identifying genomic regions associated with resistance to *S. sclerotiorum* in sunflower.

Initial QTL studies employing RFLP markers uncovered four leaf resistance loci and two capitulum resistance loci against *S. sclerotiorum* (Mestries et al. 1998). Subsequent research by Bert et al. (2002) revealed 11 QTL linked to pathogen resistance through comparative genetic analyses. Micic et al. (2004) reported 15 loci

associated with leaf lesions, stem lesions, and fungal growth rates, collectively accounting for 24.4%–33.7% of genotypic variance. Their later work (Micic et al. 2005) applied selective genotyping to confirm QTL for midstalk rot resistance. Using AFLP and SSR markers, Rönicke et al. (2005) mapped three lesion length (LL)-related and two head rot-related QTL, explaining 10.6%–17.1% phenotypic variance. Yue et al. (2008) identified 16 head rot resistance QTL, each contributing 8.4%–34.5% phenotypic variation. Amouzadeh et al. (2013) localized five resistance QTL to linkage groups 1, 3, 8, 10, and 17 using enhanced SSR/SNP maps.

High-throughput genotyping advancements facilitated more precise QTL detection. Zubrzycki et al. (2017) utilized a 384-SNP array to discover 36 main-effect and 13 epistatic QTL. Talukder et al. (2016) employed genotyping-by-sequencing to construct a 1,053-SNP map, pinpointing six QTL with two major loci (LGs 10 and 17) explaining 31.6% and 20.2% variance, respectively. Further refinements by Talukder et al. (2022a) established a 6,315 SNP/InDel map (1,420.287 cM), resolving 16 head rot resistance QTL across chromosomes 1, 2, 10, 12, 13, 14, 16, and 17. Interspecific studies by Talukder et al. (2022b, 2023) characterized 16 basal stalk rot QTL introgressed from *H. praecox* and 14 QTL from *H. petiolaris*, with wild species alleles enhancing resistance at 11 loci.

Complementary association mapping efforts have implicated candidate genes such as *HaCOI1-1*, *HaCOI1-2*, *HaRIC_B*, and *GLP15* in resistance mechanisms (Talukder et al. 2014; Fusari et al. 2012; Filippi et al. 2020, 2022). Despite these significant advancements, the genetic mechanisms underlying sunflower resistance to *S. sclerotiorum* remain incompletely understood. Many QTL with minor effects have yet to be identified, and the interactions between different resistance-related genes and QTL are still unclear. Moreover, the fine-mapping of QTL to identify the underlying causal genes has been a challenge.

Previously, our research involved using disease-sensitive (B728) and disease-resistant (C6) genotypes for a comparative transcriptomic study to investigate their responses to pathogen *S. sclerotiorum* (Zhao et al. 2025). This study aimed to achieve four objectives: (1) to identify QTL responsible for *S. sclerotiorum* resistance using an RIL population derived from B728 × C6 crosses across three experiments, (2) to pinpoint the genomic location of *qSCL2.4*, a major-effect QTL, in a narrow region using BC₁F₃ populations, (3) to locate its associated candidate gene, and (4) to develop KASP markers for *S. sclerotiorum* resistance in sunflowers. The findings from this study will enhance our knowledge of *S. sclerotiorum* resistance mechanisms and support future efforts in molecular breeding to improve disease resistance in sunflowers.

2 Materials and methods

2.1 Plant materials

This study utilized two parental oilseed sunflower inbred lines, disease-susceptible genotype B728 and disease-resistant genotype C6, which developed by Liaoning Academy of Agricultural Sciences (Zhao et al. 2025). A mapping population containing 178 F_{5:6} RILs was developed through the cross between these contrasting parental lines. Following comprehensive phenotypic evaluation and genomic characterization, RIL-5 emerged as a superior recombinant line demonstrating exceptional disease resistance. This selection was based on its unique genetic architecture, which combines the major-effect resistance QTL inherited from C6 donor parent with predominant genetic similarity to the recurrent parent B728. For fine-mapping this QTL region, RIL-5 was

backcrossed with B728 to generate the BC₁F₃ population. Additionally, 137 sunflower accessions (Table S1) were utilized to assess allele variations at the candidate gene region identified through QTL analysis.

2.2 Growth conditions

Three distinct environments (field, climate chambers and greenhouse conditions) were utilized to assess disease resistance in this study (Table 1). For RIL and parents, the disease assessment was conducted under field, climate chambers and greenhouse conditions, respectively. For BC₁F₃ populations, the disease assessment was conducted. For sunflower germplasm, the experiments were conducted under field and climate chambers conditions.

Field conditions involved sowing sunflowers at Shenyang (123.551499E, 41.816737N) in Liaoning Province during June. The experiment utilized a randomized block design replicated three times. Each plot area was 6 m², with seedlings planted at 60 cm spacing between plants and 50 cm between rows. Protection lines were consistently set up across the field, following local sunflower production standards for field management.

Climinate chambers involved seedlings grown in 3-liter plastic pots containing a sterilized vermiculite-nutrient soil mix, with three pots allocated per genotype (one plant per pot). Growth parameters were maintained at 28°C/25°C (day/night), 70% relative humidity, and 16-hour photoperiod under 4000 Lux illumination.

Greenhouse conditions involved pot-culture experiments conducted at Liaoning Academy of Agricultural Sciences in Shenyang (123.543145E, 41.824543N), Liaoning Province during March. Sunflower seedlings were cultivated in 30×30×35 cm pots containing 18.0 kg field soil per container, arranged in randomized complete blocks with three pots per genotype (three plants per pot).

2.3 Pathogen inoculation and disease assessment

S. sclerotiorum isolates were obtained from symptomatic inflorescences of sunflower (*Helianthus annuus*) plants cultivated under field conditions in Shenyang, Liaoning Province, China (Zhao et al. 2025). Fungal isolation was performed by transferring infected tissue segments onto potato dextrose agar (PDA; BD Difco™) plates, followed by purification through hyphal tip culture. A representative strain was selected and preserved on PDA slants at 4°C for long-term storage. For experimental use, a 3-mm mycelial disk was aseptically excised from the actively growing margin of a 5-day-old PDA culture (25°C, dark conditions) and transferred to fresh PDA medium. To ensure colony uniformity, ten peripheral mycelial plugs were subcultured onto new PDA plates under identical incubation parameters.

For RILs and germplasm, a modified stem inoculation protocol was implemented to assess sunflower resistance to *S. sclerotiorum* under controlled conditions. Sunflower plants at the 4-leaf developmental stage were selected for uniformity in height (25-30 cm) and stem diameter (4-5 mm). Prior to inoculation, the third stem internode was surface-disinfected with 75% ethanol and air-dried in a laminar flow hood. A hypodermic needle (23-gauge) was used to create a standardized wound (2.0 ± 0.2 mm depth) at the center of the third internode. A mycelial plug was positioned adaxial side down onto the wound to ensure direct hyphal contact with vascular tissues. The inoculation site was immediately sealed with two layers of Parafilm to maintain

humidity and prevent contamination. Lesion length (LL) was measured longitudinally along the stem axis at 3 days post-inoculation (dpi) using a digital caliper. Measurements excluded the original wound diameter (2 mm) to isolate pathogen-driven necrosis. Three technical replicates per biological sample were analyzed.

For RILs and germplasm, a standardized detached leaf assay was also conducted following modified protocols. Fully expanded sunflower leaves at the seedling stage (V4-V6) were excised from healthy plants and surface-sterilized with 70% ethanol for 30 s, followed by three rinses in sterile distilled water. Leaves were placed abaxial side up on moistened filter paper in 90-mm Petri dishes. For inoculation, two 5-mm mycelial plugs from actively growing *S. sclerotiorum* colonies were symmetrically positioned on each leaf, 10 mm apart from the central vein. In addition, for the BC₁F₃ populations, the hyphal suspensions were also prepared by homogenizing 5-day-old mycelia in sterile 0.05% Tween-20 solution (w/v) using a tissue grinder, followed by filtration through four layers of sterile cheesecloth. The suspension was adjusted to a final concentration of 1×10^5 hyphal fragments/mL using a hemocytometer. Each leaf was inoculated with 10 μ L of hyphal suspension at four equidistant points along the midvein. Petri dishes were sealed with Parafilm and incubated at $22 \pm 1^\circ\text{C}$ under a 14-hr photoperiod (4000 Lux). Lesion area (mm²) was measured at 3 dpi using ImageJ v1.53 software with threshold-based segmentation (RGB: 0-120 for necrosis). Three technical replicates per biological sample were analyzed.

2.4 Phenotypic data analyses

Statistical analyses were conducted using SPSS Statistics 19.0 for correlation coefficient calculations. Genetic variance partitioning was performed via the Analysis of Variance (ANOVA) module in QTL IciMapping software (v4.2, <http://www.isbreeding.net>) with default algorithm settings retained. Broad-sense heritability (H^2) for each trait was estimated using variance components derived from the ANOVA output according to the following mixed linear model: $H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{ge}^2 / e + \sigma_e^2 / re)$, where σ_g^2 is $(MS_f - MS_{fe}) / re$, σ_{ge}^2 is $(MS_{fe} - MS_e) / r$ and σ_e^2 is MS_e ; σ_g^2 = genetic variance, σ_{ge}^2 = genotype $\times$ environment interaction variance, σ_e^2 = error variance. MS_f , MS_{fe} and MS_e represent mean squares of genotype, genotype $\times$ environment interaction, and residual error, respectively. r = number of biological replicates ($n = 3$), and e = number of environments ($e = 3$: field, climate chamber, greenhouse).

2.5 Genotyping

Leaf samples underwent DNA extraction using an optimized CTAB method (Saghai-Maroo et al. 1984). A high-resolution SNP genotyping platform was established through Specific-Locus Amplified Fragment sequencing (SLAF-seq) across the 178 RILs and parental lines (Sun et al. 2013). Enzymatic digestion with RsaI/HaeIII (New England Biolabs, USA) generated cohesive ends, followed by 3'-adenine overhang addition and dual-index adaptor ligation (Kozich et al. 2013) for multiplexed library construction. SLAF libraries were size-selected (300-500 bp) and validated using an Agilent 2100 Bioanalyzer with DNA 1000 chips. Quantitative PCR (Kapa Biosystems) ensured optimal cluster density prior to paired-end sequencing (2 $\times$ 100 bp) on the Illumina HiSeq 2500 platform. Technical controls included parallel processing of *Oryza sativa ssp. japonica* cv. Nipponbare libraries, leveraging established rice genome annotations (MSU RGAP) for cross-species sequencing fidelity verification.

Quality-filtered sequencing reads were aligned to the *Helianthus annuus* reference genome (HA412.v2.0) using BWA-MEM (v0.7.17) with optimized parameters (Li, 2013). Sequence fragments were systematically classified into polymorphic, monomorphic, or repetitive categories through integrative analysis of allele frequency spectra and sequence divergence patterns. SNP calling was performed through parallel processing with GATK HaplotypeCaller (v4.2.6.1) and SAMtools mpileup (v1.15.1), retaining only concordant variants (McKenna et al. 2010; Li, 2009). Only consensus SNPs that were concordant across both callers were retained, followed by rigorous filtering criteria (minor allele frequency [MAF] > 0.05; missing rate < 20%) (Zhou and Stephens, 2009). Sequencing reproducibility was rigorously verified through technical replicates of 10% randomly chosen samples, demonstrating exceptional consistency (Pearson's correlation coefficient $r > 0.98$, $p < 1 \times 10^{-15}$). This stringent quality control framework ensured high-confidence variant datasets for downstream analyses.

2.6 Linkage mapping and QTL analysis

QCLiMapping 4.2 software (Meng et al. 2015) was utilized to construct genetic maps based on 6,059 SNPs across 17 chromosomes that were polymorphic between B728 and C6, controlling for genome-wide type I error using a permutation test with 1,000 iterations at $\alpha = 0.05$. The permutation analysis simulates the null distribution of no QTL effect by shuffling phenotypic data relative to genotypic data, thereby providing a stringent significance threshold ($\text{LOD} \geq 2.5$) that inherently accounts for multiple testing issues. This approach avoids the over-conservative adjustment of Bonferroni correction while maintaining statistical rigor. QTL were named using abbreviated trait names starting with "q," followed by chromosome number or location designating multiple QTL on the same chromosome.

2.7 Development of KASP markers

For fine-mapping, high-resolution genotyping was performed using kompetitive allele-specific PCR (KASP) markers. Initially, SNPs within the QTL interval, identified through whole-genome resequencing of parental lines (B728 and C6), were converted into 11 KASP markers (Table S2) for precise genotyping of 3,133 BC₁F₃ individuals. Subsequently, the additional trait-linked KASP markers (Table S3) were designed to screen allelic variations in 137 sunflower inbred lines at the candidate locus. All reactions were implemented on a 1536-well high-throughput genotyping platform (LGC Genomics, UK) following manufacturer specifications. Fluorescence signals were quantified using a Synergy H1 hybrid multimode microplate reader (BMG Labtech, Germany) with excitation/emission filters optimized for FAM™ and HEX™ dyes.

2.8 RNA isolation and qPCR analysis

To investigate dynamic transcriptional responses to *S. sclerotiorum* infection, leaf tissues bordering lesion margins (0.5 cm width) were collected from inoculated and mock-treated seedlings of B728 and C6 at five timepoints (0, 12, 24, 36, and 48 h post-inoculation) under climate chambers conditions. Total RNA was reverse-transcribed into cDNA using the PrimeScriptRT Reverse Transcription Kit with genomic DNA removal (TaKaRa, Japan). Quantitative real-time PCR (qRT-PCR) was performed on a LightCycler 480 II system (Vazyme Biotech, China) with gene-specific primers (Table S4), including actin as the endogenous control. Each biological

replicate comprised three independent plants, with four technical replicates per sample to ensure measurement precision. Relative gene expression levels were normalized to actin and calculated using the $2^{-\Delta CT}$.

3 Results

3.1 Phenotypic variation in *S. sclerotiorum* resistance across the RIL population

The resistant genotype C6 demonstrated significantly lower LL and LA compared to susceptible B728 across three experimental conditions at 3 dpi (Table 2; Fig. 1A-B). Quantitative analysis of 178 RILs revealed continuous phenotypic distributions for both traits (LL: 0.42–7.15 cm; LA: 0.16–7.32 cm²), conforming to normality, indicative of polygenic inheritance (Fig. 1C-D). More than 87% and 89% heritabilities were estimated for LL and LA, respectively. These results indicate significant genetic variation in these resistance traits, supporting the detection of QTL with large effect sizes within this population.

3.2 High-density linkage mapping and multi-environment QTL detection

Following stringent quality control (missing data <10%, segregation distortion), 6,059 high-confidence SNPs were selected to construct a genome-wide linkage map spanning 2,763 cM across 17 chromosomes, with marker density ranging from 157 (Chr8) to 658 (Chr2) loci per chromosome and an average interval of 0.52 cM (Fig. S1).

Using inclusive composite interval mapping (ICIM) with additive effects model, we detected 10 resistance-associated genomic regions distributed across chromosomes 2, 6, 8, 9, and 13 (Fig. 2). Across three environments, we identified 16 QTL for LL and 12 QTL for LA, each of these QTL explains between 1.58 and 32.86% of the observed phenotypic variation in the RIL population (Table 3). Of these, *qSCL2.4* was identified as a stable major-effect locus detected in all environments, contributing 25.62–30.22% and 23.39–28.15% of total variance of LL and LA, respectively. The *qSCL2.4* had allele conferring increased *S. sclerotiorum* resistance derived from the parent C6.

3.3 Fine-mapping of *qSCL2.4* and analyzing candidate genes

The major-effect QTL *qSCL2.4* was in an approximate 0.82 Mb interval with flanking by Chr02:136862663 and Chr02:137041016 on chromosome 2 (Fig. 3A). To precisely locate this QTL within the genome, resistant line RIL-5 was selected for backcrossing with recipient parent B728 due to their differing alleles at the *qSCL2.4* locus while sharing 71% of genetic background. A total of 3,133 offspring from the BC₁F₃ generation were genotyped using eleven KASP markers across the target region, identifying five key informative recombinants (Fig. 3B). At 3 dpi of *S. sclerotiorum*, the visible lesions appeared in RT1 and RT2 rather than in RT3, RT4 and RT5 (Fig. 3C). Finally, reducing the genomic interval of *qSCL2.4* from ~0.82 Mb to a 226.7-kb interval flanked by markers KASP8 (Chr2: 141308320) and KASP11 (Chr2: 141535027).

According to the *Helianthus annuus* reference genome *HanXRQr1.0*, this region contains two annotated genes, *LOC110907968* and *LOC110906338*. Of these, *LOC110906338* encodes unknown protein, while *LOC110907968* encodes WRKY transcription factor 48 (*HaWRKY48*) (Fig. 3D). We further found seven SNP in exon region in the DNA sequence of *HaWRKY48* between B728 and C6, which also led to the variation of amino acids (Fig. 3E). The qPCR analysis indicated that the expression level of *HaWRKY48* was significantly higher in B728 compared to C6 by *S. sclerotiorum* infection (Fig. 3F). These convergent genomic and transcriptomic evidences nominate *HaWRKY48* as the candidate underlying *qSCL2.4*-mediated resistance, though functional validation *via* allelic complementation remains imperative.

3.4 Development of KASP markers for resistance to *Sclerotinia sclerotiorum* in sunflower

Among the SNP in the *HaWRKY48* coding region between B728 and C6, a nucleotide change at position Chr02:141503867 (221 base in coding region of *HaWRKY48*) involves a substitution from T to C, as a result of amino acids variation from His to Arg. To explore the phenotypic effects of allelic variations in this SNP, a KASP marker was developed in haplotype analysis by using 137 sunflower accessions. The designed molecular markers effectively differentiate between these two genotypes. Under field condition, sunflower accessions with the Chr02:141503867-C allele exhibit a significantly reduction of 44.67% and 48.92% in LL and LA, respectively, as compared to those with the Chr02:141503867-T allele (Fig. 4A, B). Under climate chambers condition, accessions carrying the C allele also exhibited 40.06% and 41.22% reduced LL and LA, respectively (Fig. 4C, D). The favorable alleles ratio in the population of Chr02:141503867-C were 43.1% (Table S5). These findings demonstrate the potential utility of the Chr02:141503867 marker in marker-assisted selection (MAS) for improving resistance to *S. sclerotiorum* in sunflower breeding programs.

4 Discussion

4.1 Phenotypic of sunflower resistance to *S. sclerotiorum*

The accurate phenotyping of *S. sclerotiorum* resistance remains a cornerstone for genetic dissection and breeding. We employed a multi-environmental evaluation system integrating field, climate chamber, and greenhouse trials, coupled with standardized stem inoculation and detached leaf assays. This approach emphasized the necessity of controlled-environment assays to minimize environmental noise, while field trials ensure ecological relevance. In this study, the evaluation of sunflower resistance to *S. sclerotiorum* based on LL and LA in the RIL population demonstrated high heritability, with values exceeding 87% for LL and 89% for LA. These high heritability estimates suggest that the observed phenotypic variations in resistance traits are predominantly influenced by genetic factors. Similar to previous research (Micic et al. 2004; Amouzadeh et al. 2013), which also reported significant genetic variances in sunflower resistance to *S. sclerotiorum*, our results indicate that the selected traits are reliable indicators for assessing resistance. The continuous phenotypic distributions of LL and LA in the RIL population, conforming to normality, suggest a polygenic inheritance pattern. This is consistent with the findings of many previous studies (Mestries et al. 1998; Micic et al. 2004), indicating that multiple genes contribute to sunflower resistance to *S. sclerotiorum*. The high heritability and polygenic nature of these traits provide a solid foundation for further genetic analysis and QTL mapping.

4.2 Identification of the novel locus *qSCL2.4*

The identification and fine-mapping of *qSCL2.4* on chromosome 2 represents a significant advancement in sunflower resistance breeding. Previous research efforts have identified various QTL associated with sunflower resistance to *S. sclerotiorum* on chromosome 2, including *Qdi1* and *Qds1* (Yue et al. 2008), *qIP-2a* and *qDS-2a* (Zubrzycki et al. 2017), *CPY-2.1* and *CPY-2.2* (Paknia et al. 2020), *Qhr-2.1* (Talukder et al. 2022a), and *Qbsr-2.3* (Talukder et al. 2022b). By comparing the genetic position of *qSCL2.4* with previous studies, we can determine its novelty. Unlike previous QTL with modest phenotypic contributions, *qSCL2.4* is a stable major-effect locus detected in all environments, contributing a substantial proportion (25.62-30.22% for LL and 23.39-28.15% for LA) of the total variance, highlighting its potential as a breeding target.

4.3 Involvement of the *WRKY* gene family in disease resistance and prediction of candidate genes

The *WRKY* gene family has been well-documented for its crucial role in plant defense responses against diverse pathogens (Pandey and Somssich, 2009; Wani et al. 2021; Javed and Gao, 2023). In this study, the resolution of *qSCL2.4* locus to a 226.7-kb interval, achieved through BC₁F₃ population analysis and high-density KASP genotyping. The gene *LOC110907968*, encoding *WRKY* transcription factor 48, was identified as a candidate gene associated with *qSCL2.4*, which aligns with the established role of *WRKY* transcription factors in plant immunity. In oilseed rape, several *WRKY* genes have been shown to be involved in resistance to *Sclerotinia* infection. For example, *WRKY15* and *WRKY33* in oilseed rape interact and play important roles in resistance to *Sclerotinia* (Liu et al. 2018). Overexpression of *BnWRKY33* in oilseed rape enhances resistance to *S. sclerotiorum* (Wang et al. 2014), while *WRKY28* in *Brassica napus* has been found to regulate the resistance response, although its function is complex and can sometimes suppress resistance (Zhang et al. 2022). The *Arabidopsis* ortholog *WRKY48* demonstrates paradoxical regulatory behaviors, where knockout mutants paradoxically exhibit elevated *PR1* expression and improved *Pseudomonas syringae* resistance via salicylic acid pathway potentiation (Xing et al. 2008). In this study, the significant difference in the expression level of *HaWRKY48* between B728 and C6 upon *S. sclerotiorum* infection further supports its role in the resistance mechanism. Moreover, the seven nonsynonymous SNPs in *HaWRKY48* between resistant (C6) and susceptible (B728) lines, may disrupt its DNA-binding affinity or interaction with co-regulators, thereby altering defense outputs. These results suggested that *HaWRKY48* in sunflower may also play a significant role in the defense response against *S. sclerotiorum*. However, functional validation through transgenic complementation or CRISPR/Cas9 editing is essential to confirm its role, as emphasized by recent critiques of candidate gene studies.

4.4 Excellent allelic variation of *HaWRKY48*

The development of a KASP marker based on the SNP in the *HaWRKY48* coding region represents a significant advancement. This marker can effectively distinguish between the two genotypes, and sunflower germplasm carrying the Chr02:141503867-C allele exhibited notable reductions in both LL and LA, under both field and climate chamber conditions. The favorable allele ratio of Chr02:141503867-C in the population was 43.1%. This relatively high frequency is advantageous for breeders, as it implies that there is a substantial pool of genetic

material that can be exploited to enhance sunflower resistance to *S. sclerotiorum*. By using the KASP marker, breeders can efficiently screen large populations of sunflower plants to identify those carrying the favorable allele. This allows for the targeted introgression of the favorable allele into elite sunflower lines, accelerating the breeding process and increasing the efficiency of developing resistant varieties. Compared to traditional breeding methods that rely solely on phenotypic evaluation, MAS using the KASP marker offers greater precision and speed, as it can identify plants with the desired resistance traits at the genetic level, even in the early stages of plant development. Similar successes in sunflower (Talukder et al. 2014), *Brassica napus* (Roy et al. 2022), peanut (Bressano et al. 2024) and soybean (Maldonado dos Santos et al. 2019) underscore the utility of KASP platforms.

5 Conclusion

In this study, using the RIL population, sixteen QTL for LL and twelve QTL for LA were identified involved in controlling *S. sclerotiorum* resistance through individual environmental analyses. Subsequent fine-mapping employing BC₁F₃ populations delimited the major-effect QTL *qSCL2.4* to a defined 226.7-kb genomic interval containing two annotated genes. Based on its transcriptional expression differences and allele variations in the coding region, the candidate gene *LOC110907968*, encoding the WRKY transcription factor 48 (*HaWRKY48*), was ultimately selected. The Chr02:141503867-C allele for *HaWRKY48* serves as a robust molecular marker for breeding programs. The identification of *qSCL2.4* and its candidate gene *HaWRKY48* not only enriches our understanding of *S. sclerotiorum* resistance mechanisms but also provides actionable tools for combating one of the most devastating pathogens in oilseed crops.

Declarations

Acknowledgments

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Author Contribution statement

MZ conducted genotyping, pathogen inoculation and data analysis and prepared the original draft of the manuscript. DW and DS conducted population development. XL, BY, and YC conducted field experiments and phenotypic data collection. JL and LF supervised the overall research. All co-authors contributed to critical manuscript refinement through iterative peer-review discussions and provided final approval for publication.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of 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.

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Tables

Table 1 Plant materials and growth conditions for QTL analysis, fine-mapping and haplotype analysis.

Objective	Conditons (Year)	Plant materials	Pathogen inoculation	Disease assessment
QTL analysis	Field (2022), Climate chambers (2023), Greenhouse (2022)	RILs and parents	Mycelial plugs	LL and LA, 4dpi
Fine-mapping	Climate chambers (2023)	BC ₁ F ₃ and and parents	Hyphal suspension	LA, 4dpi
Haplotype analysis	Field (2024), Climate chambers (2024)	Germplasm	Mycelial plugs	LL and LA, 4dpi

LL, lesion length; LA, lesion area; RILs, recombinant inbred lines.

Table 2 Lesion length (LL) and lesion area (LA) of sunflower RIL population in different conditions.

Trait	Condition	B728	C6	Mean	SE	Min	Max	<i>h</i> b2
LL (cm)	Exp1	7.27	0.77	3.20	1.72	0.61	7.15	0.94
	Exp2	5.05	0.68	2.20	1.16	0.42	4.96	0.95
	Exp3	5.86	0.51	2.40	1.29	0.42	5.65	0.94
	Average	6.06	0.65	2.60	1.47	0.42	7.15	0.87
LA (cm ²)	Exp1	7.41	0.31	3.01	1.71	0.28	7.32	0.96
	Exp2	5.52	0.21	2.08	1.18	0.16	5.42	0.95
	Exp3	5.91	0.21	2.28	1.31	0.17	5.49	0.94
	Average	6	0.24	2.46	1.47	0.16	7.32	0.89

Table 3 QTL for lesion length and area detected in the sunflower RILs by individual environmental analyses.

QTL	Trait	Condition	Chr.	Left marker	Right marker	LOD	PVE (%)	Add
<i>qSCL2.1</i>	LL	Greenhouse	2	Chr02:33990910	Chr02:34105902	12.25	6.26	0.56
<i>qSCL2.2</i>	LL	Field	2	Chr02:35576559	Chr02:35639570	12.06	14.46	0.79
	LL	Mean	2	Chr02:35576559	Chr02:35639570	11.45	14.48	0.60
<i>qSCL2.3</i>	LL	Climinate chambers	2	Chr02:122250375	Chr02:122346990	10.35	6.32	0.44
	LA	Climinate chambers	2	Chr02:122250375	Chr02:122346990	7.37	3.76	0.41
	LA	Greenhouse	2	Chr02:122250375	Chr02:122346990	7.04	10.11	0.45
	LA	Mean	2	Chr02:122250375	Chr02:122346990	6.61	10.53	0.48
<i>qSCL2.4</i>	LL	Field	2	Chr02:136862663	Chr02:137041016	19.26	25.62	1.03
	LL	Climinate chambers	2	Chr02:136862663	Chr02:137041016	37.68	32.86	1.00
	LL	Greenhouse	2	Chr02:136862663	Chr02:137041016	40.39	30.22	1.23
	LL	Mean	2	Chr02:136862663	Chr02:137041016	19.29	27.31	0.82
	LA	Field	2	Chr02:136862663	Chr02:137041016	14.74	23.39	0.91
	LA	Climinate chambers	2	Chr02:136862663	Chr02:137041016	37.92	28.15	1.11
	LA	Greenhouse	2	Chr02:136862663	Chr02:137041016	16.23	25.71	0.71
	LA	Mean	2	Chr02:136862663	Chr02:137041016	14.85	25.42	0.73
<i>qSCL2.5</i>	LL	Climinate chambers	2	Chr02:137712745	Chr02:138547685	12.07	7.53	-0.48
	LL	Greenhouse	2	Chr02:137712745	Chr02:138547685	13.14	6.81	-0.59
	LA	Climinate chambers	2	Chr02:137712745	Chr02:138547685	11.93	6.42	-0.54
<i>qSCL6.1</i>	LA	Climinate chambers	6	Chr06:7796518	Chr06:7850983	3.77	1.76	0.36
	LA	Greenhouse	6	Chr06:7796518	Chr06:7850983	3.01	3.98	0.36
	LA	Mean	6	Chr06:7796518	Chr06:7850983	2.91	4.20	0.39
<i>qSCL6.2</i>	LA	Field	6	Chr06:7857636	Chr06:7921898	3.10	4.18	0.51
	LL	Climinate chambers	6	Chr06:41159956	Chr06:41317474	3.26	1.76	0.23
	LL	Greenhouse	6	Chr06:41159956	Chr06:41317474	3.55	1.59	0.28
<i>qSCL8</i>	LL	Climinate chambers	8	Chr08:61676635	Chr08:61900200	2.99	1.58	-0.22

<i>qSCL9</i>	LL	Climinate chambers	9	Chr09:16322350	Chr09:16387104	3.33	1.86	0.25
	LL	Mean	9	Chr09:16322350	Chr09:16387104	2.62	3.03	0.28
<i>qSCL13</i>	LL	Climinate chambers	13	Chr13:71356752	Chr13:71587496	4.18	2.29	0.28

Figures

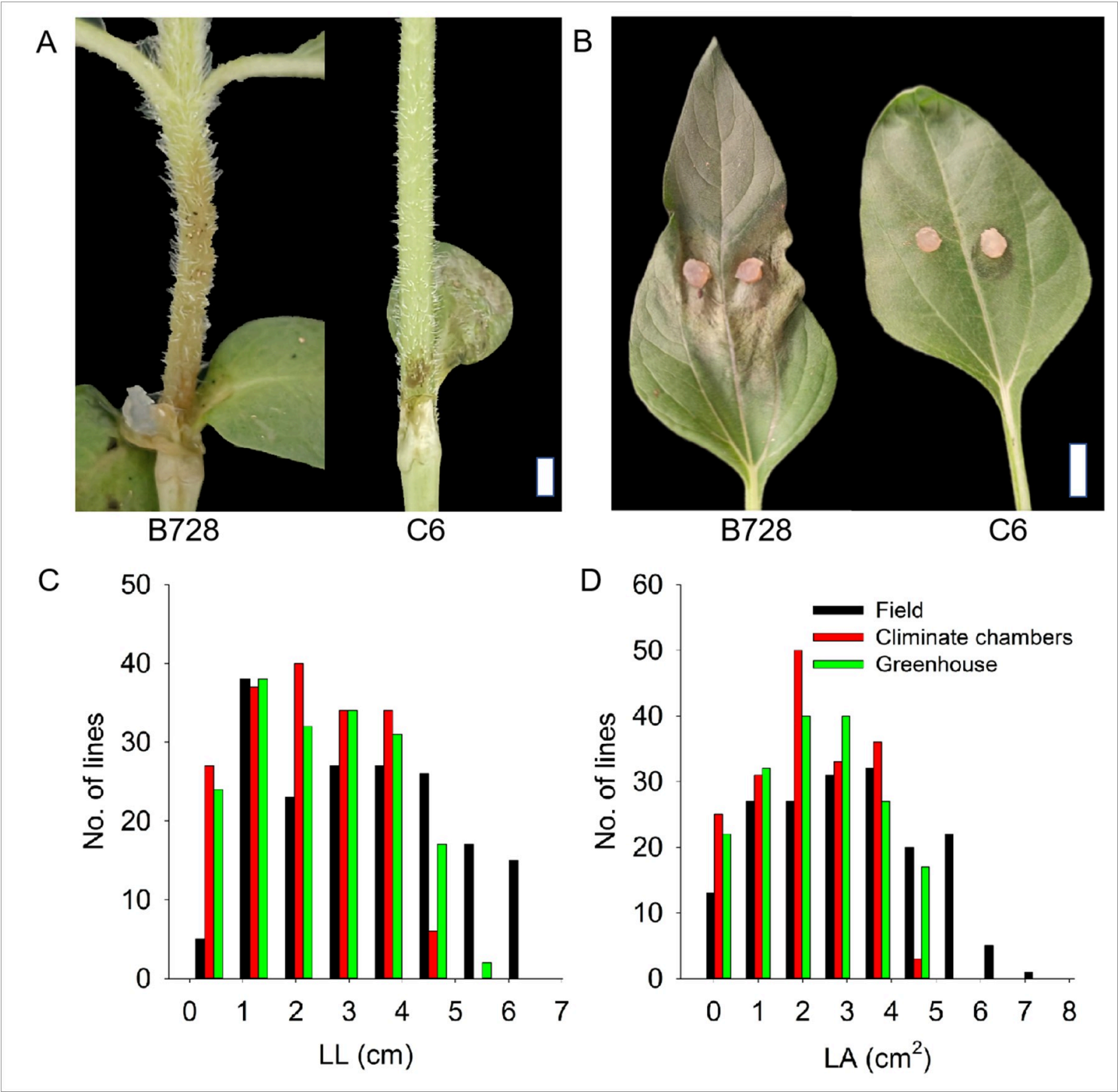


Figure 1

Difference of lesion length (LL) and lesion area (LA) between B728 and C6 (A, B) and the frequency distributions of LL and LA (C, D) in the RIL population in three experiments.

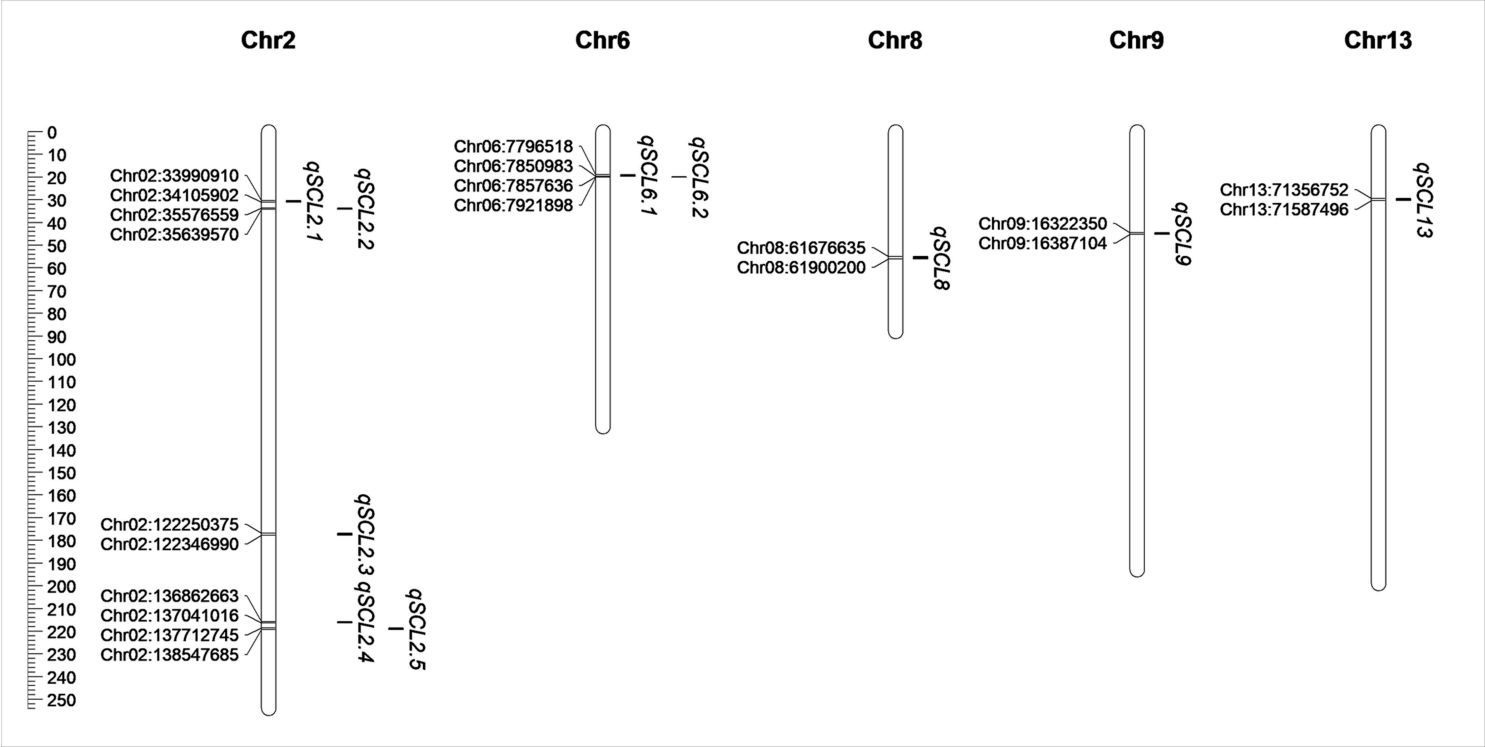


Figure 2

High-resolution genetic linkage map illustrating QTL governing lesion length (LL) and lesion area (LA) in RIL population. Black annotations denoting novel loci discovered in this investigation. Chromosomal positions reflect recombination frequencies calculated.

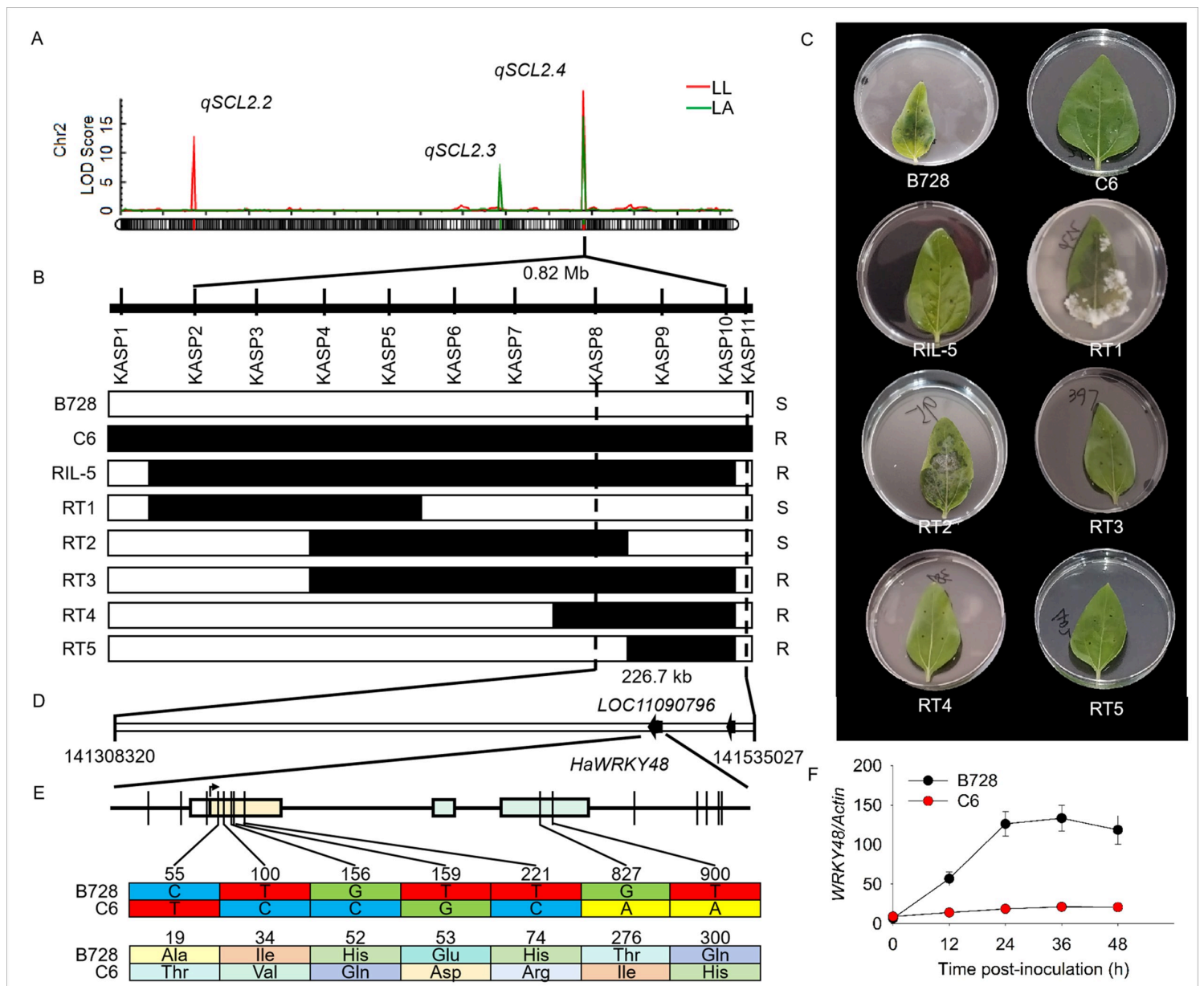


Figure 3

Molecular dissection of *qSCL2.4* locus and functional characterization of associated genes. (A) Physical map of the 226.7-kb target interval delineated by flanking molecular markers. (B) High-resolution recombination breakpoint analysis using five informative recombinants. Genotypic configurations are depicted as white (B728) and black (C6) haplotype blocks, with vertical dashed lines demarcating the critical candidate region. (C) Comparative necrotic lesion development at 3 days post-inoculation (dpi) across recombinant genotypes. (D) Genomic architecture of the refined interval showing two annotated open reading frames (ORFs), with arrow directionality indicating transcriptional orientation. (E) Allelic variation in *HaWRKY48* between B728 and C6. (F) qRT-PCR expression levels for *HaWRKY48* at different times post-inoculation.

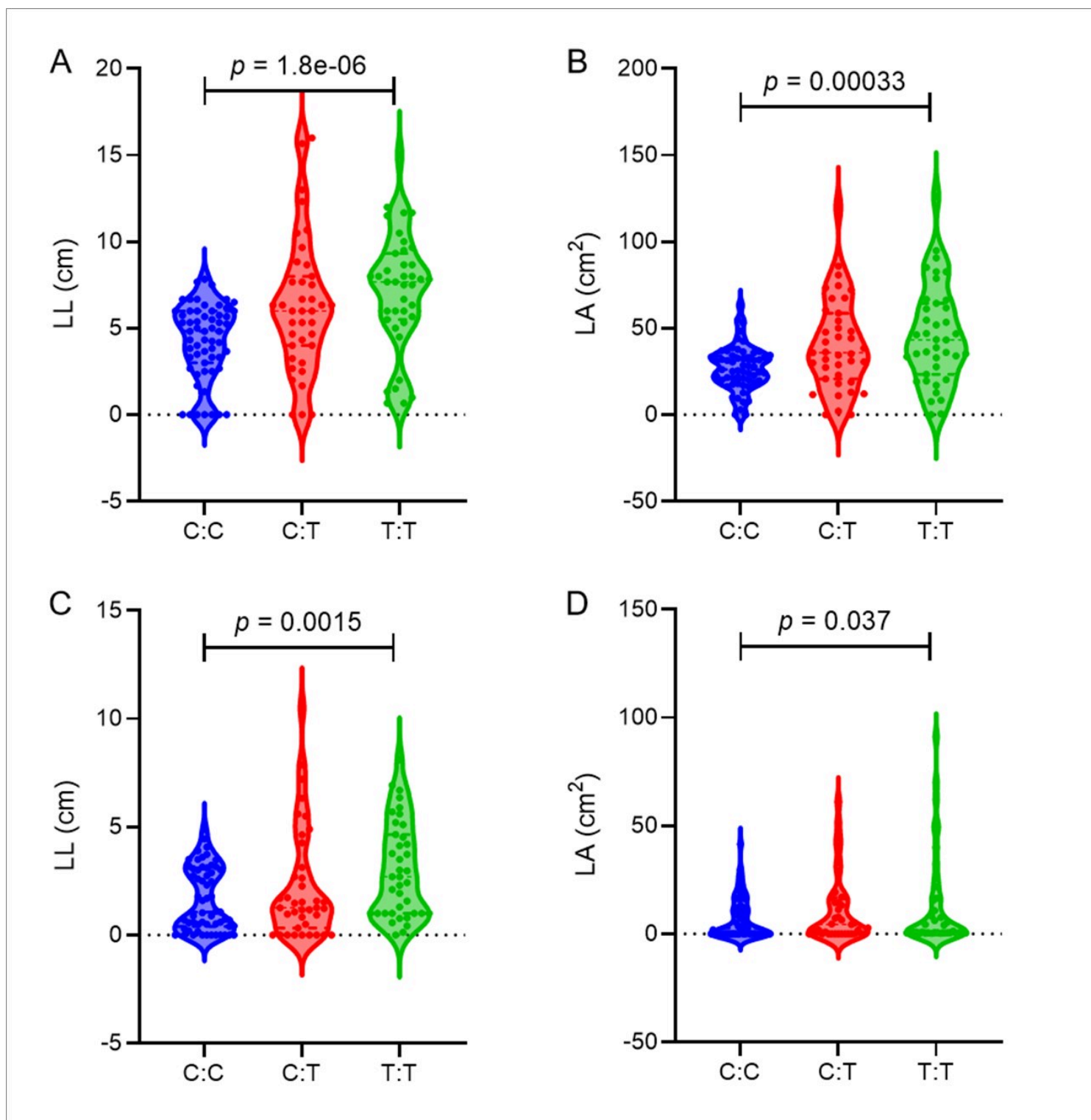


Figure 4

Haplotype analysis for SNP (Chr02:141503867) under field (A, B) and climate chambers (C, D) conditions. LL, lesion length; LA, lesion area.

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