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# Identification of a consistent QTL and development of KASP marker for stem rot resistance in Spanish-type peanut (Arachis hypogaea L.)

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**Research Article**

**Keywords:**

**Posted Date:** November 23rd, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-11672632/v1

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**Additional Declarations:** No competing interests reported.

## Abstract

Stem rot, caused by *Agroathelia rolfsii*, is a devastating soil-borne fungal disease that severely threatens peanut production worldwide. Breeding resistant cultivars is the most sustainable approach to mitigate disease damage, while the genetic basis of stem rot resistance in Spanish-type peanut remains poorly understood, and the lack of stable resistance quantitative trait loci (QTLs) and applicable markers largely restricts precision breeding for resistance in peanut. In this study, a high-density genetic map was constructed via whole-genome resequencing using a recombinant inbred line (RIL) population derived from Spanish-type peanut ZH212 and 07-3105, and a novel and environmentally stable stem rot resistance QTL *qSRA05.1*was identified, explaining the phenotypic variation of 5.4%-9.4% across three environments. Comparative transcriptomic analysis between resistant and susceptible parental lines revealed that multiple cytochrome P450 (CYP) family genes within the *qSRA05.1* interval in the resistant parent ZH212 were significantly upregulated upon *A. rolfsii* infection, and these genes are considered candidate genes for stem rot resistance in peanut. Based on the trait-linked SNPs within the target QTL interval, a locus-specific KASP marker *A05-9189944*was developed and further validated and enhance stem rot resistance by 5.31 to 6.75 in RIL population and by 7.08 to 9.72 in natural peanut germplasm population. Comparative analysis with previous studies confirmed that *qSRA05.1*is a novel resistance locus distinct from those reported in Virginia-type and wild peanuts, thereby filling the gap in resistance genetic resources for Spanish-type peanuts. This study dissect the quantitative resistance mechanism of stem rot disease in Spanish-type peanut, and provides elite resistance QTL resources, and reliable molecular markers for peanut disease resistance genetic improvement and marker-assisted pyramiding breeding.


## Key message

A stable QTL, q, for stem rot resistance was identified, and a KASP marker linked to q was developed and validated to enhance stem rot resistance by 5.31 to 6.75 in the RIL population and by 7.08 to 9.72 in the natural peanut panel.

## Introduction

Peanut (*Arachis hypogaea* L.) is a major oilseed and grain legume, cultivated across more than 100 countries in tropical, subtropical, and warm temperate regions. In China, with climate changing, continuous monocropping, raw straws returning to the field and intensive cultivation have aggravated the occurrence of soil-borne diseases, which seriously restrict the stable yield and quality improvement of peanut. Stem rot, caused by *Agroathelia rolfsii* Sacc. (syn. *Sclerotium rolfsii*), is one of the most destructive soil-borne diseases in peanut production. This pathogen mainly infects the stem base, leading to plant wilting, and pod shedding (Punja 1985; Le et al. 2012; Yan et al. 2022). Severe field outbreaks can cause yield losses exceeding 30%, and even total crop failure in extreme years (Thirumalaisamy et al. 2014; Yan et al., 2019).

At present, chemical control with fungicides remains the dominant strategy for stem rot control (Grichar 1995; Han et al. 2023; Sun et al. 2023). However, stem rot typically occurs during the mid-to late growth stage, when the dense canopy covers the soil surface, hindering uniform fungicide delivery to the stem bases and soil and resulting in poor field efficacy. Moreover, long-term application of chemical fungicides increases planting costs, causes environmental pollution (He et al. 2025), induces pathogen resistance (Shim et al. 1998; Jiang et al. 2025), and fails to provide a fundamental solution to disease recurrence caused by continuous cropping. Breeding and deploying resistant cultivars are widely recognized as the most economical, safe, and sustainable approach for managing peanut stem rot.

Resistance to stem rot in peanut is a typical quantitative trait governed by multiple genes with minor effects and strongly influenced by environmental conditions (Dodia et al. 2019; Cui et al. 2020; Yan et al. 2023). Previous studies have made considerable progress in evaluating germplasm resistance (Fan et al. 2020; Subedy et al. 2024), characterizing the genetic diversity of the pathogen (Le et al. 2012; Jebaraj et al. 2017; Yan et al. 2022), and optimizing field management practices (Minton et al. 1991; Grichar 1995; Meena et al. 2023). Several quantitative trait loci (QTLs) associated with stem rot resistance have been reported in peanut (Bera et al. 2016; Dodia et al. 2019; Cui et al. 2020; Luo et al. 2020; Agmon et al. 2022; Yan et al. 2023; Tsai et al. 2024; Veerendrakumar et al. 2025). Nevertheless, most published resistance QTLs were identified in Virginia-type peanut cultivars (Cui et al. 2020; Luo et al. 2020; Agmon et al. 2022), wild species (Tsai et al. 2024), and progeny of wild hybrids (Bera et al. 2016; Dodia et al. 2019). To date, stable QTLs for stem rot resistance derived from Spanish-type peanut have rarely been identified. The lack of elite resistance loci from Spanish-type peanut severely limits the diversity of resistance resources and hinders progress in molecular breeding of disease-resistant Spanish-type peanut cultivars, which are widely cultivated in China.

Stable major QTLs identified in different botanical varieties are essential resources for dissecting the genetic mechanisms of quantitative disease resistance and for marker-assisted breeding. To enrich genetic resources for resistance and identify reliable usable loci for improving Spanish-type peanut improvement, this study conducted multi-environment phenotypic evaluations and QTL mapping using a segregating population derived from a cross between the resistant Spanish-type peanut cultivar ‘ZH212’ and the susceptible cultivar ‘07-3105’. The objectives were to identify stable major QTLs conferring stem rot resistance in a Spanish-type background, clarify the distinct genetic basis of disease resistance in Spanish-type peanut, and provide valuable QTL/locus resources for subsequent candidate gene identification, functional validation, and the development of tightly linked molecular markers. This study fills the gap in resistance QTL resources for Spanish-type peanut and facilitates precision molecular breeding and germplasm innovation for stem rot resistance in peanut.

## Materials and methods

# Plant materials and pathogen isolate used in the experiments

In this study, the moderately resistant Spanish-type peanut accession ZH212 (Yan et al. 2019) was used as the male parent and was crossed with the susceptible Spanish-type cultivar 07-3105 to generate a recombinant inbred line (RIL) population of 242 lines by the single-seed descent (SSD) method (Song et al. 2025). Both parents and the $F_8$ to $F_{10}$ generations of each RIL were phenotyped for stem rot resistance. DNA from both parents and the $F_{10}$ generation of each RIL was used for genetic mapping. In addition, a natural peanut germplasm panel comprising 202 accessions (Yan et al. 2023) was employed to validate the Kompetitive allele-specific PCR (KASP) marker developed in the present work.

*Agroathelia rolfsii* isolate WH1 (Fan et al 2020) was used to infect the plants in the fields.

## Phenotyping evaluation of stem rot resistance

Phenotypic evaluation of the mapping population in YL2022 and YL2023 was reported in a previous publication (Song et al. 2025). In the present study, phenotypic evaluation of the RIL population in 2024 was conducted under Wuchangfield conditions, designated WC2024. The 2024 experiment was conducted using a completely randomized design with three replications, with the susceptible cultivar ZH9 as the check. The phenotypic data for the natural peanut germplasm panel were collected in 2025 in both the Yangluo and Wuchang environments. Seeds of the RIL lines were sown in rows 2.5 m long, with 33-cm row spacing and 15-cm plant spacing. Artificial inoculation was performed at 80–85 days after sowing (DAS). Each plant was inoculated by placing 2 g of oat grains infested with *A. rolfsii* WH1 around the stem base as previously reported (Yan et al. 2023). The fields were irrigated to field capacity and maintained under high moisture conditions for three consecutive days after inoculation. Disease assessment was carried out when the disease incidence in the susceptible cultivar ZH9 reached 70%. Individual plants were rated for disease severity based on a 0–5 scale according to Shokes et al. (1998), where: 0 = no visible symptoms; 1 = lesions confined to the stems; 2 = less than 25% of branches wilted; 3 = 25–50% of branches wilted; and 4 = more than 50% of branches wilted. The disease index (DI) was calculated using the formula described by Le et al. (2012). The phenotypic data for the 242 RILs and their parents obtained across the three environments were designated as YL2022, YL2023, and WC2024, respectively; those for the natural peanut panel obtained across the two environments were designated YL2025 and WC2025, respectively. The formula for disease index (DI) is as follows:

$$DI = (\sum[\text{Scale} \times \text{Number of plants in that scale}] / [\text{Total number of plants} \times \text{Maximum scale}]) \times 100$$

## DNA extraction and whole genome resequencing

Thirty days after sowing, young leaves were harvested from the $F_{10}$ RIL population and their parents for genomic DNA extraction using the cetyltrimethylammonium bromide (CTAB) method described by Wang et al. (2018). DNA quality was assessed by 1% agarose gel electrophoresis, and DNA concentration was quantified with a NanoDrop spectrophotometer. DNA libraries were constructed following the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® protocol and sequenced on an Illumina HiSeq 2500 platform

(Illumina, Inc., San Diego, CA, USA) according to the manufacturer's instructions. Raw reads were processed with fastp (v0.23.0) for adapter trimming and low-quality filtering, and clean reads (> 50 bp) were retained. The clean reads were then aligned to the *Arachis hypogaea* "Tifrunner" reference genome (https://www.peanutbase.org/data/public/Arachis_hypogaea/Tifrunner.gnm2.J5K5/) using the Burrows-Wheeler Aligner (BWA, v0.7.15-r1140) (Li and Durbin 2009). Genetic variants, including single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels), were called with the Genome Analysis Toolkit (GATK v3.7).

## Genetic linkage map construction and QTL analysis

The genetic linkage map was constructed following the protocol described by Xie et al. (2010). Markers were grouped into linkage groups (LGs) at a logarithm of odds (LOD) threshold of 5.0 and ordered using the Recombination Counting and ORDering (RECORD) algorithm (Van Os et al. 2005). Genetic distances between markers were calculated using the Kosambi mapping function. Quantitative trait locus (QTL) analysis was performed using QTL iciMapping(version) to identify loci associated with stem rot resistance based on disease index (DI) data across different environments. The inclusive composite interval mapping (ICIM) method was employed (Li et al. 2007). The significance threshold for QTL detection was determined via a permutation test with 1,000 iterations ($P = 0.05$). QTL nomenclature followed the standard convention, in which the prefix 'q' is followed by the trait abbreviation (SR) and the LG designation. When multiple QTLs were identified on the same LG, a sequential number was appended to the name. QTLs with a phenotypic variance (PVE) > 10% were classified as major QTLs, whereas the others were considered minor. The genetic linkage map and QTL positions were visualized using MapChart 2.3 software (Voorrips, 2002).

## RNA-seq and data processing

The two parental lines, ZH212 and 07-3105, were grown in pots in a greenhouse. At 70 days after sowing (DAS), plants were inoculated with *A. rolfsii* isolate WH1 using infested oat grains as described previously (Yan et al. 2023). Stem samples were collected at 0, 72, 96, and 120 hours post-inoculation (hpi). Total RNA was extracted from the stem samples using TRIzol reagent (DP 424, TIANGEN, China) according to the manufacturer's instructions. RNA concentration was measured with a Nanodrop spectrophotometer, and the integrity of all RNA samples was assessed using an Agilent 2100 Bioanalyzer. RNA-seq libraries were constructed from the RNA samples and sequenced on an Illumina HiSeq platform. After removal of low-quality reads, adapter sequences, and ambiguous bases using Trimmomatic (Bolger et al. 2014), high-quality clean reads were obtained. The clean reads were aligned to the reference genome of the cultivated peanut 'Tifrunner' (*Arachis_hypogaea*.Tifrunner.gnm2.ann1.4K0L.genome.fa) using HISAT2 software (Kim et al. 2019). Differentially expressed genes (DEGs) between different stem samples were identified using DESeq2 (Love et al. 2014). Transcript abundance was normalized and expressed as fragments per kilobase of transcript per million mapped reads (FPKM). DEGs were defined using the following criteria: FPKM ≥ 1,

|log2 fold change| ≥ 1, and false discovery rate (FDR) < 0.05. Gene expression heat maps were generated using TBtools (Chen et al. 2020).

# Statistical Analysis of the phenotyping data

Analysis of variance (ANOVA) for the disease index among the RIL population in each environment was performed using SPSS 24 software (IBM Corporation). The broad sense heritability (H2) for the disease index was calculated using the equation $H^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{g\times e}/n + \sigma^2_e/rn)$, where $\sigma^2_g$ is the genetic variance component, $\sigma^2_{g\times e}$ is the genotype-environment interaction variance component, and $\sigma^2_e$ is the residual (error) variance component. The variable *n* and *r* represent the number of environments and replications, respectively. The distribution of disease index was visualized using distribution and box plots genetrated by OriginPro 2021 (OriginLab Originpro 2021).

## Results

# Phenotypic variation

The phenotypic data (disease index) of the RIL population and their parents in YL2022 and YL2023 were described in a previously published study (Song et al 2025). The phenotypic data were newly evaluated in the Wuchang environment in 2024 (WC2024) in the present study (Fig. 1A, 1B). In the WC2024 environment, the disease index of ZH212 was 43.26 and that of 07-3105 was 71.81 (Fig. 1C, Table 1). For the RIL population, the disease index ranged from 33.70 to 96.67, with a mean value of 58.39 (Table 1). The disease index exhibited continuous variation in the RIL population. Consistent with the results obtained in YL2022 (Fig. 1D) and YL2023 (Fig. 1E), the population exhibited a normal frequency distribution in WC2024 (Fig. 1F), with skewness of 0.28 and kurtosis of 0.49 (Table 1). These results suggest that peanut resistance to stem rot is controlled by quantitative loci. The broad-sense heritability for the disease index across three years was estimated at 0.72, indicating that resistance to *A. rolfsii* is predominantly governed by genetic factors. Variance analysis was implemented using the general linear model (GLM) across three environments, the results revealed that genetic, environments and their interaction significantly influenced resistance of peanut to stem rot in the RIL population ($P < 0.001$) (Table 2).

# Sequence data and SNP calling

Whole-genome resequencing of the RIL population and their parents generated a total of 2.32 Tb of raw sequencing data. After stringent data filtering and quality control, 81.81 billion clean reads were obtained, with mean sequencing depths of 3.61× for the RIL population, 10.76 ×for the male parent ZH212, and 9.81× for the female parent 07-3105 (Table S1). Quality assessment indicated high reliability of the sequencing data, with Q30 values ranging from 92.6% to 97.9% and a mean value of 96.3%. The GC content of the population ranged from 35.55% to 38.68%, with a mean of 36.71%.

The clean reads were aligned to the cultivated peanut 'Tifrunner' reference genome using BWA (v. 0.7.15-r1140). Following SNP filtering based on standardized genotyping criteria, a total of 275,821 high-confidence genomic variations were discovered between the two parents, ZH212 and 07-3105, comprising 246,047 single nucleotide polymorphisms (SNPs) and 29,774 insertion and deletion (InDel) variants (Fig. S1). These variants were widely distributed across all 20 chromosomes of the peanut genome, with 2,204–36,049 variants per chromosome. Chromosome B09 harbored the most variants, whereas chromosome B02 harbored the fewest. Genome-wide density analysis revealed a mean SNP density of 96.94 SNPs/Mb (range: 16.04–201.36 SNPs/Mb), and a mean InDel density of 11.73 InDels/Mb (range: 2.25–25.05 InDels/Mb) (Table S2).

# Construction of genetic linkage map and QTL identification

Recombination breakpoints of individual RILs were identified using a sliding window approach, and the 275,784 high-quality genomic variants were further grouped into 4,448 bin markers (Table 3, Fig. S2). Based on these bin markers anchored to the 20 chromosomes, a high-density genetic linkage map was constructed with a total map length of 1,564.27 cM (Fig. S3). Chromosome map lengths ranged from 57.12 cM (A04) to 111.62 cM (B03), and the number of bin markers per chromosome varied from 88 (B10) to 375 (B03). The distribution of bin intervals differed markedly among chromosomes, with a mean interval of 0.38 cM per locus. The minimum and maximum average bin intervals were 0.27 cM (B09) and 0.81 cM (B10), respectively, while the maximum single inter-bin interval reached 11.41 cM on chromosome B10 (Table 3). To evaluate the quality of the constructed genetic map, we performed a collinearity analysis by aligning the genetic positions of marker loci with their corresponding physical positions on the peanut reference genome. The results revealed high genome-wide collinearity for the mapped markers. Markers on most chromosomes showed well-ordered linear arrangements and consistent positions. The newly constructed genetic map showed high coverage and positional accuracy, thereby providing a reliable foundation for subsequent gene localization and genetic studies. (Fig. S4).

QTL mapping for stem rot resistance was performed using the high-density genetic map combined with disease index of stem rot obtained from 242 RILs across three environments from 2022 to 2024. In total, three QTLs for stem rot resistance were identified using QTL Cartographer (v1.17j), with LOD score > 3.0 (Fig. 2a, 2b). These three QTLs were located on chromosomes A04 and A05, and explained 5.4–9.4% of the phenotypic variation (PVE) across the three environments (Table 4). One QTL on chromosome A05, *qSRA05.1*, was consistently detected in all three environments, with LOD scores of 3.88–5.72 and PVE of 5.4% − 9.4%. The additive effect of *qSRA05.1* ranged from − 3.12 to -2.91, suggesting that resistance allele was derived from the resistant parent ZH212. *qSRA05.1* spanned a 3.98 Mb physical interval flanked by makers of C05b001 and C05b013. The other two QTLs, *qSRA04* and *qSRA05.2*, had LOD scores of 5.96 and 4.25 and explained 5.7% and 8.1% of the phenotypic variation, respectively. These two QTLs were environment-specific minor-effect QTLs and were identified in only one environment (Table 4). The resistant alleles for these two QTLs were − 3.63 and − 3.06, respectively, implying the resistance alleles also derived from the resistance parent ZH212. *qSRA04* was located in the physical

interval 118.03–120.64 Mb, flanked by the markers C04b122 and C04b132. The physical interval of *qSRA05.2* was 113.17–113.90 Mb, flanked by makers C05b222 and C05b232.

**Putative candidate gene for QTL qSRA05.1**

Within the physical interval of the candidate region for qSRA05.1 on chromosome A05, a total of 702 SNPs and 93 InDels were identified. Genomic annotation indicated that 426 SNPs and 59 InDels were located in intergenic regions, 156 SNPs and 24 InDels were located within genic regions, and the remaining 120 SNPs and 10 InDels were distributed in upstream and downstream regulatory regions of genes (Table S3).

Based on the positions of the two flanking markers (C05b001 and C05b013), the physical interval of the *qSRA05.1* locus spanned 6.33–10.31 Mb on chromosome A05. A total of 288 genes resided in the QTL interval, 112 of which harbored SNPs according to the gene annotation in the reference genome (Table S4). Among these SNPs-carrying genes, 26 possessed exonic variants: one with a stop-gain variant, one with a frameshift insertion, one with a frameshift deletion, ten harboring nonsynonymous SNPs, and the remainder carrying synonymous SNPs. According to the annotation of the genes in the *qSRA05.1* interval, several candidate genes associated with fungal disease resistance were identified, including receptor-like protein kinase 1, peroxidase superfamily protein, cytochrome P450 superfamily protein, AP2-like ethylene-responsive transcription factor and phloem A10-like protein (Table S5).

To further identify genes associated with resistance to *A. rolfsii* infection, transcript expression patterns were analyzed by comparing gene expression between the resistant parent ZH212 and the susceptible parent 07-3105. The symptoms of ZH212 differed from those of the susceptible parent 07-3105. The disease rating of ZH212 was lower than that of the susceptible parent 07-3105 at all three sampling time points (Fig. 3a).

The transcriptomic data yielded a total of 10.67 Gb clean data and 0.35 billion clean reads, and 69.74% to 96.92% of clean reads were successfully mapped to the reference genome (Table S6). Principal component analysis (PCA) was performed based on the normalized gene expression data of three biological replicates at each sampling time point to assess the overall transcriptomic similarity and divergence among samples. The first two principal components (PC1 and PC2) explained 74% and 15% of the total variance, respectively. Samples of ZH212 at the three time points were closely distributed with limited temporal separation, and they were distant from the control group. The three time-point samples of 07-3105 also clustered tightly and separated from the control. Furthermore, ZH212 and 07-3105 clusters were well resolved from each other, demonstrating distinct transcriptional signatures between the two genotypes (Fig. 3b). Differentially expressed genes (DEGs) were identified between the two parents at different time points. A total of 1,728 DEGs were detected at 0 h, including 991 upregulated and 737 downregulated genes. At 72 hpi, 2,724 DEGs were obtained, with 1,554 upregulated and 1,170 downregulated genes. At 96 hpi, 1,273 DEGs were identified, of which 874 were upregulated and 399 were downregulated. At 120 hpi, 2,420 DEGs were found, consisting of 1,233 upregulated and 1,187 downregulated genes (Fig. 3c). WGCNA was performed using genes with FPKM > 1; the results

revealed a scale-free network with 18 co-expression modules. The number of genes among 18 modules ranged from 36 to 10,907. To characterize the key modules associated with DI of peanut stem rot in the two parents, the module-trait relationships (MTRs) were assessed. As shown in Fig. 3d, the yellow ($r$ = 0.93, $p < 0.001$) and salmon ($r$ = 0.79, $p < 0.05$) modules were significantly positively correlated with DI, while the turquoise ($r$ = − 0.73, $p < 0.05$) and the lightgreen ($r$ = − 0.72, $p < 0.05$) modules was significantly negatively correlated with DI.

Of the 288 genes located in the *qSRA05.1* interval, 134 genes were expressed, while only 17 genes were differentially expressed between the resistant and susceptible parents at 72 hpi, 96 hpi and 120 hpi (Fig. 3e). Specifically, Ten DEGs (five upregulated and five downregulated) were detected at 72 hpi, six DEGs (one upregulated and five downregulated) at 96 hpi, and twelve DEGs (nine upregulated and three downregulated) at 120 hpi. No upregulated DEGs overlapped among all three time points. Further SNP annotation of these upregulated DEGs revealed no exonic variations, while the detected SNPs were distributed in upstream, 3' UTR, and downstream. Among them, *Arahy.0K456L* and *Arahy.F6Z6ZJ* both encode cytochrome P450 superfamily protein and contain a SNP within the upstream and 3’ UTR, respectively (Table S7).

To further identify resistance-associated genes induced by pathogen infection, the transcriptomic profiles of the resistant parent at 72, 96, and 120 hpi were compared with those at 0 hpi control. A total of 18 infection-responsive DEGs were identified, including 15 DEGs (six upregulated and nine downregulated) at 72 hpi, 16 DEGs (seven upregulated and nine downregulated) at 96 hpi, and 14 DEGs (seven upregulated and seven downregulated) at 120 hpi. Six genes (*Arahy.SR30KW, Arahy.TQQ6DC, Arahy.F2XVK1, Arahy.LW6UI8, Arahy. VPW2G6, and Arahy.P6VX4D*) were consistently upregulated across all three inoculation time points, *Arahy.SR30KW* encoded a peroxidase superfamily protein with an intergenic SNP. *Arahy.LW6UI8, Arahy.VPW2G6* and *Arahy.F2XVK1*, annotated as cytochrome P450 superfamily proteins, *arahy.LW6UI8* and *Arahy.VPW2G6* both harbored a nonsynonymous SNP, while *arahy.F2XVK1* possessed a synonymous SNP (Fig. 3f, Table S8).

# Validation of QTL effect in RIL population and natural germplasm panel

To assess the potential utility of three DEGs carrying exonic SNPs within the *qSRA05.1* interval for stem rot resistance breeding, three SNP markers (A05-7583678, A05-9189944, and A05-9221214) located in *Arahy.LW6UI8*, *Arahy.VPW2G6*, and *Arahy.F2XVK1*, respectively, were selected for KASP marker development. A05-9189944 was succeeded, while A05-7583678, A05-9221214 failed. The KASP marker A05-9189944 showed high resolution in distinguishing lines within the RIL population (Fig. S5). The elite allele of *qSRA05.1* derived from the resistant parent ZH212 was designated as the “CC” genotype, whereas the alternative allele from susceptible parent 07-3105 was defined as the “GG” genotype. Genotypic comparison within the RIL population with A05-9189944 revealed that lines carrying the “CC” genotype had a significantly lower disease index than those homozygous for “GG”. Relative to the “GG”

genotype, the “CC” genotype showed disease index decreases of 6.75, 5.31, and 5.72 in YL2022, YL2023, and WC2024, respectively (Fig. 4a).

Marker A05-9189944 was subsequently used to genotype a panel of 202 germplasm accessions previously reported by Yan et al. (2023). As described above, the elite allele was designated as “CC”, whereas its homozygous allele was defined as the “GG”. Among these peanut accessions, 151 accessions carried the “GG” genotype, 44 accessions harbored the “CC” genotype, two accessions had heterozygous genotype and five accessions carried missing SNP data (Table S9). Consistent with the results in RIL population, accessions harboring the “CC” genotype exhibited reduced disease indices by 6.84, and 5.21 relative to those with the “GG” genotype across WC2025 and YL2025, respectively (Fig. 4b). Collectively, these results showed that the “CC” genotype confers enhanced stem rot resistance across diverse genetic backgrounds. Among accessions carrying the CC genotype, the panel included Spanish-type, Virginia-type, and intermediate-type accessions (Table S9), 93.18% of these accessions were Spanish-type. This finding suggest that most Chinese accessions carrying the resistance-associated CC genotype belonged to the Spanish type. The developed KASP marker was validated as efficient and showed considerable potential for marker-assisted selection in breeding peanut varieties resistant to stem rot.

# Discussion

Stem rot, caused by the necrotrophic fungus *Agroathelia rolfsii*, is one of the most destructive soil-borne diseases that severely limit peanut yield and quality worldwide. Breeding and cultivating resistant cultivars represents the most economical, sustainable, and environmentally friendly strategy for managing this disease. However, the genetic dissection of peanut stem rot resistance remains challenging due to its quantitative inheritance, strong environmental plasticity, and the limited resolution of traditional genetic maps. In the present study, we constructed a high-density genetic map via whole-genome resequencing using an RIL population derived from a cross between two Spanish-type peanut parents and identified a stable and reproducible QTL *qSRA05.1* conferring stem rot resistance. Integrated with transcriptome profiling of resistant and susceptible parental lines, candidate resistance genes showing significantly upregulated expression were identified within the stable QTL interval. Furthermore, the KASP marker A05-9189944 was successfully developed based on SNPs in the *qSRA05.1* QTL region and validated in both the RIL mapping population and a natural germplasm population. These integrated genomic and transcriptomic results provide robust genetic resources and molecular tools for dissecting the molecular mechanisms of peanut stem rot resistance and accelerating marker-assisted breeding of peanut.

High-density genetic mapping is a prerequisite for the precise QTL localization and candidate gene mining for complex quantitative traits. Previous studies on QTL mapping for peanut stem rot resistance QTL mapping mainly relied on low-throughput molecular markers, such as simple sequence repeat (SSR), and low-density of SNP markers. For example, Bera et al. (2016) constructed a genetic linkage map with 22 SSR markers. Dodia et al. (2019) constructed a genetic linkage map comprising 585 SNP

markers. Luo et al. (2020) constructed a linkage map integrating 1,451 SNPs and 73 SSR markers. Agmon et al. (2022) reported a genetic map consisting of 1,833 SNP markers, and Tsai et al. (2024). generated a linkage map with 1,926 SNP markers for peanut stem rot resistance studies. In this study, we constructed a high-resolution genetic map with 4,448 bin markers encompassing 275,784 genomic variants. The high-density map generated in this present study provides a more solid genomic basis for fine-scale identification and functional validation of QTLs conferring stem rot resistance in peanut.

Through a comparative genomic analysis of previously reported peanut stem rot resistance loci and the QTLs identified in this study, we found that QTL *qSRA04* identified in here overlapped in genomic interval with a previously reported resistance locus. The previously reported SNP marker A04-120110031, which is associated with stem rot resistance (Yan et al. 2023), was located within the physical interval of *qSRA04* (118.03–120.64 Mb) detected in the current work, suggesting that *qSRA04* is a stable and reliable locus for marker-assisted selection. In contrast, the other two QTLs on chromosome A05 were confirmed as novel resistance loci. Previous studies identified a consensus stem rot resistance QTL on chromosome A05, within a physical interval of 1.4–1.8 Mb by Cui et al. (2020) and Luo et al. (2020). Significant physical distance exists between this previously reported locus and the two A05 QTLs identified in our study, *qSRA05.1* located within 6.33–10.31 Mb interval and *qSRA05.2* within 113.17–113.90 Mb interval, verifying the novelty of these two resistance loci. Among the three identified QTLs in this study, *qSRA05.1*, which was stably and repeatedly detected across all three experimental environments with a PVE of 5.4% − 9.4%, is a stable resistance locus with considerable potential for future breeding of resistant peanut cultivars.

Notably, the RIL population utilized in the present study was developed from two Spanish-type peanut accessions, indicating that the newly detected stem rot resistance QTLs are genetically derived from Spanish-type peanuts. To date, most documented stem rot resistance QTLs have been identified in Virginia-type peanut cultivars (Cui et al. 2020; Luo et al. 2020; Agmon et al. 2022; Veerendrakumar et al. 2025), wild *Arachis species* (Tsai et al. 2024), and interspecific progeny derived from crosses between wild and cultivated *Arachis* accessions (Bera et al. 2016; Dodia et al. 2019). In contrast, the genetic architecture and resistance loci underlying stem rot resistance in Spanish-type peanut remain largely unexplored and underutilized in peanut resistance breeding. Of particular importance, the stable stem rot resistance QTL *qSRA05.1* identified in our study fills this research gap, expands the existing pool of peanut disease-resistance genetic resources, and provides a valuable novel locus for improving stem rot resistance in peanut breeding programs.

Integration of QTL mapping and transcriptome profiling represents a powerful and efficient strategy for mining functional candidate genes responsible for quantitative disease resistance in crops (Huai et al. 2024; Ontoy et al. 2026). In this study, comparative transcriptomic analysis between resistant and susceptible peanut parents demonstrated that multiple genes located within the stable stem rot resistance QTL *qSRA05.1* were significantly upregulated in the resistant genotypes upon *A. rolfsii* infection, suggesting their critical involvement in peanut defense responses against stem rot disease. Notably, several cytochrome P450 (CYP) family genes exhibited marked pathogen-induced upregulation

within the *qSRA05.1* interval, and this upregulation was tightly associated with stem rot resistance. The co-occurrence of genetically mapped resistance loci and pathogen-responsive differential gene expression strongly supports the conclusion that P450 superfamily members serve as core functional candidates underlying this quantitative resistance locus. Plant cytochrome P450 proteins are well-established key regulators of defense against diverse necrotrophic pathogens across plant species. For instance, heterologous overexpression of the soybean gene *GmCYP82A3* in tobacco significantly enhanced plant resistance to *Botrytis cinerea* and *Phytophthora parasitica* (Yan et al. 2016). Tandem-duplicated P450 genes located in the blackleg resistance QTL *BLMR2* have been proven to mediate plant basal defense against fungal infection (Zhang et al. 2021). The rice P450 gene *BSR2* (*CYP78A*) confers broad-spectrum resistance to *Rhizoctonia solani* (Maeda et al. 2019), and cotton *GhP450-94C1* acts as a positive regulator of resistance to Verticillium wilt (Hu et al. 2022). Consistently, the sustained transcriptional upregulation of the CYP genes identified in the present study strongly implies their potential role as positive regulators of peanut stem rot resistance. Nevertheless, further genetic functional validation, including gene overexpression and gene silencing assays, is essential to elucidate the precise biological functions and molecular mechanisms of these P450 genes in peanut defense against this destructive necrotrophic pathogen.

KASP technology has been widely applied in crop molecular breeding due to its high efficiency, accuracy, and low cost in SNP genotyping. In this study, we developed the locus-specific KASP marker A05-9189944 based on key SNPs in the stable resistance QTL interval and validated it in two populations: the RIL population and natural germplasm population. The results showed that the developed KASP A05-9189944 marker had high genotyping accuracy and significant phenotypic association efficiency in both populations, indicating that these markers are tightly linked to stem rot resistance and has considerable application potential. Most previously reported QTLs for peanut stem rot resistance lack effective markers and large-scale validation, which has limited their practical use in breeding. The successfully validated KASP markers in this study fills the gap in functional molecular markers for Spanish-type peanut stem rot resistance loci, and can be directly used for marker-assisted selection, germplasm resistance identification, and pyramiding breeding of southern blight resistance in peanut.

In summary, the present study integrated high-throughput whole-genome resequencing-based QTL mapping with comparative transcriptome analysis to dissect the genetic basis of stem rot resistance in Spanish-type peanut. We successfully identified a stable and novel resistance QTL *qSRA05.1*, that is distinct from previously reported resistance loci derived from Virginia-type and wild peanut backgrounds, filling a critical gap in the genetic resource repository of peanut stem rot resistance. Transcriptomic profiling further pinpointed pathogen-inducible cytochrome P450 family genes within *qSRA05.1*, which represent promising candidate genes and are consistent with the conserved defensive roles of P450 proteins against necrotrophic fungal pathogens in multiple plant species. Moreover, the locus-specific KASP marker A05-9189944 developed from trait-linked SNPs exhibited high genotyping accuracy and a reliable association with phenotype in both the RIL segregating population and a natural germplasm population, providing a robust and practical molecular tool for practical resistance breeding. Compared with previous studies relying on single genetic mapping or transcriptomic analysis, our multi-omics

strategy combined stable genetic loci, functional candidate genes, and validated molecular markers to systematically elucidate the quantitative resistance mechanisms underlying peanut stem rot disease. The novel QTL resource, candidate defense genes, and functional KASP marker identified in this study provide valuable insights into the molecular regulatory mechanisms of peanut–*A. rolfsii* interaction and lay a solid foundation for future functional validation of genes and marker-assisted pyramiding breeding for durable stem rot resistance in peanut.

## Declarations

### Conflict of interest

The authors declare no conflict of interest.

### Ethical approval

The authors state that all experiments in the study comply with the ethical standards.

# Supplementary information

Table S1 Details of WGRS quality, coverage, and depth for RILs and their parents

Table S2 Detail information on genome-wide variants

Table S3 Variants within the candidate physical interval of *qSRA05.1*

Table S4 Detail information of genes carrying variants within the physical interval of *qSRA05.1*

Table S5 Detail information of genes carrying exonic variants within *qSRA05.1* interval

Table S6 Summary of quality control of RNA-seq data of resistant and susceptible parents

Table S7 Differentially expressed genes (DEGs) that differ between resistant and susceptible parents

Table S8 Differentially expressed genes (DEGs) within *qSRA05.1* carrying SNPs that differ between the infection stages and control

Table S9 Genotypes of accessions in natural peanut panel distinguished by KASP marker A05-9189944.

# Funding

This work was supported by the National Key R&D Program of China (2023YFD1202800), the Hubei Provincial Key R&D program (2026BBB012), the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Science (CAAS-ASTIP-2026-OCRI), Central Public-interest

Scientific Institution Basal Research Fund (1610172024001), the earmarked fund for CARS-13 and Innovation Team of Hubei Agricultural Science and Technology Innovation Center (2026620000001031).

# Author Contribution

L.Y, and Y.L conceived and designed the experiments. L.Y, and D.Y analyzed the data and performed the bioinformatics analysis. D.Y, W.S, X.L, Y.K and Q.W performed the experiments. Y.L and L.Y wrote the manuscript. Z.W, D.H, Y.C, X.W revised the manuscript and improved the English writing. All authors read and approved the final manuscript.

# Acknowledgments

Not applicable

# Data Availability

The clean re-sequencing data of the RIL population and transcritptome data in this study are available at the BioProject database at https://ngdc.cncb.ac.cn/ under the BioProject ID: PRJCA074400 and PRJCA075248

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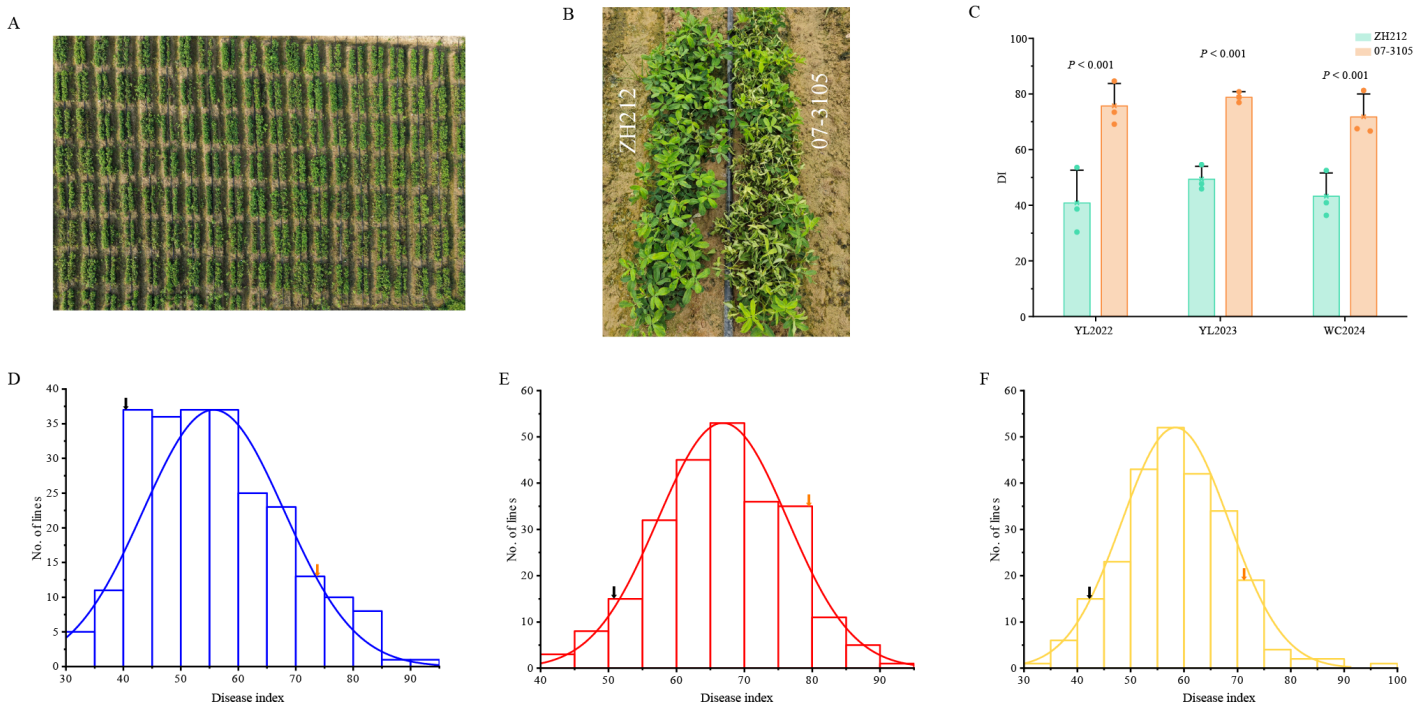
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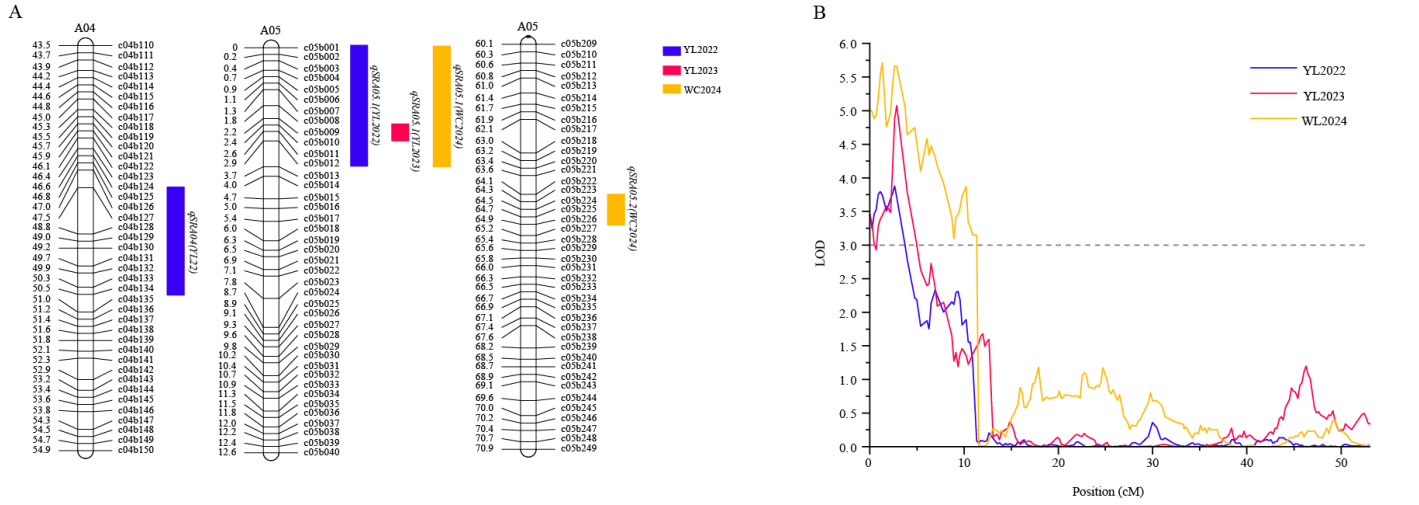
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# Figures




## Figure 1

Symptom and phenotypic variation of disease index in the two parents and RIL population across three environments.




## Figure 2

QTL for peanut stem rot resistance identified across three environments

A, Whole genome QTL for peanut stem rot resistance detected using QTL Cartographer in the RIL population. B, Distribution of QTLs for peanut stem rot resistance on A04 and A05 chromosomes across three environments

*YL2022* phenotypic data collected in 2022 at Yangluo experiment station, *YL2023* phenotypic data collected in 2023 at Yangluo experiment station, *WC2024* phenotypic data collected in 2024 at Wuchang experiment station.

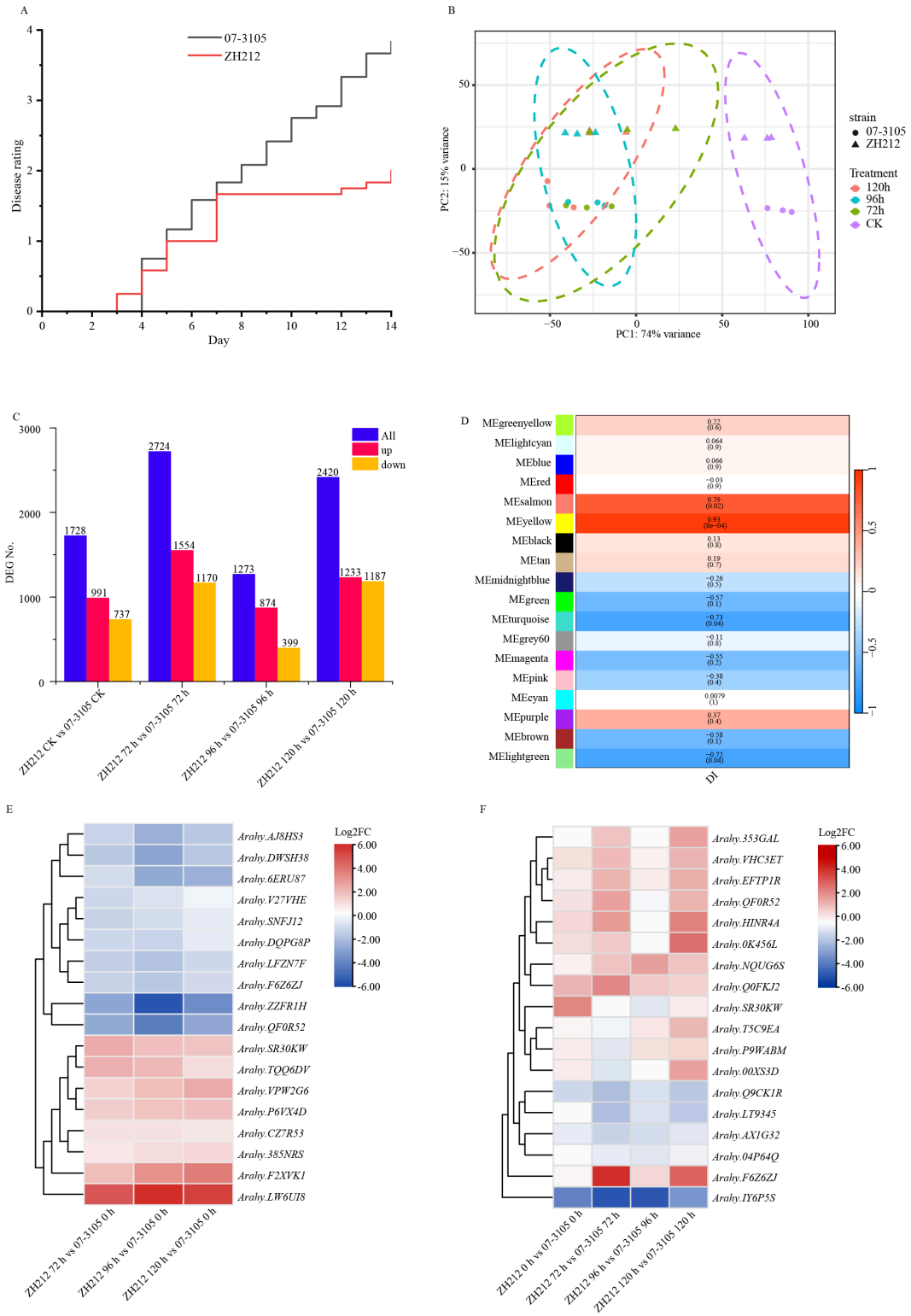



**Figure 3**

Analysis of disease index and transcriptome profiles in stems infected by *A. rolfsii* in ZH212 and 07-3105.

A, Disease index variation with time in ZH212 and 07-3105. B, PCA analysis of those stem samples of ZH212 and 07-3105 collected at 0, 72, 96 and 120 hpi. C, the number of upregulated and downregulated genes in ZH212 as compared with 07-3105. D, correlation analysis between co-expression modules and disease index. Boxes contained Pearson correlation coefficients and *P* values. E, Heat map DEGs revolved in stem rot resistance between ZH212 and 07-3105 in *qSRA05.1*. The color indicates the $log_2$fold-change of gene expression value in ZH212 to that in 07-3105 at the same infection stage. Bold indicated upregulated genes within *qSRA05.1* carrying variation. F, Heat map DEGs, within *qSRA05.1* harboring SNP, revolved in stem rot resistance between ZH212 at different stage to control. The color indicates the $log_2$fold-change of gene expression value in ZH212 72, 96, 120 hpi to that in ZH212 at 0 hpi.

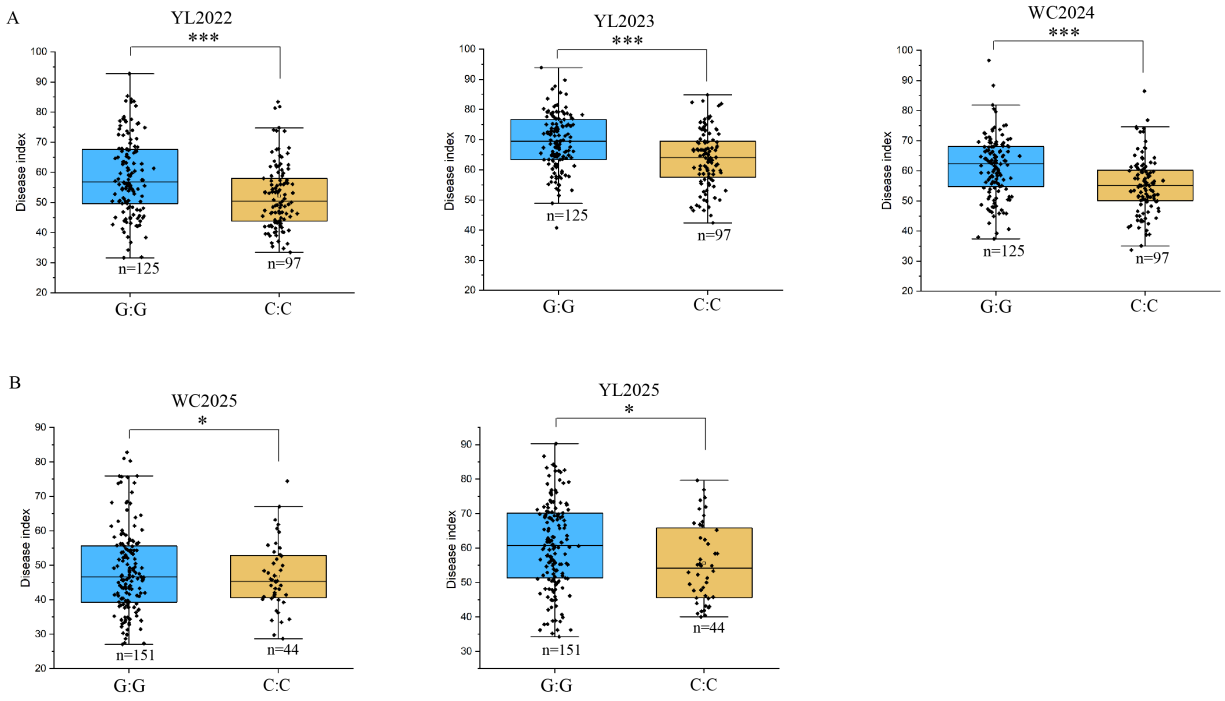



**Figure 4**

Phenotypic differences between two genotypes with KASP marker A05-9189944 for RIL population and peanut germplasm accessions. A Phenotypic difference of between two genotypes for 242 lines in RIL population. B Phenotypic difference between two genotypes for 195 peanut germplasms.

The alleles of *qSRA05.1* derived from ZH212 were designated as “CC”, while the alleles from 07-3105 was designated as “GG”, respectively. ****P*< 0.001.

## Supplementary Files

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

- SupplementaryFigures.docx
- attachmentTablefiles.xlsx
- Table1PhenotypicvariationofRILstostemrotinfectioninfiveenviroments.xlsx
- Table4QTLsidentifiedbyQTLmappingacrossthreeenvironments.xlsx
- Table2Analysisofvarianceforstemrotresistanceacrossmultipleenvironments.xlsx
- Table3Detailinformationofbinsonthelinkagegroups.xlsx