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Targeting and mapping resistance to *Cercospora sojina* in two elite soybean (*Glycine max* L.) populations

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Frogeye Leaf Spot (FLS) is a soybean disease caused by the fungus Cercospora sojina. It is distinctly signified by red-brown circular lesions on the leaves that can move to the stems, pods, and seeds in severe infections. QoI inhibitor fungicides had been priorly used to control C. sojina, but resistance was quickly developed. Without adequate control, yield can be reduced to 40% when environmental conditions are conducive. Therefore, genetic host resistance is key to managing the disease. To this end, the 'Forrest' by 'Williams 82' and the 'Flyer' by 'Hartwig' soybean populations were screened in the greenhouse for FLS resistance and genotyped using the BARCSoySNP6K BeadChip array. The Rcs15-01 and Rcs15-02 were identified in the Forrest by Williams 82 population on chromosomes (Chr.) 6 and 11, respectively, whereas Rcs15-03 was identified on Chr. 6 in the Flyer by Hartwig population. Although Rcs15-01 and Rcs15-03 were previously mapped on Chr. 6 in the same disease resistance gene-rich region near Satt079, Rcs15-02 was identified as a novel QTL. Overall, our data will help breeders implement FLS resistance into high-yielding lines quickly and efficiently using marker-assisted selection.

Key words: Frogeye leaf spot, *Cercospora sojina,* quantitative trait loci, marker assisted selection, disease resistance.

INTRODUCTION

Frogeye Leaf Spot (FLS) is a soybean disease caused by the fungus *Cercospora sojina* K. Hara that can cause significant yield loss in warm and humid soybean producing countries (Mian et al., 2008). The disease is signified by circular red-brown lesions that grow as infection continues. The disease usually occurs on the foliage, but the lesions can also spread to stems, pods, and seeds in severe infections (Phillips, 1999). It can be observed throughout the growing season but is most seen after flowering. If infection reaches the seed, it acts as an inoculum in the following growing season (Carmona et al., 2009; Dorrance and Mills, 2011; Malvick, 2018). FLS reduces yield by 40% in conducive environmental conditions, affecting the final product and

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profit (Byamukama et al., 2019).

Since its discovery in the United States of America in 1925, FLS has been particularly problematic in the southern states where the weather is hot and humid during the growing season. Consequently, soybean yield losses due to FLS consistently ranked in the top 5 most damaging soybean diseases from 2010 to 2014 in the southern states (Lehman, 1928; Philips and Boerma, 1981; Allen et al., 2017). FLS has also been reported in the midwestern states, including Iowa (Yang et al., 2001b), Wisconsin (Mengistu et al., 2002), and Ohio (Dorrance et al., 2010).

The QoI inhibitor fungicides (also known as FRAC Group 11) were originally used as a control method for the disease, but *C. sojina* developed resistance by 2010 (Zhang et al., 2012). This resistance has only spread throughout the United States in the past decade. In a recent study, 14 soybean producing states reported QoI fungicide-resistant *C. sojina*, making genetic host resistance the more sustainable and cost-effective option for long-term control (Mian et al., 2008; Sharma and Lightfoot, 2014; Zhang et al., 2018).

In the United States, five *C. sojina* races were identified in early studies: *Rcs1* (resistant to *C. sojina* race 1), which derived its resistance from 'Lincoln,' conferring resistance to race 1; *Rcs2*, which derived its resistance from 'Kent,' conferring resistance to race 2; and *Rcs3*, which derived its resistance from 'Davis,' conferring resistance to race 5 and broad resistance to all other known races (Mian et al., 2008). Mian et al. (2008) established a *C. sojina* race system for races 5–15 by screening the collection of 93 *C. sojina* isolates from the University of Georgia with 12 differential cultivars. In recent years, two additional resistance alleles, *Rcs*(PI 594891) and *Rcs*(PI 594774), were identified and approved by the Soybean Genetics Committee (Hoskin, 2011; Pham et al., 2015). Nevertheless, it is still unknown the exact quantitative trait loci (QTL) that are associated with each resistance gene. This information would make the implementation of resistance genes more feasible for breeding projects.

Resistance genes are not localized to one area of the genome. Single dominant *Rps* (resistance to *Phytophthora sojae*) were centralized on chromosomes (Chr.) 7, 13, 16, and 18 (Gordon et al., 2006). Of genes conferring resistance to FLS, *Rcs3* was mapped near Satt244 and Satt547 on Chr. 16 (Mian et al., 1999; Missaoui et al., 2007). The Satt114 on Chr. 13 was identified to be resistance gene-rich region that contains the *Rcs* (PI 594891), *Rcs* (PI 594774), and *Rsp8*. The Satt114–Sct_033 and Satt663–Satt114 intervals were subsequently used to identify genes that confer resistance to FLS (Hoskin, 2011; Pham et al., 2015). The *Rcs2* (American type culture collection [ATCC] strain number 44531) was mapped on Chr. 6 at the Satt319–Satt079 and the Satt632–A2D8 intervals (Sharma and Lightfoot, 2014). However, these are not the only areas known for disease resistance, and areas of interest were detected throughout the genome. The *Rhg* (resistance to *Heterodera glycines*) was detected on 12 different chromosomes (Concibido et al., 2004; Chang et al., 2016). *C. sojina* races are diverse, and thus, it is likely the existence of novel *Rcs* currently unmapped.

Quantitative trait loci and their closely linked single nucleotide polymorphism (SNP) markers can be utilized to screen hundreds of lines for a resistance gene at a time. The use of marker-assisted selection is generally cost-efficient, time saving, and more precise than large phenotypic assays (Yousef and Juvik, 2001; Mammadov et al., 2012). The BARCSoySNP6K BeadChip array is a low cost, highly efficient, and highly qualitative assay developed for soybean genetics and breeding research (Song et al., 2013; Song et al., 2020). The array was used to construct linkage genetic maps (Lee et al. 2015) and map or confirm QTL/genes that confer resistance to soybean diseases such as soybean sudden death syndrome (Wen et al., 2014; Luckew et al., 2017; Lee et al., 2018), charcoal rot resistance (Vinholes et al., 2019), *P. sojae*, *Pythium irregulare*, and *Fusarium graminearum* resistance (Stasko et al., 2016; Million et al., 2019), and FLS (McAllister et al., 2021).

In this study, 'Forrest' and 'Williams 82' were chosen as parental lines for their well-documented genomes. Forrest is a historic Southern germplasm, while Williams 82 is a historic Northern germplasm (Wu et al., 2010). Forrest is considered the first soybean line developed with built-in resistance to soybean cyst nematode (SCN) and is estimated to have saved farmers billions of dollars in yield loss (Lightfoot, 2008). Williams 82 is a *P. sojae*-resistant version of the high-yielding 'Williams' line developed in 1982 (Wilcox and Christmas, 1996). Williams 82 is also the model line used in the SoyBase database and utilized by many breeders to understand modes of resistance for various diseases (Grant et al., 2010; Brown et al., 2021). Forrest is partially susceptible to FLS, whereas Williams 82 does not have a well-documented FLS rating (Sharma and Lightfoot, 2014). 'Flyer' derived from the cross of 'Asgrow A3127' (4) × Williams 82, it was released for its high seed yield, good lodging resistance, and multi-race resistance to *Phythopthora megasperma* f. sp. glycines (McBlain et al., 1990). 'Hartwig' derived from the cross of Forrest (3) × PI 437654, it was released for its resistance to SCN, root-knot nematode (*Meloidogyne incognita* [Kofoid & White] Chitwood), and reniform nematode (*Rotenlenchulus reniformis* Linford & Oliviera) (Anand, 1992). The *Rcs3* was not reported in Hartwig and is susceptible to the 15 *C. sojina* isolates (Missaoui et al., 2007). Resistance in FLS for Flyer is not well documented.

The objectives of this study were to create genetic linkage maps for the Forrest by Williams82 (F × W) and Forrest by Hartwig (F × H) populations, identify resistance

across the populations in a greenhouse study, and identify QTL of interest associated with FLS resistance.

MATERIALS AND METHODS

Plant

The F × W population was created by crossing Forrest with Williams 82. Afterwards the F_1 seeds were advanced to F_2, and each F_2 plant was advanced to F_7 by the single seed descent (SSD) method. In the F_8 generation, the $F_{2:7}$ seeds were bulked in 1-m rows to create 1,025 $F_{2:7}$ recombinant inbred lines (RILs) for genetic mapping (Wu et al., 2011). Of these lines, 190 were randomly selected and maintained at Southern Illinois University-Carbondale. The F × H population was created by crossing Flyer with Hartwig. The F_2 plants were advanced to the F_5 generation with SSD. A total of 92 lines were selected from the 739 F_5 plants to be used for QTL studies. The population was increased every two years and maintained at Southern Illinois University-Carbondale (Kazi et al., 2007).

Greenhouse screening

Greenhouse studies were conducted at the Horticulture Research Center, Southern Illinois University-Carbondale, where the greenhouse has a north-south orientation. The assay began by planting 190 F × W and 92 F×H RILs in six-inch plastic nursery pots filled with Berger BM1 growing medium. The plants were allowed to experience ambient conditions and no supplemental lighting was used. Plants were watered according to environmental need, approximately 2-3 times a week, using tap water. The parental lines Forrest, Williams 82, Flyer, and Hartwig, the resistant control Kent, and the susceptible controls Lincoln and 'Blackhawk' were added into the greenhouse study. A randomized complete block design was utilized to create two blocks per experiment, and the experiment was replicated twice in time. After seeds emerged, the pots were thinned to one plant per pot. Plants were inoculated between the V2 and V5 stages with a *C. sojina* solution and then inoculated two more times with a week between inoculations.

C. sojina Race 15 used in this study was kindly provided by Dr. Fakhoury, Southern Illinois University-Carbondale. *C. sojina* was cultured in petri dishes using clarified V8 (CV8) solid medium. CV8 medium was prepared by mixing 1 g calcium carbonate with 100 ml of commercially available V8 juice. The CV8 solution was centrifuged at 10,000 g for 10 min at 25°C. A total of 50 ml of CV8 supernatant was collected, mixed with 950 ml of deionized water and 18 g of agar, and then autoclaved (Salas et al., 2007). The fungus was allowed to grow for 15 to 25 days in a growth chamber at 25 ± 2°C and 80 to 90% relative humidity. Upon spore maturity, the petri dishes were flooded with 0.1% Tween 20. A sterilized metal spatula was used to knock spores into the solution, and approximately eight petri dishes with seven colonies were used to make 300 ml of solution. The solution was thoroughly mixed using a stir plate for 5 min. This solution was then filtered through a cheese cloth and poured into a spray bottle to be used for immediate inoculation.

Lines were sprayed to dripping with the *C. sojina* solution, and then the pots were covered and sealed with a gallon plastic bag to create a 90 to 100% relative humidity microenvironment for 72 h. For the remainder of the experiment, the plants were left under a humidity tent created with plastic sheeting and a humidifier. Humidity was maintained at 80 to 90%, and temperature at 28 ± 2°C throughout the experimentation period.

Two weeks after the first inoculation, FLS disease severity (DS) ratings were taken for each line using the Newman Scale. This scale ranges from 1 to 10, with 1 indicating 1 to 10% of the leaf surface showing disease symptoms, and 10 indicating 90 to 100% of the leaf surface showing symptoms. Defoliation due to disease was counted as a 10. Six ratings were taken in total over two weeks.

DNA isolation and SNP genotyping

The F×W and F×H soybean lines were germinated in a dark room, and 50 mg of plant tissues was kept at -80°C until DNA isolation. Samples were thawed, flash frozen with liquid nitrogen, and crushed using a mortar and pestle. DNA isolation was conducted using the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. To test the DNA quality, 5 µL of DNA was electrophoresed horizontally for 20 min on a 1.5% agarose gel stained with ethidium bromide. DNA quantification was performed using the NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). DNA aliquots were used for SNP genotyping using the BARCSoySNP6K BeadChip at the Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MA.

Data analysis

The sixth FLS DS rating for each line was used for analyzing the distribution of FLS resistance. Lines that had lower FLS scores than the resistant parent were recorded as "resistant," whereas lines with higher scores than the susceptible parent were recorded as "susceptible." Data were analyzed with JMP 15 (SAS Institute, Raleigh, NC, USA).

BARCSoySNP6k data were analyzed using the GenomeStudio Genotyping Module 2.0 (Illumina, San Diego, CA, USA). Non-segregating homozygous markers were removed, and the minor allele frequency was set at 5% (Luckew, 2018). The remaining markers were used to construct the genetic map and QTL analysis using the r/QTL package for R (Broman et al., 2003; Broman and Sen, 2009; Arends et al., 2010; R Core Team, 2020). The sixth greenhouse rating for each line was selected for QTL analysis. Single marker analysis and interval mapping were used to identify chromosomes of interest (data not shown). The Cim() function was used for CIM interval mapping, whereas the Fitqtl() function for estimating the variance of the QTL of interest. A 1,000-permutation test was run to determine approximate logarithm of the odds (LOD) significance thresholds using the 'omperm.ag' function. The F×W and F×H critical LOD thresholds were 4.48 and 4.22, respectively.

Gene ontology (GO) of candidate QTL were analyzed using the SoyBase database (Wm.82 version 2) to discover proteins coded for within the QTL. The UniProt Consortium database was used to understand the function proteins in plants. Potential candidate genes were recorded.

RESULTS

The distribution of F×W FLS DS scores was positively skewed, suggesting segregation during the initial cross has contributed to more lines with resistance to *C. sojina*. This could suggest that a FLS resistance is the dominant allele. The mean FLS DS score was relatively low at 3.19 ± 1.02 with scores ranging from 1.00 to 6.33. Forrest had an FLS DS score of 2.25, whereas Williams 82 an FLS

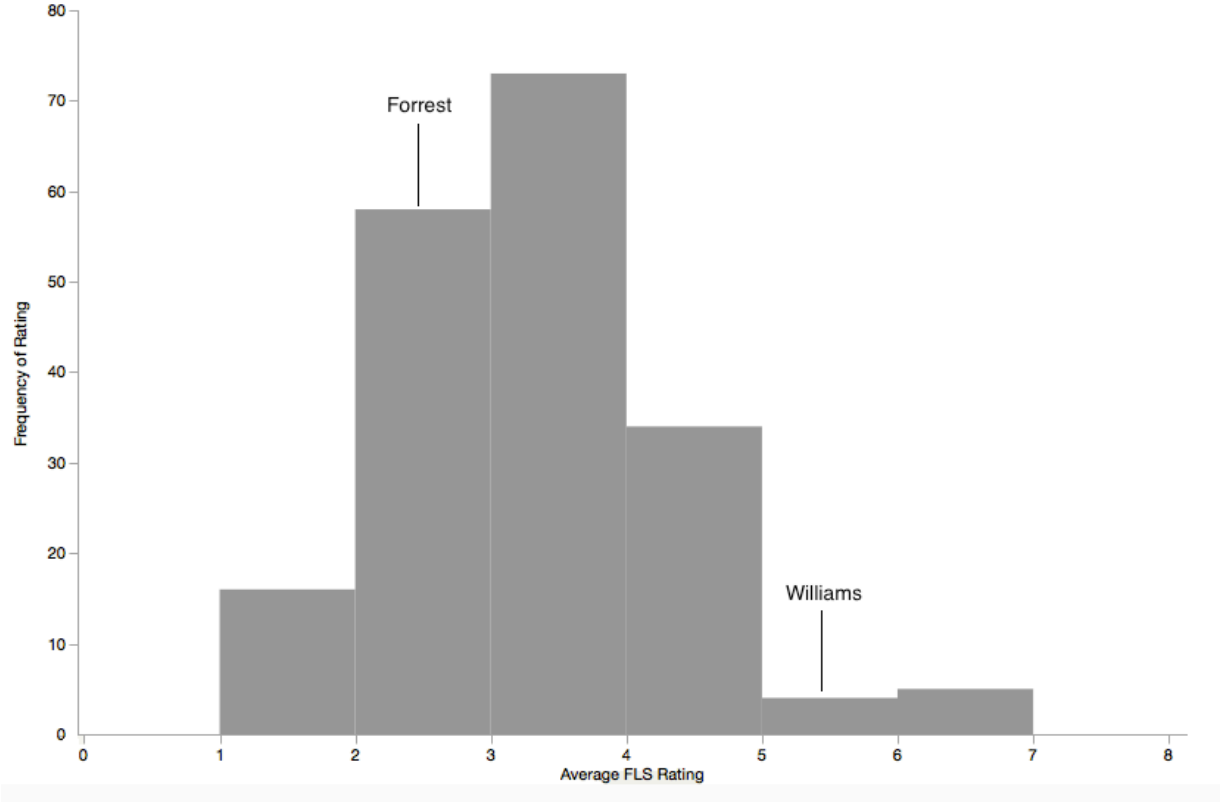

Figure 1. Histogram visualizing FLS scores across the Forrest × Williams 82 population.

score of 5.00. The FLS DS scores for the resistant check Kent was 1.00, the susceptible checks Lincoln was 2.50, and Blackhawk was 5.50, respectively. We identified 26 lines with FLS scores lower than Forrest and seven lines with FLS scores higher than Williams 82. The distribution of F×H FLS DS score was normal (P = 0.0728) (Figure 1). The mean FLS DS score was 3.69 ± 1.42 with scores ranging from 1.00 to 6.50. Flyer had an FLS DS score of 1.00, whereas Hartwig had an FLS DS score of 4.00. The resistant check Kent had an FLS score of 1.00, the susceptible checks Lincoln had and FLS score of 3.00, and Blackhawk had an FLS score of 5.0, respectively. We identified three lines that were as resistant as Flyer and 31 lines that were more susceptible than Hartwig (Figure 2)

Across 20 chromosomes, 2,186 markers were identified for the F×W. The map was 2,105.23 cM in length with an average distance of 0.97 cM (Table 1). The largest gap between markers was 74.35 cM with 97.16% of gaps being < 5 cM. The average chromosome length was 105.26 cM. The longest chromosome was Chr. 18 at 137.47 cM with 164 markers, whereas the shortest was Chr. 16 at 83.40 cM with 73 markers (Table 1). Across 20 chromosomes, 2,031 markers were identified for the F×H. The map was 2,133.49 cM in length with an average distance of 1.05 cM (Table 2). The longest chromosome was Chr. 18 at 135.77 cM with 148 markers, whereas the shortest chromosome Chr. 16 at 85.15 cM with 81 markers (Table 2).

Two QTL were identified to underlie FLS DS resistance in F×W. *Rcs15-01* was located on Chr. 6 (LG C2) at the ss715594329–ss715594474 interval (Position 87.11–99.97 cM; Physical Position: 39.19–43.69 Mbp). One peak was noted in this interval at ss715594440 (Position: 64.04 cM; Physical Position: 43.46 Mbp) with an LOD score of 5.16 (Table 3). *Rcs15-01* explained 5.16% of the phenotypic variation and derived from Williams82. *Rcs15-02* was located on Chr. 11 (LG B1) at the ss715610717–ss715610843 interval (Position 9.90–13.04 cM; Physical Position: 4.34–5.25 Mbp) with a peak at ss715610720 (Position 9.94 cM; Physical Position: 4.35 Mbp). *Rcs15-02* explained 6.75% of the phenotypic variation and derived from Williams 82 (Table 3). Interaction effects of the two QTL were insignificant (P = 0.14). *Rcs15-02* was mapped at the ss715610717–ss715610843 interval (Position 9.90–13.04 cM; Physical Position: 4.34–5.25 Mbp).

A single QTL was identified to underlie FLS DS in F×H. *Rcs15-03* was located on Chr. 6 at the interval ss715594497–ss715594771 (Position: 101.77–108.10

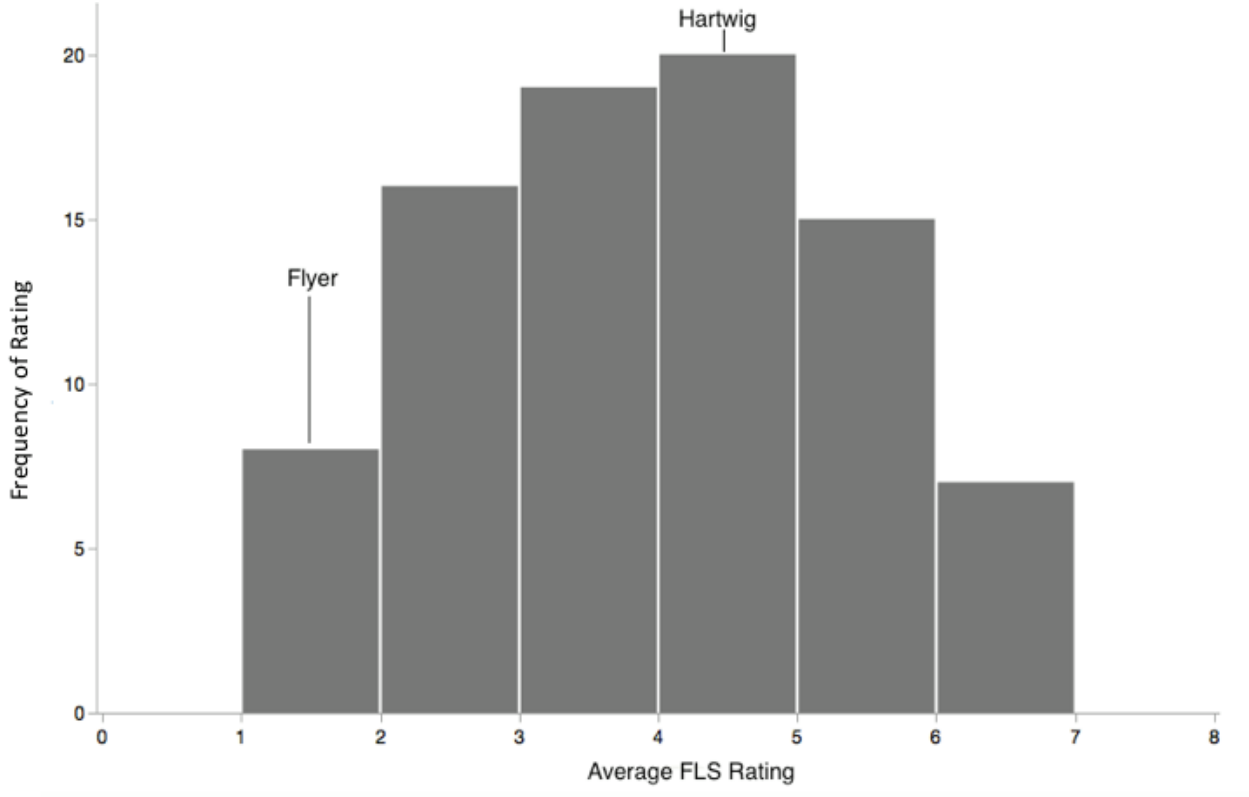

Figure 2. Histogram visualizing FLS scores across the Flyer × Hartwig population.

Table 1. Characteristics of the Forrest×Williams 82 genetic linkage map.

Chromosome	Linkage Group	Number of Markers	Coverage (cM)	cM per Marker
Chr. 1	D1a	105	121.60	1.15
Chr. 2	D1b	155	117.21	0.75
Chr. 3	N	109	108.05	0.99
Chr. 4	C1	62	112.24	1.81
Chr. 5	A1	97	92.39	0.95
Chr. 6	C2	116	114.33	0.95
Chr. 7	M	123	98.00	0.79
Chr. 8	A2	151	100.39	0.66
Chr. 9	K	123	93.27	0.75
Chr. 10	O	110	114.12	1.03
Chr. 11	B1	86	88.67	1.03
Chr. 12	H	72	88.77	1.03
Chr. 13	F	152	91.36	1.26
Chr. 14	B2	61	111.40	1.82
Chr. 15	E	107	115.74	1.08
Chr. 16	J	73	83.40	1.14
Chr. 17	D2	101	94.97	0.94
Chr. 18	G	164	137.47	0.83
Chr. 19	L	125	115.39	0.92
Chr. 20	I	94	106.46	1.13
Total		2186	2105.23	0.97

Table 2. Characteristics of the Flyer × Hartwig genetic linkage map.

Chromosome	Linkage Group	Number of Markers	Coverage (cM)	cM per Marker
Chr. 1	D1a	66	121.92	1.85
Chr. 2	D1b	91	117.33	1.29
Chr. 3	N	92	108.44	1.18
Chr. 4	C1	56	111.80	2.00
Chr. 5	A1	94	95.52	1.02
Chr. 6	C2	131	115.46	0.88
Chr. 7	M	113	102.11	0.90
Chr. 8	A2	127	105.02	0.83
Chr. 9	K	85	100.4	1.18
Chr. 10	O	98	113.81	1.16
Chr. 11	B1	88	88.71	1.01
Chr. 12	H	66	88.80	1.35
Chr. 13	F	177	97.57	0.55
Chr. 14	B2	75	112.37	1.50
Chr. 15	E	153	116.39	0.76
Chr. 16	J	81	85.15	1.05
Chr. 17	D2	102	95.81	0.94
Chr. 18	G	148	135.77	0.92
Chr. 19	L	98	114.53	1.17
Chr. 20	I	100	106.58	1.07
Total		2031	2133.49	1.05

Table 3. Location of *Rcs15-01* on Chromosome 6 and *Rcs15-02* on Chromosome 11 mapped in Forrest × Williams 82.

Interval	LG/Chr	Position of Interval (cM)	Position (cM)	LOD	R^2 (%)	FLS mean	
						Forrest	Williams 82
ss715594329 –ss715594474	C2/6	87.11-99.97	97.72 (ss715594440)	5.16	6.01	3.11± 1.04	3.04 ± 0.89
ss715610717–ss715610843	B1/11	9.90-13.04	9.94 (ss715610720)	3.39	6.75	3.29 ± 1.10	3.02 ± 0.92

cM; Physical position: 45.05–47.77 Mbp). A single peak was located at ss715594534 (Position: 104.73 cM; Physical position: 46.30 Mbp) with an LOD score of 5.78. *Rcs15-03* explained 14.07% of the phenotypic variation and derived from Flyer (Table 4). Candidate genes were inferred in this study. At *Rcs15-01*, *Glyma.06g241500* and *Glyma.06g247200* encoded for a WD domain repeat protein family; *Glyma.06g243800* encoded

Table 4. Location of the *Rcs15-03* on Chromosome 6 mapped in Flyer × Hartwig.

Interval	LG/Chr	Position of Interval (cM)	Position (cM)	LOD	R^2 (%)	FLS Mean	
						Flyer	Hartwig
ss715594497– ss715594771	C2/6	101.77-108.10	104.73 (ss715594534)	5.78	14.07	3.22±0.22	4.58±0.26

Table 5. Potential candidate genes in *Rcs15-01*

Glycine max gene ID (Wm82.a2.v1)	Protein family	Protein general function
Glyma.06g241500	PF00400 (WD domain, G-beta repeat)	WD40 REPEAT PROTEINPRL1/PRL2-RELATED
Glyma.06g242200	PF03106 (WRKY DNA -binding domain)	WRKY family transcription factor family protein
Glyma.06g243800	PF07714 (Protein tyrosine kinase) PF11721 (Di-glucose binding within endoplasmic reticulum)	LEUCINE-RICH REPEAT RECEPTOR-LIKE PROTEIN KINASE
Glyma.06g244100	PF00069 (Protein kinase domain) PF07714 (Protein tyrosine kinase)	LEUCINE-RICH REPEAT RECEPTOR-LIKE PROTEIN KINASE
Glyma.06g247200	PF00400 (WD domain, G-beta repeat)	WD REPEAT DOMAIN 44
Glyma.06g254300	PF00931 (NB-ARC domain)	LEUCINE-RICH REPEAT-CONTAINING PROTEIN
Glyma.06g255900	F07714 (Protein tyrosine kinase) PF01453 (D-mannose binding lectin) PF08276 (PAN-like domain)	LEUCINE-RICH REPEAT RECEPTOR-LIKE PROTEIN KINASE

for a WRKY DNA-binding domain family; *Glyma.06g243800*, *Glyma.06g244100*, and *Glyma.06g255900* encoded for leucine-rich-repeat receptor-like protein kinase; *Glyma.06g254300* encoded a leucine-rich-repeat containing protein (Table 5). At *Rcs15-02*, *Glyma.11g058800* encoded a serine/threonine kinase protein; *Glyma.11g058900*, and *Glyma.11g059000* encoded a leucine-rich repeat serine/threonine-protein kinase 1; *Glyma.11g060700*, *Glyma.11g063100*, *Glyma.11g063200*, and *Glyma.11g067200* encoded a leucine-rich-repeat receptor-like protein kinase (Table 6). At *Rcs15-03*, *Glyma.06g264100*, *Glyma.06g264200*, *Glyma.06g264300*, *Glyma.06g264400*, *Glyma.06g265200*, and *Glyma.06g268600* encoded a leucine-rich-repeat containing protein (Table 7).

An analysis of the GO at *Rcs15-01* identified that the AK246052.1 and AB331959 genes are the closest to the peak of this CIM interval. These genes code for the peroxisomal 3-hydroxyacycl-CoA dehydrogenase-like protein. Nearest to the *Rcs15-02* QTL are the BT094200 and AF004806.1 genes, which are associated with the 24 kDa seed maturation protein.

DISCUSSION

The disease pressure for the F×W screening assay was moderate, the FLS DS scores ranged from 1.00 to 6.33. The FLS DS score for the

Table 6. Potential candidate genes in *Rcs15-02*.

Glycine max gene ID (Wm82.a2.v1)	Protein family	Protein general function
Glyma.11g058800	PF00069 (Protein kinase domain)	SERINE/THREONINE KINASE
Glyma.11g058900	PF05659 (Arabidopsis broad-spectrum mildew resistance protein RPW8)	LEUCINE-RICH REPEAT SERINE/THREONINE-PROTEIN KINASE 1
Glyma.11g059000	PF00560 (Leucine Rich Repeat) PF00931 (NB-ARC domain)	LEUCINE-RICH REPEAT SERINE/THREONINE-PROTEIN KINASE 1 LEUCINE-RICH REPEAT-CONTAINING PROTEIN
Glyma.11g060700	PF00069 (Protein kinase domain)	LEUCINE-RICH REPEAT RECEPTOR-LIKE PROTEIN KINASE
Glyma.11g063100	PF07714 (Protein tyrosine kinase) PF00069 (Protein kinase domain)	LEUCINE-RICH REPEAT RECEPTOR-LIKE PROTEIN KINASE
Glyma.11g063200	PF01476 (LysM domain) PF00069 (Protein kinase domain)	LEUCINE-RICH REPEAT RECEPTOR-LIKE PROTEIN KINASE
Glyma.11g067200	PF07714 (Protein tyrosine kinase)	LEUCINE-RICH REPEAT RECEPTOR-LIKE PROTEIN KINASE

Table 7. Potential candidate genes in *Rcs15-03*.

***Glycine max* gene ID (Wm82.a2.v1)**	**Protein family**	**Protein general function**
Glyma.06g264100	PF01582 (TIR domain)	LEUCINE-RICH REPEAT-CONTAINING PROTEIN
Glyma.06g264200	PF00560 (Leucine Rich Repeat) PF00931 (NB-ARC domain)	LEUCINE-RICH REPEAT-CONTAINING PROTEIN
Glyma.06g264300	PF01582 (TIR domain) PF00931 (NB-ARC domain)	LEUCINE-RICH REPEAT-CONTAINING PROTEIN
Glyma.06g264400	PF00560 (Leucine Rich Repeat)	LEUCINE-RICH REPEAT-CONTAINING PROTEIN
Glyma.06g265200	PF01582 (TIR domain)	LEUCINE-RICH REPEAT-CONTAINING PROTEIN
Glyma.06g268600	PF00931 (NB-ARC domain) PF01582 (TIR domain)	LEUCINE-RICH REPEAT-CONTAINING PROTEIN

parental lines Forrest was 2.25 and Williams 82 was 2.25 and 5.00. The FLS DS score for the resistant control Kent was 1.00, the susceptible control Lincoln was 2.50, and Blackhawk was 5.50. Forrest appeared to be partially resistant in this study, results that disagreed with those in Sharma and Lightfoot (2014) and McAllister et al. (2021) where Forrest was partially susceptible to *C. sojina* race 15. Of the 190 lines tested, 26 lines appeared to be more resistant than the Forrest whereas 7 lines appeared to be more susceptible than the Williams 82. Similar results were observed in the F×H screening assay. The disease pressure for this experiment was considered as moderate, the FLS FS score ranged from 1.00 to 6.50. The FLS DS score for the parental lines Flyer was 1.00 and Hartwig was 4.00. The FLS DS score for the resistant control Kent was 1.00, the susceptible control Lincoln was 3.00, and Blackhawk was 5.00. Of the 92 lines, 3 lines were as resistant as Flyer whereas 31 lines were more susceptible than Hartwig. The F×W and F×H resistant lines identified in this study could be used in selection for *C. sojina* race 15 resistance in future breeding programs.

Two QTL were identified to be associated with FLS DS in F×W. The two QTL, *Rcs15-01* and *Rcs15-02* were mapped on Chr.6 and Chr.11. *Rcs15-01* was mapped at the ss715594329–ss715594474 interval (Position 87.11–99.97 cM) (Physical Position: 39.19–43.69 Mbp). Two QTL were mapped on Chr.6 in this study, *Rcs15-01* was mapped in the F×W population and *Rcs15-3* was mapped in the F×H population. The two QTL were located near each other and were also near the *Rcs2* previously mapped in the E×F population (Sharma and Lightfoot, 2014). *Rcs15-01* which was flanked by the ss715594329–ss715594474 interval (Physical Position: 39.19- 43.69 Mbp) overlapped with *Rcs2* (Satt319–Satt079 interval) (Physical Position: 38.05–44.50 Mbp), *Rcs15-03* was located near *Rcs2* and *Rcs15-01*. Since *Rcs15-01*, *Rcs15-03*, and *Rcs2* were mapped at the same region in different soybean populations near Satt, this could indicate that there was a novel *Rcs* conferring resistance to multiple *C. sojina* races (race 2 and 15). *Rps4* (resistant to *P. sojae*) like proteins were identified at *Rcs15-01* and *Rcs15-03*, this was similar to *Rcs3*, which was mapped on Chr.16 near a cluster of disease resistance genes (Webb, 1997; Bachman et al., 2001; Mian et al., 2008). Interestingly, Pham et al. (2015) reported that the *Rcs*(PI 594891) and *Rcs*(PI 594774) were located near the Satt114, which was also a disease resistance gene rich region that contained the resistance gene *Rps8*. *Rcs15-02* was mapped on Chr.11 and was not reported in previous studies, multiple potential disease resistance genes were also identified in the interval.

Prior studies indicate that the peroxisomal 3-hydoyacyl-CoA protein associated with *Rcs15-01* is connected to many different cellular functions (Arai et al., 2008). Fatty acid β-oxidation, the glyoxylate cycle, and stress response mechanisms are all influenced by this protein, and future research should be done to distinguish the connection between these peroxisomes and FLS resistance.

The 24kDa seed maturation protein that is associated with *Rcs15-02* can be detected in the final stages of seed maturation in the parenchyma and aleurone cells of the seed coat. The genes associated with its production are highly expressed in vegetative tissues when wounded by pathogens. This suggests that it plays some roll in wound response, possibly to seal the wounded tissue off from healthy tissues (Dhaubhadel et al., 2005). The connection between 24 kDa seed maturation protein and FLS resistance is not well understood, and future studies should be done to solidify the link.

Conclusions

Three QTLs were mapped in this study. *Rcs15-01* and *Rcs15-02* were mapped in the F×W population and *Rcs15-03* was mapped in the F×H population. *Rcs15-01* and *Rcs15-03* were mapped in a disease-resistance gene rich region on Chr.6 in the same region as *Rcs2*, a QTL previously mapped in the E×F population. This confirms that there are QTL conferring resistance to *C. sojina* on Chr.6 since the same region has been mapped in three different soybean populations. The *Rcs15-02* was mapped on Chr.11 and appeared to be novel. These QTL can provide soybean breeders a new source of resistance to *C. sojina*. Future studies should be conducted to identify the genes that confer resistance to *C. sojina*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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