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RESEARCH ARTICLE OPEN ACCESS

Genomic Markers Associated With Soybean Resistance to the Stem Borer, *Dectes texanus* (Coleoptera: Cerambycidae)

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Received: 29 October 2024 | **Revised:** 18 December 2024 | **Accepted:** 30 December 2024

Funding: This work was supported by Nebraska Soybean Board (NSB) (1744).

Keywords: Dectes stem borer | insect resistance | quantitative trait loci | soybean

ABSTRACT

The Dectes stem borer, *Dectes texanus* LeConte (Coleoptera: Cerambycidae), can significantly reduce yields by causing significant lodging in soybean. While this stem borer has not been considered a major pest of soybean, damage from it is increasing in the United States Midwest region with no current elite cultivars found resistant. Our objective was to map quantitative trait loci (QTL) that reduce girdled stems caused by Dectes stem borer infection and infestation of Dectes stem borer. A genome-wide association study (GWAS) using 50,000 single nucleotide polymorphisms was used to analyze data from a population of maturity group (MG) V to VII soybean accessions grown in North Carolina, which had been scored for Dectes stem borer larvae infestation and girdled stems caused by Dectes stem borer infestation. The GWAS identified 3 QTL with reduced larvae infestation and 4 QTL for reduced girdled stems. Allele effects ranged from 1% to 9% reduced larvae infestation or girdled stems. The QTL identified and germplasm containing the beneficial alleles can be used for improving resistance to the damage caused by the Dectes stem borer in elite soybean cultivars.

1 | Introduction

The Dectes stem borer, *Dectes texanus* Leconte (Coleoptera: Cerambycidae), is native to eastern North America and can infect soybeans, cultivated sunflowers, ragweed, and cocklebur (Buschman and Sloderbeck 2010). The Dectes stem borer has not been considered a major pest of soybean in the United States, but damage has been increasing in both frequency and geographic range (Jeschke 2018). In soybean, yield loss caused by Dectes stem borer mainly occurs through increased lodging and stem breakage due to internal girdling caused by infection of a single larva reaching the base of a soybean stem (Richardson 1975). Currently, no elite cultivars have been found resistant to the stem borer (Buschman and Sloderbeck 2010; Seiter 2018).

The life cycle of the Dectes stem borer has made it difficult to produce large-scale cultivar screens and deploy effective biocontrol measures. In the soybean field, the overwintered adult beetle emerges from plant stubble around June to mate and oviposit on a mid-canopy petiole (Rystrom 2015). The female *D. texanus* can mate in 5 days from emergence with a mating ritual that requires a contact female-producing sex pheromone(s) (Crook et al. 2004). Larva tunnel inside the petiole and stem with the only visible indication of infestation being a small oviposition mark found on the petiole. The larvae are also cannibalistic, so by the end of the season, only one larva survives to travel down to the plant base, girdle the stem, and make a safe home for itself with a frass plug in preparation for diapause. Once girdled, slight pressure can cause the whole plant to topple over, which will reduce yield during harvest (Adkisson et al. 1960).

For biocontrol, aerial pesticide is not recommended since there is a long period of egg laying, larvae are protected inside the plant, and only one larvae needs to survive for plant lodging (Campbell and Van Duyn 1977; Michaud, Grant, and Jyoti 2007; Sloderbeck and Buschman 2011). Host resistance could potentially reduce infestation of Dectes stem borer or be used to reduced girdling caused by the larvae. A screen of 618 plant introduction (PI) accessions within maturity groups (MG) V–VII were previously screened for larvae infestation and girdling in the southeast United States (Richardson 1975). Four accessions were found with less than 10% girdled stems and 12 accessions with less than 30% larvae infestation. In this study, using the phenotype data from these 618 accessions along with recently obtained SNP data on these accessions, we aimed to identify regions within the soybean genome that provides resistance to infestation and girdled stems due to the Dectes stem borer. These associated genomic loci can be used to develop increased resistance in response to potential infestation by *D. texanus*.

2 | Materials and Methods

2.1 | Phenotype Data

Phenotype data for the late maturity groups (V–VII) were obtained from Richardson (1975). Briefly, 618 accessions were tested in 1972 for larva infestation and girdled stems in a historically infested North Carolina field. To ensure high insect pressure, *D. texanus* infested stubble was spread throughout the field and cage tests. The field design included one replication for each accession and plots 3-m long row with approximately 40 plants per row. Each cage experiment contained 15 rows, each 0.5-m long with approximately 10 plants per individual plot within the cages. After the initial screen, 185 accessions were chosen to plant in 1973, 3-m long, three replications with approximately 30 plants each. Cage experiments in 1973 were similar size as 1972 but each plot was three replicates each 1-m long. A few accessions were selected to test in 1974, 6 m, three replications. Of the 618 accessions, 379 had been genotyped with the SoySNP50k iSelect Beadchip (Song et al. 2015) and had sufficient replicated phenotype data from the field and cage screening. Best linear unbiased estimations (BLUEs) for each of accession was calculated using ASReml-R (Butler et al. 2017).

2.2 | Genome Wide Association Study (GWAS)

2.2.1 | Best Linear Unbiased Estimates

The phenotypic values for larvae infestation and girdling were estimated by models incorporating known sources of variation in ASReml-R, such as environment (field or cage and year), replications, and maturity group as a covariate (Butler et al. 2017). Since Richardson (1975) does not provide the raw phenotypic data, BLUEs were created by using line, year, and type (field or cage) as fixed effects and maturity group as a covariate.

Genome Wide Association of traits to markers on the SoySNP50k iSelect BeadChip.

The marker association for the best linear estimates for larvae infestation and girdle stems was conducted using the Fixed and random model Circulating Probability Unification (FarmCPU) method ran through the software Genomic Association and Prediction Integrate Tool (GAPIT) in R studio (Liu et al. 2016; Wang and Zhang 2021). Briefly, FarmCPU is a Mixed Linear Mixed Model (MLMM) but divided in two by a Fixed Effect Model (FEM), containing the testing markers one at a time and using multiple associated markers as covariates, and a random effect model (REM), which estimates the associated markers by using them to define kinship (Liu et al. 2016). The minor allele frequency threshold was set to MAF > 0.05 and heterozygotes along with markers mapping only to scaffolds were removed from the marker data. Scaffolds may not have a mapping location so they were eliminated from analysis. For the 379 later maturing accessions GWAS, 35,019 markers were included after filtering. Linkage disequilibrium around significant SNPs was calculated for these SNPs (Table S1) on a 25 SNP sliding window using Tassel 5.2.94 (Bradbury et al. 2007). Based on the principal component analysis using eigenvalue charts and data representation in 2D and 3D models, principal component analysis (PCA) was set to four on all analyses (Figure S1). Q-Q plots were created for each GWAS analysis to help determine if population structure and kinship analysis were being controlled in the analysis (Figures S2 and S3). The effect is a standardized difference of the means. The Bonferroni correction for significance was applied using $\alpha = 0.05$.

3 | Results and Discussion

Statistical and computation tools have improved since the original publication of the Richardson (1975) data. The authors that originally collected and analyzed the phenotypic data noted a negative correlation with maturity and girdled stems. The original analysis observed an average infestation rate of 48.7% for the 1972 screen, which also included 26.1% girdled stems. The original analysis also demonstrated that infestation decreased as maturity of the line increased with average infestation for Maturity Group (MG) V, VI, and VII at 56.5%, 49.4%, and 41.2%, respectively. Girdled stems also showed a decrease with an increase in maturity with MG V, VI, and VII having girdled stem values of 36.9%, 27.4%, and 15.4%, respectively (Richardson 1975). Despite this observed negative correlation of infestation and girdling with maturity, the authors were not able to incorporate maturity into their statistical model.

For our reanalysis of the phenotype data, maturity was included as a covariate. When maturity was included as a covariate, the 618 lines had an average BLUE for larvae infestation of 60% and the percentage of girdled stems was 46% (Figure 1, Table S2). Using a significance threshold of $p < 0.05$, four accessions were found to have significantly less larvae infestation and five accessions with significantly less girdling (Table 1). PI 165989, PI 175195, and PI 319525 were significantly less for both traits while FC 03719 was significantly less for only larvae. PI 219698 and PI 171437 were only significant for fewer girdled stems. Both traits had moderate heritability at 0.51 for larvae infestation and 0.45 for girdled stems.

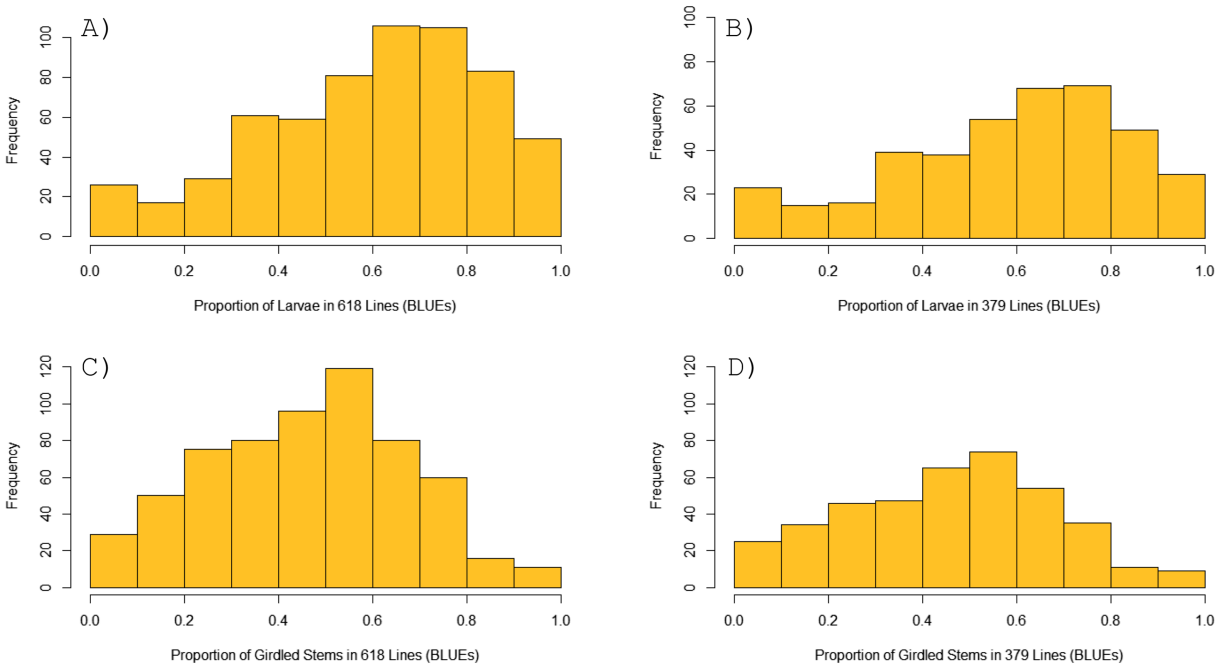

FIGURE 1 | Phenotypic histograms of the Best Linear Unbiased Estimates (BLUEs) of larvae count for (A) all lines and (B) lines genotyped for the SoySNP50k and of girdled stems for (C) all lines and (D) lines genotyped for the SoySNP50k.

TABLE 1 | Soybean accessions with significantly fewer larvae or girdled stems.

Accessions	Larvae (mean ± SE, %)	Girdled stems (mean ± SE, %)	Phenotypic screens
Accessions with significantly fewer larvae			
PI 165989	0 ± 0	0 ± 0	1972 Field, 1972 Cage, 1973 Field
PI 175195	5 ± 2	4 ± 2	1972 Field, 1972 Cage, 1973 Field
PI 319525	6 ± 4	4 ± 4	1972 Field, 1972 Cage, 1973 Field
FC 031719	14 ± 3	13 ± 3	1972 Field, 1972 Cage, 1973 Field
Accessions with significantly fewer girdled stems			
PI 175195	5 ± 2	4 ± 2	1972 Field, 1972 Cage, 1973 Field
PI 319525	6 ± 4	4 ± 4	1972 Field, 1972 Cage, 1973 Field
PI 165989	0 ± 0	0 ± 0	1972 Field, 1972 Cage, 1973 Field
PI 219698	12 ± 9	9 ± 9	1972 Field, 1972 Cage, 1973 Field
PI 171437	21 ± 9	9 ± 9	1972 Field, 1972 Cage, 1973 Field

Many of the late maturity plant introduction accessions screened by Richardson (1975) are still contained within the USDA soybean germplasm collection and available to breeders for use as a source of germplasm for breeding for resistance to Dectes stem borer. The USDA soybean germplasm collection has also been genotyped with the SoySNP50 iSelect Beadchip (Song et al. 2015). Out of the 618 accessions tested by Richardson 1975, there are 379 accessions with SoySNP50 SNP data that also had sufficient replicated phenotypic data from the 1975 screen. Since the Richardson (1975) thesis contained data from their original screen, this allowed us to take the historical phenotype data with our reanalysis and BLUE estimates and perform a GWAS with the SoySNP50 SNP data using the 379 accessions (Table S2).

With the 379 accessions, the SoySNP50 Beadchip provided 35,019 quality SNPs that were included in the GWAS. Using a Bonferroni correction for significance level, there were three significant markers (p-value < 0.05) associated with reduced larvae infestation on chromosomes 7, 12, and 13 (Figure 2, Table 2). For reduced girdled stems, there were four significantly associated markers on chromosomes 1, 7, 11, and 12. The additive allelic effects ranged from 3% to 9% (Figure 3). The largest QTL located on chromosome 12 saw the minor allele reduced larvae infection from 67% down by 9% to 58% infestation (Figure 3).

For each significant marker, the surrounding interval was checked using Soybase (www.soybase.org) to look for any

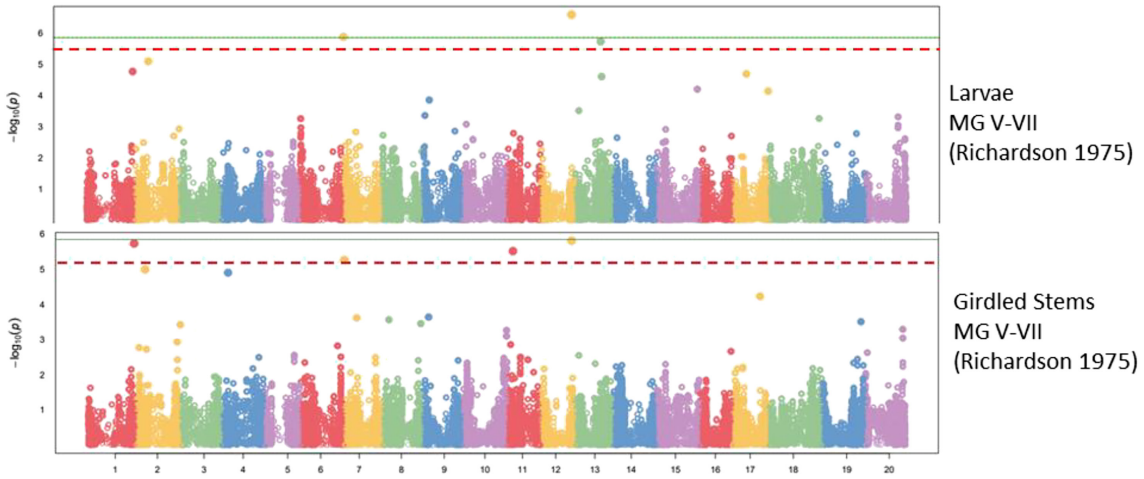

FIGURE 2 | Manhattan plots for (A) larvae and (B) girdled stems from 379 accessions analyzed for the GWAS analysis. Green line is the Bonferroni correction at $p = 0.01$, and the red dashed line is at $p = 0.05$.

TABLE 2 | Significant markers for larvae and girdled stems.

dbSNP name	Chromosome	Position Wm. a1	Position Wm. a2	*p*	MAF	Effect (%)	−log10 (*p*)
Larvae							
ss715597107	7	308,906	313,803	1.34E-06	0.49	−3	5.87
ss715612627	12	35,998,785	35,989,537	2.61E-07	0.11	9	6.58
ss715615024	13	29,524,129	30,724,301	1.88E-06	0.21	−4	5.73
Girdled stems							
ss715580443	1	53,365,928	54,250,600	1.86E-06	0.09	5	5.73
ss715597107	7	308,906	313,803	5.49E-06	0.49	−3	5.26
ss715611045	11	6,890,057	6,900,048	3.06E-06	0.45	−3	5.51
ss715612626	12	35,982,867	35,973,619	1.55E-06	0.11	7	5.81

Note: Negative effects infer that the minor allele statistically has the lower larvae or girdled stem phenotype.

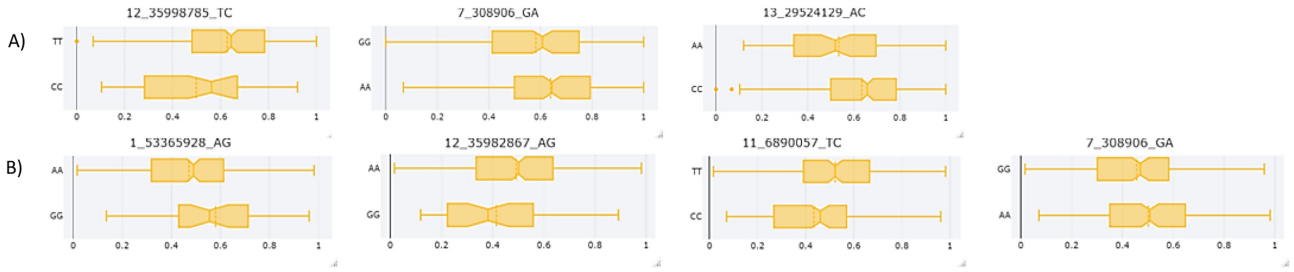

FIGURE 3 | Box and whisker plots of markers found significant in GWAS with (A) larvae and (B) girdled stems data. The solid line signifies the median while the dashed line is the mean. The boxes are notched as a comparison interval around the median value. The marker label is formatted as chromosome_position (soy genome a1) then major allele listed first and minor allele listed second. The major allele in the SNP name was originally determined using the 50 k SNP chip data genotyped with over 20,000 accessions.

previous identified QTL that might be related to the novel QTL discovered in this study. To determine the interval for QTL, the nearest marker found on the Glyma.1.0 physical map was identified and 1 cM on each side of that marker using the Composite 2003 genetic map was considered the surrounding 'interval'. The nearest marker information on the genetic and physical maps along with previously known QTL listed in Soybase, which fall within that interval are listed in Table S3. The QTL that overlap with the significant markers for larvae infestation include QTL for *P. sojae* resistance, sudden death syndrome (SDS) resistance, lodging resistance, drought tolerance, flood tolerance, and pubescence density (Table S2). For girdling, significant markers

overlap with whitefly resistance, drought tolerance, and lodging resistance. Lodging resistance was a common QTL across larvae infestation and girdled stem resistant QTL. The infestation and girdling QTL being associated with lodging resistance QTL are especially of interest since the main loss for yield from Dectes stem borer is due to increased lodging and stem breakage (Richardson 1975).

The marker on chromosome 7, position 313,803 bp, was significant for both Dectes stem borer traits but did not overlap any known QTL for insect resistance or other traits of potential interest. The marker on chromosome 12 for larvae is located 15,918 bp from the significant marker on chromosome 12 for girdled stems and both intervals had overlap with drought tolerance related QTL.

Significant QTL identified here and overlapping QTL for insect and disease resistance along with architectural traits could give further insight into the mechanism of resistance to *D. texanus*. As this pest becomes more prevalent in the Midwest, there is renewed interest in breeding for resistant lines through finding germplasm and QTL that can increase resistance. Future work should entail phenotyping in more environments to confirm QTL discovered in this study and fine mapping of highly resistant vs susceptible accessions to provide a better understanding of the genetics involved in the resistance to Dectes stem borer. As future work begins to find additional resistance to this emerging pest, the ability to gain insight into resistance using phenotype data collected 50 years ago on accessions that have been genotyped with SNPs along with these accessions being still available to breeders today demonstrates the utility and value of having public resources such as the USDA germplasm collection characterized with molecular resources. The soybean accessions identified as having low infestation and/or stem breakage, along with the putative QTL identified here, are potential germplasm sources and genomic targets for improving resistance to the damage caused by the stem borer in elite soybean cultivars.

Author Contributions

S.J. and D.H. designed the research, S.J. performed the research, S.J. and D.H. analyzed data, S.J. wrote the manuscript and D.H. edited the manuscript.

Acknowledgments

The research reported in this publication was supported by the Nebraska Soybean Board project #1744. This work was completed utilizing the Holland Computing Center of the University of Nebraska, which receives support from the Nebraska Research Initiative.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Genotype data for all accessions is available at www.soybase.org. Original phenotypic data is available from Richardson, 1975. All other data supporting the findings are contained within the manuscript.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.