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Identification of Quantitative Trait Loci Conferring Resistance to *Rotylenchulus reniformis* in Soybean Germplasm from Maturity Groups IV and V

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Abstract

Key message: In a genome-wide association study of 202 soybean accessions, three SNPs and five candidate genes associated with *Rotylenchulus reniformis* resistance were identified on chromosome 18, providing resources for resistance breeding.

Abstract: *Rotylenchulus reniformis* is an economically important nematode of soybean in the southern United States. While host resistance is one of the most effective management strategies, the genetic basis of resistance remains poorly understood, and only a few resistant soybean varieties have been developed. The aim of this study was to utilize a genome-wide association study (GWAS) to identify genomic regions associated with *R. reniformis* resistance in soybean germplasm from maturity groups IV and V. A panel of 202 soybean accessions was grown under controlled greenhouse conditions in 2024 and 2025. Resistance was assessed using nematode reproduction index (RI = P_f/P_i; P_f, final nematode population density; P_i, initial inoculation density). Best linear unbiased predictions (BLUPs) were estimated across years for GWAS analysis. Significant phenotypic variation for *R. reniformis* resistance was observed among the soybean accessions, with BLUP-derived RI values displaying a continuous distribution

consistent with quantitative inheritance. The GWAS analysis utilizing 34,303 SNP markers identified three significant SNPs associated with resistance on chromosome 18. Allelic analysis revealed that accessions carrying the favorable allele at SNP ss715632853 exhibited reduced nematode reproduction. Candidate gene analysis identified genes associated with plant defense, signaling, and metabolic processes. The chromosome 18 region identified in this study represents a candidate region associated with resistance to *R. reniformis,* providing molecular resources for soybean improvement. These findings improve the understanding of the genetic basis of *R. reniformis* resistance and will facilitate the development of resistant soybean varieties through marker-assisted and genomic selection approaches.

Keywords: *Glycine max*, *Rotylenchulus reniformis*, Genome-wide association study, quantitative trait loci, host resistance, Single-nucleotide polymorphism

1 Introduction

Soybean (*Glycine max* [L.] Merr.) is among the most economically important crops in Louisiana. Soybean production faces several biotic constraints, such as insect pests, pathogens, and plant-parasitic nematodes, which account for substantial crop yield reductions (Temple et al. 2013; Allen et al. 2021; Crop Protection Network 2025). Among these, plant-parasitic nematodes remain a major yield-limiting factor in soybean production systems across the United States. In the southern United States, the most economically important nematodes affecting soybean production include the soybean cyst nematode (*Heterodera glycines*), southern root-knot nematode (*Meloidogyne incognita*), and reniform nematode (*Rotylenchulus reniformis*) (Allen et al. 2021; Sikora 2021). Among these nematodes, *R. reniformis* is recognized as an important constraint in Louisiana soybean fields (Overstreet 2009; Overstreet and McGawley 1998, 2000).

Recently, yield losses due to *R. reniformis* in Louisiana alone have been estimated at 1.25%, which translated into a loss of $5,790,909 in 2025 (Crop Protection Network 2025).

The use of resistant soybean varieties is one of the most effective management strategies for *R. reniformis*-infested soybean fields (Kiarie et al. 2025). However, only a few resistant commercial soybean varieties are currently available. Quantitative trait loci (QTL) associated with resistance to *R. reniformis* have been mapped across the soybean genome, suggesting a quantitative nature of resistance. Major QTL have been previously reported on chromosomes 11 and 18 (Ha et al. 2007; Jiao et al. 2015; Lee et al. 2016; Wilkes et al. 2020). Genome-wide association studies (GWAS) have become a powerful tool for identifying genomic loci associated with complex traits such as nematode resistance in soybean. Numerous GWAS have successfully identified loci associated with resistance to *H. glycines*, revealing both major genes and minor-effect QTL distributed across the soybean genome (Han et al. 2015; Vuong et al. 2015; Zhang et al. 2017; Tran et al. 2019). In addition, GWAS approaches have also been used to investigate the genetic basis of resistance to *M. incognita*, with significant marker-trait associations identified in diverse soybean germplasm (Passianotto et al. 2017). However, in contrast to the extensive body of work on *H. glycines*, relatively few GWAS have focused on resistance to *R. reniformis*. Therefore, given the limited understanding of the genetic basis of resistance to *R. reniformis*, the use of GWAS in diverse soybean germplasm provides an opportunity to identify novel loci associated with resistance. The objective of this study was to identify genomic regions associated with resistance to *R. reniformis* in soybean germplasm from maturity groups IV and V using a genome-wide association approach. Identifying such loci will help facilitate the development of resistant soybean varieties and support long-term management of *R. reniformis* in infested soybean fields.

2 Materials and Method

2.1 Plant material

A total of 202 geographically diverse soybean germplasm from maturity groups IV and V were selected from the United States Department of Agriculture, Agricultural Research Service (USDA, ARS), Soybean Germplasm Collection available through GRIN-Global (https://npgsweb.ars-grin.gov/gringlobal/) for greenhouse phenotyping against *R. reniformis*. Most of these accessions originated from China, Japan, and Korea (Table S1). Soybean lines PI 548655 (Forrest) and PI 543795 (Hartwig) were included as resistant checks, whereas PI 54865 (Braxton) and PI 595362 (Magellan) were used as susceptible checks (Robbins et al. 2002; Jiao et al. 2015; Usovsky et al. 2021).

2.2 Source of inoculum and soil preparation

A population of *R. reniformis* was collected from a cotton field at the Northeast Research Station, Louisiana. Nematodes were isolated from soil samples and maintained on tomato cultivar 'Rutgers' under greenhouse conditions for six months prior to inoculation. Mixed vermiform stages of *R. reniformis* were extracted from soil using the modified Baermann tray technique and incubated in tap water for 48 h at room temperature (Viaene et al. 2021). The nematodes were collected using a 25 μm sieve (No. 500), and the suspension was transferred into 1000 mL Corning® PS storage bottles following the same-day inoculation.

The field soil (loam and sand) collected from the LSU AgCenter Doyle Chambers Research Station in Baton Rouge, Louisiana, was autoclaved at 121°C for 90 min. A 1:1 soil mixture consisting of autoclaved loam soil and sand was prepared, and 500 cm^3 of the mixture was filled into 0.5-gallon square plastic pots (12.7 cm top diameter × 14.6 cm height).

2.3 Phenotyping soybean resistance to *Rotylenchulus reniformis*

Greenhouse experiments were conducted in 2024 and 2025 at the LSU AgCenter Plant Materials Center in Baton Rouge, Louisiana. Greenhouse temperatures were maintained between 28 and 34°C. One seed from each soybean genotype, including resistant and susceptible checks, was planted in the center of each pot. Each genotype was replicated three times in a randomized complete block design (RCBD). Plants were inoculated at the V2 growth stage (second trifoliolate leaf) with 1,000 mixed vermiform stages of *R. reniformis* suspended in 10 mL of water. The inoculum was applied into two holes approximately 2-3 cm deep around the stem base using a pipette. The holes were covered immediately with soil, followed by light watering to facilitate nematode distribution in the pot. Plants were hand-watered as needed to maintain appropriate moisture content for proper plant growth and nematode reproduction. Plants were fertilized weekly throughout the study.

Twelve weeks after inoculation, watering was stopped for one week before experiment termination. The mineral and organic fractions from each pot were separated, and a homogenized 250 cm^3 soil subsample was collected into Ziploc® bags and stored in a cold room before nematode extraction. Nematodes were extracted using a semi-automatic elutriator followed by sucrose centrifugal flotation (Jenkins 1964; Byrd et al. 1976). Extracted nematodes were stored in 20 mL glass vials at 4°C and counted under a compound microscope at 40x magnification to estimate the final population densities.

2.4 Reproduction index, normalization, and resistance rating

The reproduction index (RI) was calculated as: $RI = P_f / P_i$, where P_f represents final nematode density, and P_i is the initial inoculation density (Oostenbrink 1966). The RI values were calculated from the average of three replicates for each soybean accession. To improve normality, RI values were transformed ($\log_{10}$ (x). Because experiments were conducted across

two years (2024 and 2025), best linear unbiased predictions (BLUPs) were estimated using the linear mixed model:$y = X\beta + Zu + e$ (Henderson 1975; Panter Jr 1991). In this model, y represents the vector of observed RI values, X is the design matrix for the fixed effects, β is the vector of fixed effects (overall mean and year effects), Z is the design matrix for random effects, u is the vector for the random genetic effects associated with soybean genotypes, and e represents the residual error. The BLUPs were used to obtain adjusted phenotypic estimates across years by minimizing environmental variation.

The resistance rating was based on the baseline RI (RI = 1); accessions with RI < 1 were classified as resistant, while those with RI > 1 were classified as susceptible. Since RI values were $\log_{10}$-transformed prior to analysis, the threshold of RI = 1 corresponded to 0 on the transformed scale; therefore, transformed values below 0 were considered resistant, whereas values above 0 were considered susceptible. The RI calculations were performed in Microsoft Excel, while log transformation and BLUP analyses were conducted using R version 4.3.2 (R Foundation for Statistical Computing) in RStudio software.

2.5 Genotypic data, quality control, and association analysis

Single-nucleotide polymorphism (SNP) genotypic data for 202 soybean germplasm were obtained from the Illumina Infinium SoySNP50k iSelect BeadChip (Song et al. 2013; Song et al. 2015) available through the Soybase database (https://www.soybase.org/tools/snp50k/). The quality control filtering was performed using TASSEL software version 5.0 (Bradbury et al. 2007). The SNP markers with more than 10% missing data and minor allele frequency (MAF) values less than 0.05 (< 0.05) were removed from further analysis. Following quality control filtering, 34,303 SNPs were retained and used for subsequent genome-wide association analysis.

Genome-wide association analysis was performed in TASSEL using both the general linear model (GLM) and the mixed linear model (MLM). In the GLM, principal component analysis (PCA) was included as a covariate to account for population structure, while the MLM incorporated both PCA and the kinship matrix to account for population structure and genetic relatedness among accessions (Bradbury et al. 2007). The GLM and MLM outputs were subsequently used for further analysis in RStudio software. The Manhattan and quantile-quantile (QQ) plots were plotted in R using the qqman package (Turner 2018). A significance threshold of $-\log_{10}(P\text{-value}) = 4$ was used to identify significant SNP markers associated with resistance to *R. reniformis*, consistent with thresholds used in previous GWAS studies (Jiang et al. 2023; Yu et al. 2022; Pruthi et al. 2024). Candidate genes within ±50 kilobase (kb) flanking region surrounding significant SNPs, following the approach described by Zhang et al. (2016) and Shi et al. (2025), were identified based on the *G. max* reference genome Williams 82 assembly Wm82.a2.v1 available in SoyBase (https://www.soybase.org). Functional annotations of these candidate genes were further examined to assess their potential biological relevance in *R. reniformis* resistance. Allelic effects of significant SNP loci were evaluated by comparing BLUP-based $\log_{10}$-transformed RI values between allelic groups and visualized using boxplots. Linkage disequilibrium (LD) analysis and LD heatmap visualization were performed using Haploview software version 4.2 (Barrett et al. 2005).

3 Results

3.1 Reproduction index of *Rotylenchulus reniformis* and distribution across soybean accessions

Significant variation in the reproduction index (RI) was observed among the 202 soybean accessions evaluated (Fig. 1). The BLUP-based $\log_{10}$-transformed RI values ranged from

approximately −0.4 to 0.4. Both resistant and susceptible accessions were observed within the population, with values distributed on both sides of the resistance threshold of 0 on the $\log_{10}$-transformed scale.

3.2 Genome-wide association analysis of *Rotylenchulus reniformis* resistance

The Manhattan plot derived from the mixed linear model (MLM) revealed significant single-nucleotide polymorphism (SNP) markers on chromosome 18 associated with resistance to *R. reniformis* (Fig. 2a). Three significant SNPs exceeded the genome-wide significance threshold ($-\log_{10} P > 4$): ss715632853, ss715632857, and ss715632859. The two significant SNPs, ss715632857 and ss715632859, were located closely together on the chromosome and therefore appeared as a single peak in the plot. The quantile-quantile plot (QQ plot) for MLM showed that the observed $-\log_{10}(P)$ values followed the expected distribution (Fig. 2b). A slight upward deviation was observed from the diagonal line at higher $-\log_{10}(P)$ values (around 3.5), whereas most markers closely followed the expected distribution.

3.3 Genomic associated region and linkage disequilibrium

Regional association analysis was performed to characterize the genomic area surrounding the significant SNP markers on chromosome 18 associated with *R. reniformis* resistance. Three significant SNP markers were identified at physical positions 9,066,013 bp (ss715632853), 9,100,326 bp (ss715632857), and 9,114,287 bp (ss715632859) (Fig. 3a, Table 1). All three markers showed significant association, with *P*-values ranging from 4.99×10^{-5} to 8.85×10^{-5} ($-\log_{10}(P) = 4.05 – 4.30$). Each SNP explained between 8% and 9% of the phenotypic variation ($R^2 = 0.08 – 0.09$) (Table 1). Linkage disequilibrium (LD) analysis revealed that these markers (ss715632853, ss715632857, and ss715632859) were in strong LD, forming a single 88 kb LD

block. Pairwise LD relationships between the three markers are illustrated in the heatmap in Fig. 3b.

3.4 Allelic effects and candidate genes in the associated region

The allelic effects of the three significant SNP loci on chromosome 18 were determined by comparing the BLUP-based $\log_{10}$-transformed reproduction index (RI) values between allelic groups (Fig. 4). A significant difference in RI was observed between two alleles of SNP ss715632853 (A/G; $P = 0.0004$), where accessions carrying allele A had lower RI compared to accessions carrying allele G. In contrast, no significant differences were found between the alleles of SNP ss715632857 (C/T; $P = 0.0729$) and SNP ss715632859 (A/G; $P = 0.0572$).

Candidate genes within a ±50 kb window flanking the significant SNP were identified using the *G. max* Williams 82 reference genome (Wm82.a2.v1). A total of five candidate genes were located within this region on chromosome 18: *Glyma.18G090900*, *Glyma.18G091000*, *Glyma.18G091100*, *Glyma.18G091200*, and *Glyma.18G091300*. Their corresponding functional annotations are provided in Table 2.

4 Discussion

Although *R. reniformis* is among the most problematic nematodes for soybean production in the southern United States, the genetic architecture of resistance to this pathogen remains poorly understood. To bridge this knowledge gap, we evaluated 202 diverse soybean germplasm accessions from maturity groups IV and V and performed a GWAS to identify loci associated with resistance. The wide variation in the reproduction index among accessions suggested that resistance is quantitative. Furthermore, the RI values exhibited a nearly continuous distribution, even after $\log_{10}$ transformation, consistent with resistance being controlled by multiple loci with small-to-moderate effects. These results are in agreement with the previous studies that reported

quantitative resistance to *R. reniformis* in soybean (Ha et al. 2007; Jiao et al. 2015; Lee et al. 2016). Overall, highly resistant accessions were relatively uncommon within the germplasm tested, highlighting the importance of continued screening efforts to identify additional sources of resistance for soybean improvement.

Three significant SNPs associated with resistance to *R. reniformis* in the present study were localized within a narrow interval (approximately 9.06 to 9.11 Mb) on chromosome 18. Chromosome 18 (linkage group G) is widely recognized as a genomic region enriched with disease resistance loci in soybean. It harbors resistance hotspots against various pathogens, including nematodes such as *Heterodera glycines* (Webb et al. 1995; Concibido et al. 1996, 1997; Chang et al. 1997; Cregan et al. 1999b) and *Meloidogyne incognita* (Tamulonis et al. 1997), as well as the fungus *Fusarium solani* f. sp. *glycines* (Njiti et al. 1998). Additionally, major loci controlling *R. reniformis* resistance have independently been mapped to chromosome 11 (LG B1) and chromosome 18 (LG G) (Ha et al. 2007; Jiao et al. 2015; Lee et al. 2016; Wilkes et al. 2020). Although the specific locus detected in our GWAS does not overlap with *rhg1*, an extensively studied locus for *H. glycines* resistance (Cook et al. 2012, 2014; Lee et al. 2015; Patil et al. 2019), it lies within a broader 5.8–12.0 Mb genomic region previously linked to multi-race *H. glycines* resistance (Zhang et al. 2016). This spatial overlap highlights the biological significance of the QTL identified, indicating that this region may harbor resistance genes to multiple nematode species. Further research by Usovsky et al. (2021) revealed that resistance to *H. glycines* and *R. reniformis* might be controlled by the same genomic location, implying pleiotropy where the same locus confers resistance to both nematodes. Taken together, our findings define an ideal candidate target window for future breeding efforts to develop *R. reniformis*-resistant soybean varieties.

The observed co-localizations of resistance regions may be further explained by the utilization of soybean germplasm initially selected for resistance against *H. glycines*, which may already have favorable resistance alleles on chromosome 18. Another probable reason could be the similarities between the parasitic mechanisms of *H. glycines* and *R. reniformis*, where both nematodes induce the formation of specialized feeding structures (syncytia) in the roots of their hosts (Jones et al. 2013; Mitchum et al. 2013). These similarities may lead to overlapping host defense pathways, which could result in co-localization of resistance-associated regions. Nonetheless, additional research is needed to determine whether this region contains new resistance genes or alternative alleles of previously characterized resistance loci.

All three SNPs evaluated in this study exhibited high linkage disequilibrium (LD) and clustered into a single LD block of approximately 88 kb. This strong association indicates that these SNPs likely tag a single QTL. The moderate levels of phenotypic variance explained by these markers (8% to 9%) are consistent with loci governing partial, quantitative resistance, where individual loci typically account for a relatively small proportion of total phenotypic variation (Collard et al. 2005; Bernardo 2010). This high degree of LD is consistent with the self-pollination habit of soybean, which reduces the frequency of crossing-over and thus leads to a large block of linkage (Hyten et al. 2007).

Among the candidate genes identified in the present study, three are promising as they are linked to functions related to plant defense mechanisms, regulation of gene expression, and metabolic processes, and thus may collectively help confer resistance against *R. reniformis*. The first candidate, *Glyma.18G090900*, which encodes a eukaryotic aspartyl protease, is particularly promising because proteases have been shown to contribute greatly to the defense mechanisms of plants, especially in the processing of proteins and activation of immune responses such as the

hypersensitive response (Xia et al. 2004; Simoes and Faro 2004; Figueiredo et al. 2021). The second potential candidate is *Glyma.18G091000*, which has been annotated as a CCCH-type zinc finger protein containing a G-patch domain. Considering that CCCH-type zinc finger proteins play an essential role in stress-responsive pathways and can regulate plant immunity through the modulation of mRNA turnover during pathogen infection, this gene likely functions in RNA binding and post-transcriptional regulation (Pomeranz et al. 2010; Tang et al. 2023; Chen et al. 2025). The third gene *Glyma.18G091300* encoding NAD(P)-binding Rossmann-fold protein is involved in redox reactions, including cellular metabolism. Thus, this gene may play a critical role in plant defense via reactive oxygen species (ROS) signaling (Apel and Hirt 2004; Marttinen et al. 2023).

5 Conclusion

Overall, the SNP markers identified in this study associated with resistance to *R. reniformis* provide a valuable resource for enhancing soybean resistance. These markers can also be utilized in marker-assisted selection (MAS) breeding programs to expedite the development of resistant soybean varieties. However, because these loci explain only a moderate proportion of the phenotypic variance, resistance is likely controlled by multiple genes with small to moderate effects. Consequently, integrating the identified markers into genomic selection (GS) frameworks may yield higher genetic gains by capturing the cumulative effects of multiple loci associated with resistance (Meuwissen et al. 2001). The presence of resistant and susceptible lines within the set of genotypes tested confirms that positive alleles exist within the available soybean germplasm, facilitating stacking of genes for effective resistance. While this study successfully identified genomic regions linked to resistance, further research, including fine

mapping, candidate gene analysis, and the assessment of genotype-by-environment interactions, is important to validate the identified regions.

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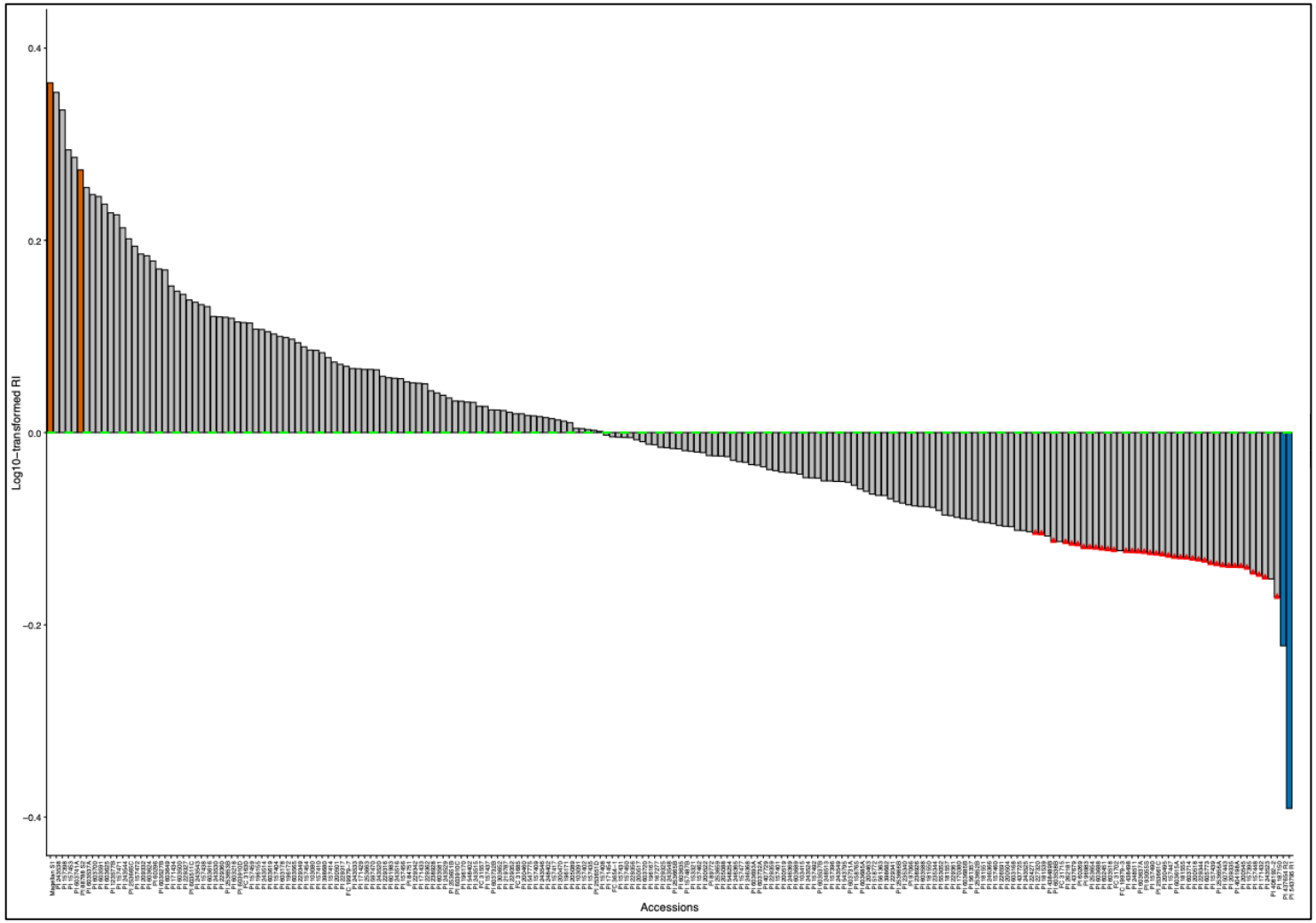


Fig. 1 Log_{10} transformed reproduction index (RI = P_f/P_i) of *R. reniformis* for 202 soybean accessions tested over two years. (Fig. 1 continued)

Each bar represents the mean reproduction index calculated using the Best Linear Unbiased Prediction (BLUP) method for each accession. The orange bars represent susceptible controls, while the blue bars represent resistant controls. The dashed green line at 0 represents the resistance threshold on the $\log_{10}$ scale (RI = 1), with values less than 0 indicating resistance and values greater than 0 indicating susceptibility. The red triangles are accessions that have favorable alleles for significant SNPs identified through GWAS analysis.

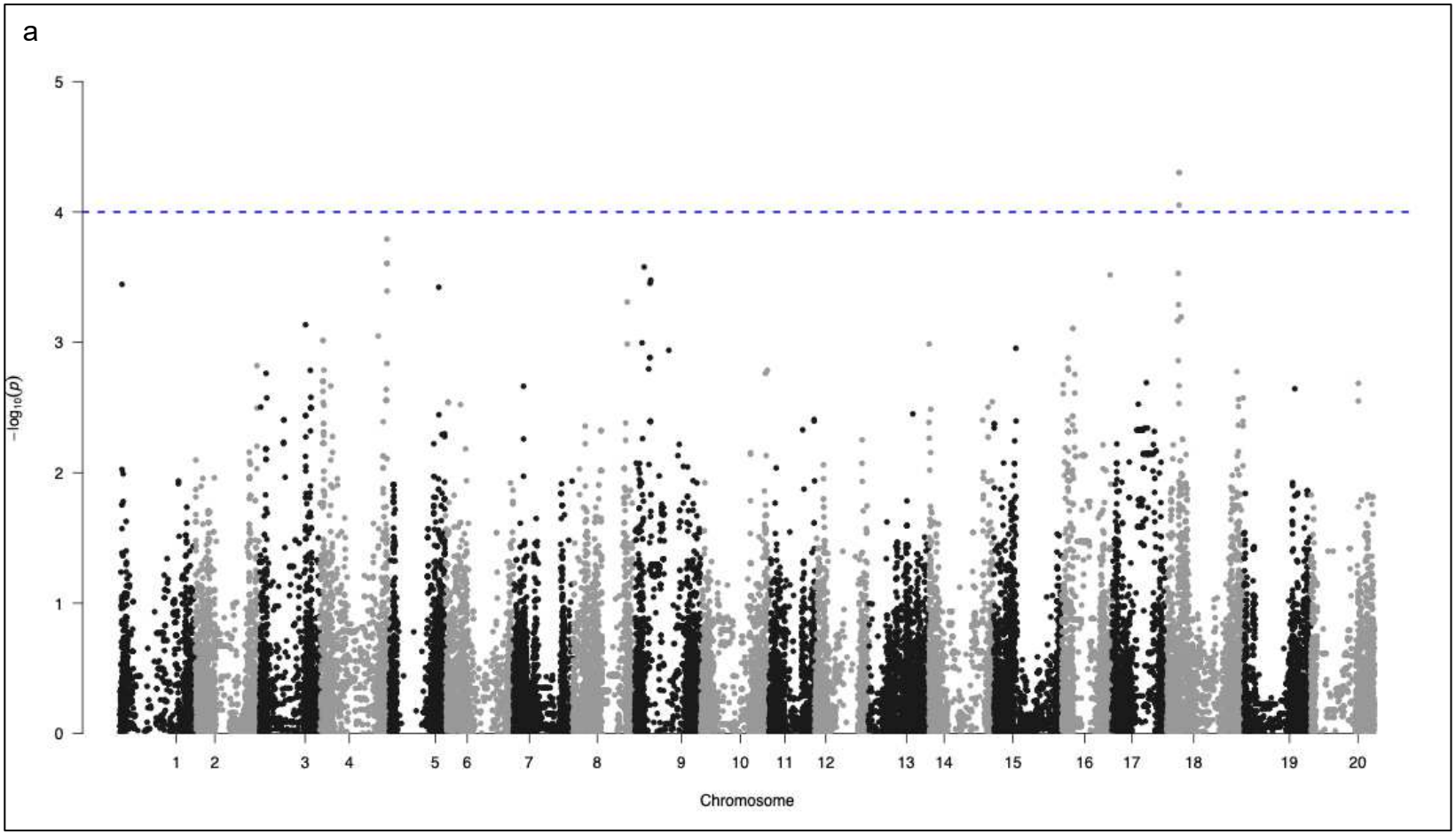
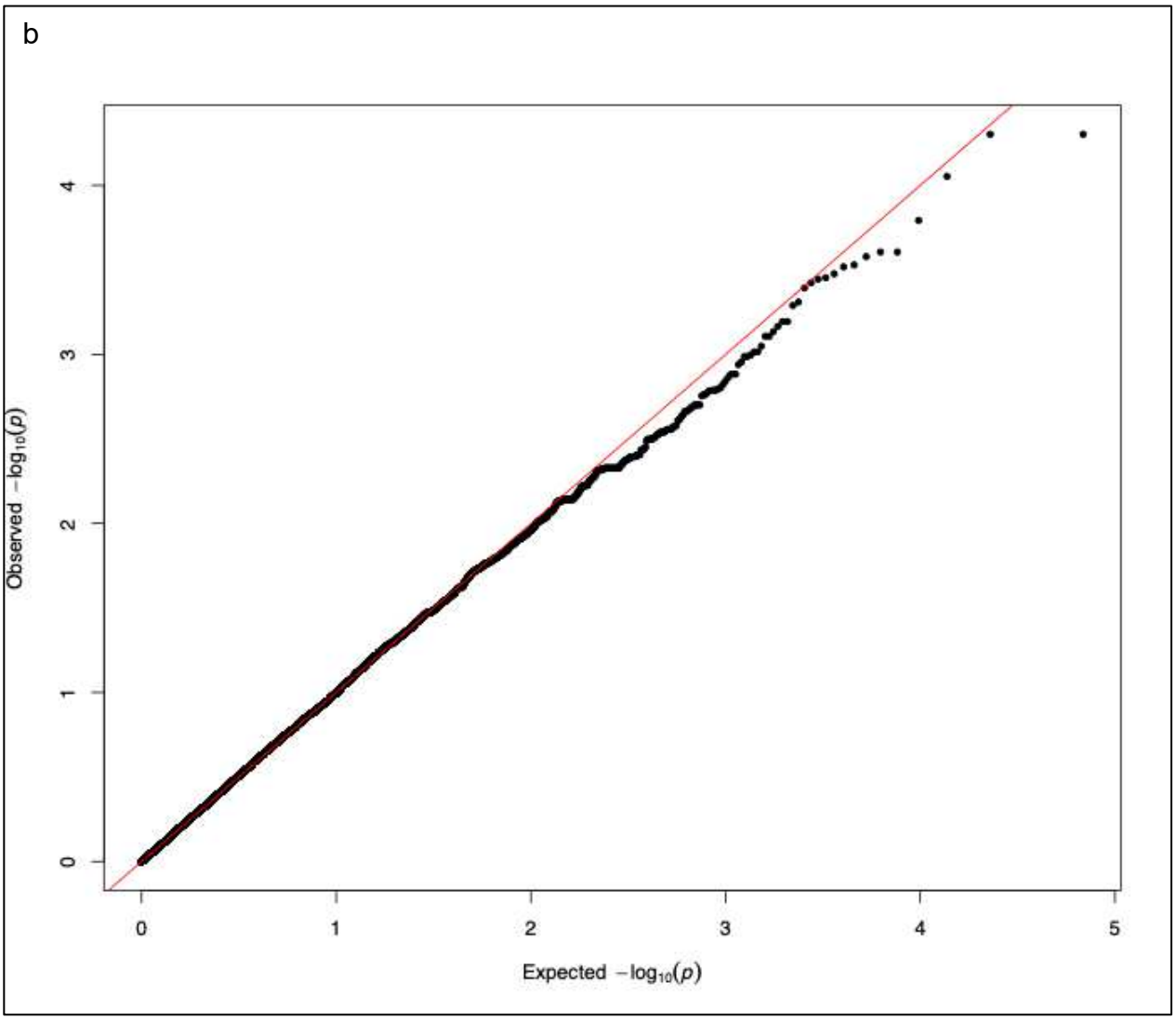


Fig. 2 Genome-wide association analysis of *R. reniformis* reproduction index in soybean. **a** Manhattan plot of the association results for the mixed linear model (MLM), where the distribution of the SNPs is shown. The horizontal blue dashed line represents the genome-wide significance threshold ($-\log_{10}(P\text{-value}) = 4$). Three SNPs (ss715632853, ss715632857, and ss715632859) on chromosome 18 surpass this threshold and were found to be significantly associated with reduced reproduction index. The association signals for ss715632857 and ss715632859 overlap due to their close physical proximity. (Fig. 2 continued)



b Quantile-quantile (QQ) plot of the observed (y-axis) and expected (x-axis) distribution of the $-\log_{10}$ P-values for the MLM.

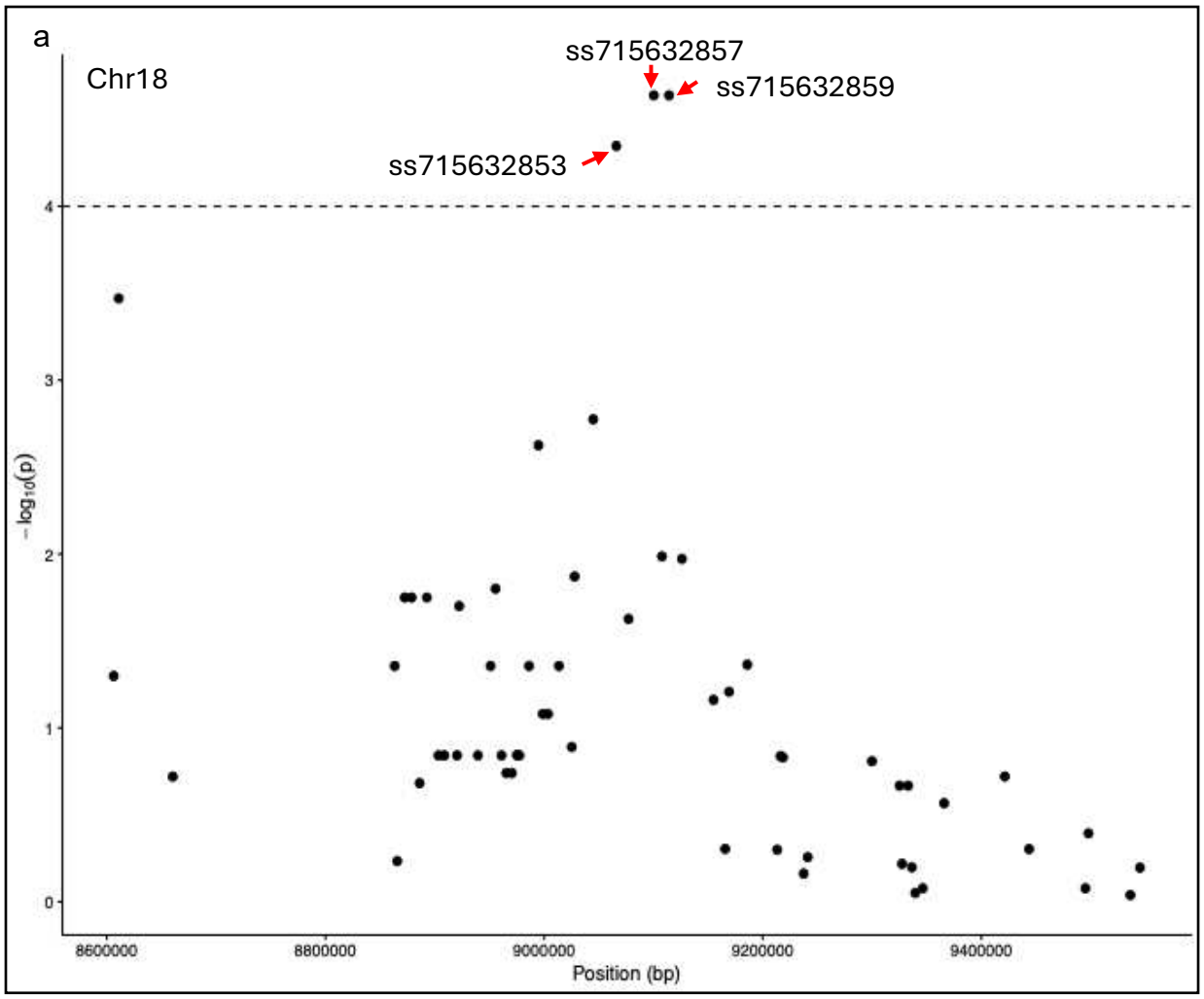
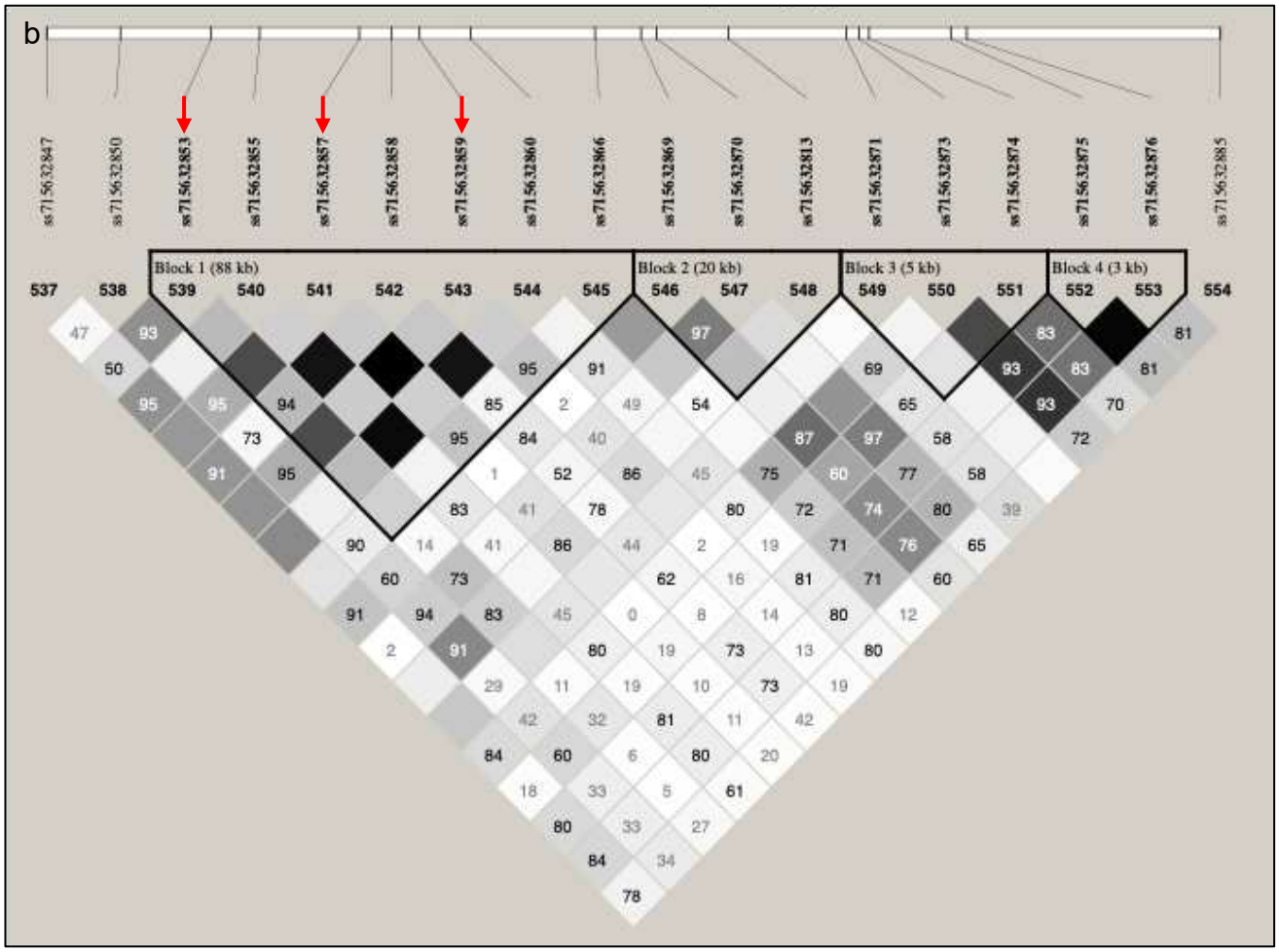


Fig. 3 Regional association plot of the significant locus on chromosome 18. **a** The regional Manhattan plot with the significance threshold represented as a horizontal dashed line ($-\log_{10}$ (*P*-value) = 4). (Fig. 3 continued)



b The LD heatmap of the region surrounding the three significant SNPs (ss715632853, ss715632857, ss715632859; highlighted within the LD block). Strong LD is observed within the genomic region.

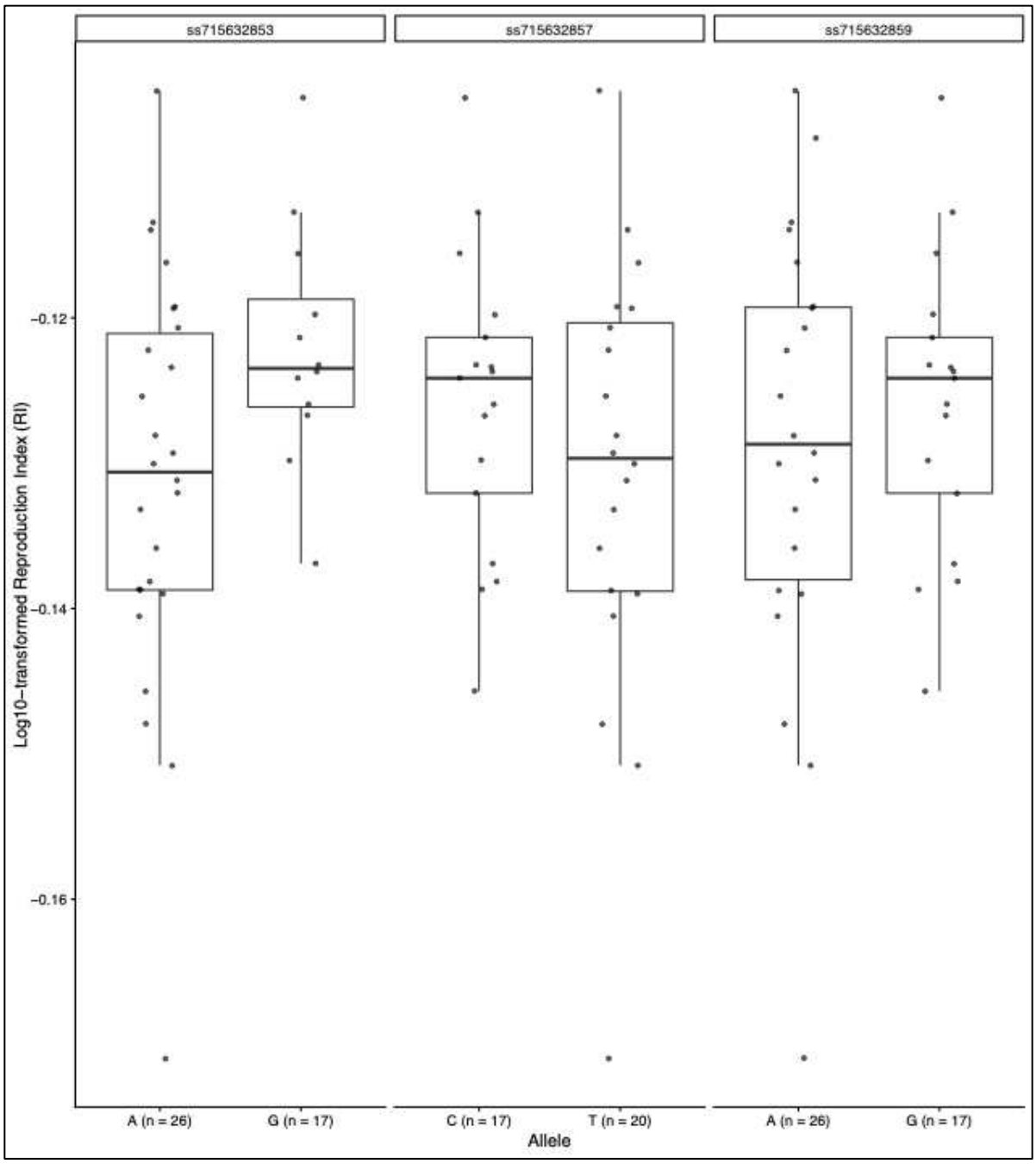


Fig. 4 Boxplots showing the distribution of $\log_{10}$-transformed BLUP-based reproduction index (RI) values for soybean accessions grouped by alleles at the three significant SNPs identified on chromosome 18: ss715632853 (A/G; $P = 0.0004$), ss715632857 (C/T; $P = 0.0729$), and SNP ss715632859 (A/G; $P = 0.0572$).

Table 1: Significant SNP markers associated with resistance to *R. reniformis* identified by genome-wide association analysis

Marker	**Chromosome**	**Physical Position**	**Allele Present**	**MAF**	***P* value**	**−log (*P* value)**	**Marker R^2**
ss715632853	18	9,066,013	A/G	0.39	8.85E-05	4.05	0.08
ss715632857	18	9,100,326	T/C	0.48	4.99E-05	4.30	0.09
ss715632859	18	9,114,287	A/G	0.47	4.99E-05	4.30	0.09

[a] MAF, minor allele frequency.

[b] −log (*P* value), negative logarithm (base 10) of the *P* value.

[c] Marker R^2, proportion of phenotypic variance explained by the SNP marker.

Table 2: Candidate genes found in a region within a ±50 kb window associated with *R. reniformis* resistance.

Chr	*Glycine max* gene ID	Physical Position	Description
18	Glyma.18G090900	9,044,375 - 9,050,844	Eukaryotic aspartyl protease family protein;
18	Glyma.18G091000	9,060,662 - 9,066,712	Zinc finger CCCH-type with G patch domain protein
18	Glyma.18G091100	9,081,698 - 9,083,625	3-oxoacyl-acyl-carrier protein synthase I, chloroplastic-like isoform X3
18	Glyma.18G091200	9,110,189 - 9,111,657	Magnesium transporter NIPA2-like isoform X1
18	Glyma.18G091300	9,117,664 - 9,125,559	NAD(P)-binding Rossmann-fold superfamily protein

[a] Chr, chromosome

[b] Gene IDs correspond to the *Glycine max* reference genome (Williams 82), assembly version Wm82.a2. v1.

[c] Physical positions are based on the Williams 82 reference genome available at SoyBase (https://www.soybase.org).

[d] Candidate genes were identified within ±50 kb of peak SNP positions detected by GWAS.

Supplementary Files

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

- SupplementaryTable1.xlsx