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ORIGINAL RESEARCH

Mining germplasm panels and phenotypic datasets to identify loci for resistance to *Phytophthora sojae* in soybean

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Abstract

Phytophthora sojae causes Phytophthora root and stem rot of soybean and has been primarily managed through deployment of qualitative *Resistance to P. sojae* genes (*Rps* genes). The effectiveness of each individual or combination of *Rps* gene(s) depends on the diversity and pathotypes of the *P. sojae* populations present. Due to the complex nature of *P. sojae* populations, identification of more novel *Rps* genes is needed. In this study, phenotypic data from previous studies of 16 panels of plant introductions (PIs) were analyzed. Panels 1 and 2 consisted of 448 *Glycine max* and 520 *G. soja*, which had been evaluated for *Rps* gene response with a combination of *P. sojae* isolates. Panels 3 and 4 consisted of 429 and 460 *G. max* PIs, respectively, which had been evaluated using individual *P. sojae* isolates with complex virulence pathotypes. Finally, Panels 5–16 (376 *G. max* PIs) consisted of data deposited in the USDA Soybean Germplasm Collection from evaluations with 12 races of *P. sojae*. Using these panels, genome-wide association (GWA) analyses were carried out by combining phenotypic and SoySNP50K genotypic data. GWA models identified two, two, six, and seven novel *Rps* loci with Panels 1, 2, 3, and 4, respectively. A total of 58 novel *Rps* loci were identified using Panels 5–16. Genetic and phenotypic dissection of these loci may lead to the characterization of novel *Rps* genes that can be effectively deployed in new soybean cultivars against diverse *P. sojae* populations.

Abbreviations: Chr, Chromosome; CMLM, compressed mixed linear model; FarmCPU, Fixed and random model Circulating Probability Unification; GRIN, Germplasm Resources Information Network; GWA, Genome-wide association; LD, linkage disequilibrium; MLMM, multi-locus mixed model; NLR, nucleotide-binding, leucine-rich repeat; PIs, plant introductions; QDR, quantitative disease resistance; QTL, quantitative trait locus; SCN, soybean cyst nematode; SDS, soybean sudden death syndrome; SNP, single nucleotide polymorphism.

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1 INTRODUCTION

Phytophthora sojae, a soil-borne pathogen, causes Phytophthora root and stem rot of soybean [*Glycine max* (L.) Merr.], one of the major diseases that affects soybean yield world-wide (Barreto, Stegman de Garfinkel, & Fortugno, 1995; Costamilan et al., 2013; Cui et al., 2010; Dorrance, 2018a; Kang et al., 2019; Qin et al., 2017; Ryley, Obst, Irwin, & Drenth, 1998; Sans, Rodriguez, Silva, & Stewart, 2017; Sugimoto et al., 2006; Xue et al., 2015; Yang et al., 2019; S. Zhang et al., 2010). Phytophthora root and stem rot can cause 100% loss on highly susceptible cultivars when poorly drained fields are combined with abundant rainfall and warm soil temperatures (Dorrance et al., 2009; Matthiesen et al., 2016; Robertson, Cianzio, Cerra, & Pope, 2009).

Resistant soybean cultivars with single qualitative resistance genes (*Rps* genes) have been the most effective method for managing *P. sojae*, but the rise in pathotype diversity has made utilizing single *Rps* genes less effective in many regions (Anderson, Walch, & Kurle, 2012; Costamilan et al., 2013; Dorrance et al., 2016; Grau, Dorrance, Russin, & Bond, 2004; Kaitany, Hart, & Safir, 2001; Ryley et al., 1998; Schmitthenner, Hobe, & Bhat, 1994; Stewart, Abeysekara, & Robertson, 2014; Yan & Nelson, 2019). Along with the recently identified *Rps* genes, *RpsGZ* and *RpsX* (Jiang et al., 2020; Zhong, Li, Sun, Duan, & Zhu, 2019), more than 30 *Rps* genes/alleles have been proposed and mapped to nine chromosomes (Anderson & Buzzell, 1992; Athow & Laviolette, 1982; Athow, Laviolette, & Mueller, 1980; Buzzell & Anderson, 1981, 1992; Dorrance, 2018b; Gordon, St. Martin, & Dorrance, 2006; Kilen, Hartwig, & Keeling, 1974; Lin et al., 2013; Ping et al., 2016; Ploper, Athow, & Laviolette, 1985; Sahoo, Abeysekara, Cianzio, Robertson, & Bhattacharyya, 2017; Sugimoto et al., 2012; Sun et al., 2011, 2014; Wu et al., 2011a; Yu et al., 2010; Zhang et al., 2013a, 2013b; Zhu, Huo, Wang, Huang, & Wu, 2007), with many located on chromosome (Chr) 3 (Supplemental Table S1). Although more than 30 *Rps* genes/alleles have been identified, only a few, *Rps1a*, *Rps1b*, *Rps1c*, *Rps1k*, *Rps3a*, and *Rps6,* have been deployed in soybean cultivars (Abney et al., 1997; Dorrance, 2018b; Grau et al., 2004; Slaminko, Bowen, & Hartman, 2010). These deployed genes/alleles represent those that were more effective towards the *P. sojae* populations at the time (Dorrance, 2018b; Ping et al., 2016). Because of high pathotypic and genetic diversity as well as rapid adaptation of *P. sojae* (Arsenault-Labrecque et al., 2018; Costamilan et al., 2013; Dorrance et al., 2016; Matthiesen et al., 2016; Robertson et al., 2009; Stewart et al., 2014; Sugimoto et al., 2012; Yang et al., 2019), *Rps* genes have a limited life span of 8 to 20 years (Grau et al., 2004). While quantitative disease resistance (QDR) including partial resistance has also been used in combination with *Rps* genes to manage *P. sojae*, breeders have primarily focused on *Rps* genes, likely due to the relative ease of introgression of single, dominant genes (Dorrance, Berry, Bowen, & Lipps, 2004; Mideros, Nita, & Dorrance, 2007; Tooley & Grau, 1984). Thus, though there are still losses in high disease environments, identification of more novel *Rps* genes will help to broaden resistance against *P. sojae*.

Linkage mapping has been the primary method to identify and locate *Rps* genes. Demirbas et al. (2001) identified simple sequence repeat markers linked to *Rps1* through *Rps6* genes (ten *Rps* alleles: *Rps1*, *Rps1b*, *Rps1c*, *Rps1d*, *Rps1k*, *Rps2*, *Rps3*, *Rps4*, *Rps5*, and *Rps6*) using eleven parental near-isogenic lines. *Rps1a* and *Rps7* were mapped on Chr 3 with an F_2 population derived from a cross between OX281 and Mukden (Weng, Yu, Anderson, & Poysa, 2001). Also, $F_{2:3}$ families derived from bi-parental crosses were used for mapping *Rps8* on Chr 13 (Gordon et al., 2006), *Rps9* on Chr 3 (Wu et al., 2011a), and *Rps10* on Chr 17 (Zhang et al., 2013a). This same approach was used to map *Rps* genes, *Rps11* on Chr 7, *Rps12* on Chr 18, *RpsHC18* on Chr 3, *RpsZS18* on Chr 2, *RpsUN1* on Chr 3, and *RpsUN2* on Chr 16, from sources of resistance of plant introductions (PIs) and Chinese soybean cultivars (Li et al., 2016; Lin et al., 2013; Ping et al., 2016; Sahoo et al., 2017; Zhang et al., 2013a, 2013b; Zhong et al., 2018b; Zhong, Sun, Li, Duan, & Zhu, 2018a).

Association mapping was also used to identify *Rps* genes within natural germplasm panels based on the correlation between genetic markers and resistance response. A total of 14 SNPs were associated with resistance towards *P. sojae* through the screening of a soybean mini-core collection of 224 Chinese germplasms with 1,645 SNP markers and eleven *P. sojae* isolates (Huang et al., 2016). Qin et al. (2017) carried out genome-wide association (GWA) mapping using race-specific phenotypic data, publicly available from USDA GRIN (http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?51), in a diverse soybean germplasm panel of PIs from maturity groups IV and V, originating from 10 countries. Across more than 30,000 SNPs, this study detected 32 SNPs significantly associated with *P. sojae* resistance from the disease response data from *P. sojae* races 1, 3, 7, 17, and 25.

The objective of our study was to identify novel *Rps* alleles suitable for the development of new soybean cultivars with Phytophthora resistance by using various sources of *P. sojae* isolates and soybean PIs. We applied three strategies to identify novel and previously known *Rps* genes via GWA analyses. First, we used 448 *G. max* and 520 *G. soja*, known as a good source for genetic improvement in cultivated soybean (Hyten et al., 2006), accessions evaluated with a pool of three *P. sojae* isolates (Matthiesen et al., 2016). Secondly, we used two panels of 429 and

460 *G. max* PIs, examined with two highly virulent *P. sojae* isolates, respectively (Rolling, Schneider, Dorrance, & McHale, 2020). Finally, we collected publicly available data from USDA GRIN on the resistance phenotypes of *G. max* accessions against twelve races of *P. sojae* (Dorrance & Schmitthenner, 2000; Kyle, Nickell, Nelson, & Pedersen, 1998; Lohnes, Nickell, & Schmitthenner, 1996). These phenotypic data were each combined with genotypic data from the SoySNP50K iSelect BeadChip and three analytic methods, compressed mixed linear model (CMLM), multi-locus mixed model (MLMM), and Fixed and random model Circulating Probability Unification (FarmCPU), were utilized to identify *Rps* loci (genes and QTL). In this study, a total of 75 new *Rps* loci were identified for *P. sojae* resistance among 16 panels comprised of a total of 1,813 PIs.

Core Ideas

- Sixteen soybean panels were used to identify novel *Rps* genes against *P. sojae*.
- Panels each comprised of 109 to 520 PIs for a total of 1,813 PIs were screened.
- Data from a total of 4,302 disease reactions were used in GWA analyses.
- GWA analyses identified a total of 75 novel *Rps* loci from the 16 soybean panels.

2 | MATERIALS AND METHODS

2.1 | Plant materials and phenotypic evaluations

A total of 1,813 PIs comprised of sixteen soybean germplasm panels were evaluated in this study (Table 1). Some accessions were included in multiple panels resulting in a total dataset of 4,302 disease reactions. The first two panels were described in Matthiesen et al. (2016). Briefly, 448 *G. max* (Panel 1) and 520 *G. soja* (Panel 2) accessions from USDA GRIN were selected for screening *P. sojae* reaction with pooled inoculum from a combination of three isolates of *P. sojae*: PT2004 C2.S1 (pathotype 1a, 1b, 1c, 1d, 1k, 2, 3c, 4, 6, 7, 8), R7-2a (pathotype 1d, 2, 3a, 5, 6, 7), and 1005-2.9 (pathotype 1a, 1b, 1c, 1k, 3b, 7) (Supplemental Table S2). The level of resistance of the Panel 1 and Panel 2 accessions to the pooled *P. sojae* inoculum was assessed as a percentage of dead plants in a hypocotyl assay tested in the greenhouse (Matthiesen et al., 2016) and the percentages of dead plants were used as phenotypic data for GWA analyses (Supplemental Table S3).

Panels 3 and 4 were comprised of 429 and 460 PIs from USDA GRIN, respectively. The groups were respectively inoculated with one of two highly complex isolates, OH.12108.6.3 (OH.121) from Ohio (vir. 1a, 1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8) and PT2004 C2.S1 (vir. 1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8) (Supplemental Table S2), as described in Rolling et al. (2020) by the hypocotyl assay tested in the greenhouse. For GWA analyses, susceptible phenotype was assigned as "0," and resistance phenotype was assigned as "1". Two replicates of data were collected for each PI included in *Rps*-gene testing.

In addition to the four soybean panels, we utilized data previously deposited in the USDA GRIN database (Dorrance & Schmitthenner, 2000) for 376 *G. max* accessions with resistance to 12 *P. sojae* races which differed from those used for Panels 1 to 4 (Table 1). This provided 12 additional soybean panels, each assayed with one of the following races: 1, 3, 4, 5, 7, 10, 12, 17, 20, 25, 30T, and 31 (Table 1). Similar to the Panels 3 and 4, susceptible/resistant was assigned as "0" or "1", respectively, for analyzing phenotypic data with three GWA models.

2.2 | Genotypic data and linkage disequilibrium (LD) block establishment

Publicly available SNP marker data (https://soybase.org/snps/) of all PIs was obtained from the Soy50K SNPs data repository (Song et al., 2013, 2015). We followed the same criteria for filtering SNPs as Lee et al. (2019), briefly described as: i) removing SNPs due to monomorphism or low minor allele frequency (< 0.05), ii) eliminating additional SNPs due to higher than 10% of missing genotypes (i.e., undefined genotypes) or undetermined chromosomal position and iii) imputing by TASSEL 5.0 software (Bradbury et al., 2007) with LD k-nearest neighbors imputation option (Money et al., 2015). After filtering, the total number of SNPs used for each GWA was dependent on the specific germplasm panel and each *P. sojae* isolate (Supplemental Table S4).

Haploview 4.2 (Barrett, Fry, Maller, & Daly, 2005) was used to determine haplotype blocks by the four-gamete method (Wang, Akey, Zhang, Chakraborty, & Jin, 2002) with a Hardy-Weinberg cutoff of $\alpha = 0.01$. A SNP was excluded in the haplotype block if addition of the SNP to the block resulted in a recombinant allele at a frequency exceeding 1%. Also, if adjacent blocks were separated by less than 10 kb, these blocks were combined (Schneider et al., 2016).

TABLE 1 Soybean panels evaluated in this study by *Phytophthora sojae* isolate/race and its phenotype

Panel	Species	*Phytophthora sojae* isolate/race	Resistant[a]	Heterogeneous[a]	Susceptible[a]	Number of lines	Heritability[b]	References
1	*Glycine max*	PT2004 C2.S1, R7-2a, 1005-2.9	80 (17.9%)	79 (17.6%)	289 (64.5%)	448	0.60	Matthiesen et al. (2016)
2	*G. soja*	PT2004 C2.S1, R7-2a, 1005-2.9	60 (11.5%)	83 (16.0%)	377 (72.5%)	520	0.21	Matthiesen et al. (2016)
3	*G. max*	OH.12108.6.3	46 (10.7%)	0 (0.0%)	383 (89.3%)	429	0.42	Rolling et al. (2020)
4	*G. max*	PT2004 C2.S1	22 (4.8%)	0 (0.0%)	438 (95.2%)	460	0.87	Rolling et al. (2020)
5	*G. max*	race 1	310 (85.4%)	8 (2.2%)	45 (12.4%)	363	1.00	USDA Germplam Resource Information Network (GRIN)[c]
6	*G. max*	race 3	229 (90.5%)	7 (2.8%)	17 (6.7%)	253	1.00	GRIN
7	*G. max*	race 4	191 (76.4%)	23 (9.2%)	36 (14.4%)	250	0.73	GRIN
8	*G. max*	race 5	138 (81.2%)	13 (7.6%)	19 (11.2%)	170	0.43	GRIN
9	*G. max*	race 7	253 (82.4%)	5 (1.6%)	49 (16.0%)	307	0.54	GRIN
10	*G. max*	race 10	84 (75.7%)	5 (4.5%)	22 (19.8%)	111	1.00	GRIN
11	*G. max*	race 12	23 (20.7%)	23 (20.7%)	65 (58.6%)	111	0.16	GRIN
12	*G. max*	race 17	207 (77.2%)	7 (2.6%)	54 (20.1%)	268	0.84	GRIN
13	*G. max*	race 20	39 (37.8%)	2 (1.8%)	71 (63.4%)	112	0.54	GRIN
14	*G. max*	race 25	187 (69.5%)	23 (8.6%)	59 (21.9%)	269	0.55	GRIN
15	*G. max*	race 30T	102 (83.6%)	0 (0.0%)	20 (16.4%)	122	0.35	GRIN
16	*G. max*	race 31	91 (83.5%)	0 (0.0%)	18 (16.5%)	109	1.00	GRIN

[a]Resistant, > 70%; Heterogenous, 30–70%; Susceptible < 30% of survival rate after *P. sojae* inoculation.
[b]Estimated heritability was extracted from the optimal compression output of Genome Association Prediction Tool (Lipka et al., 2012).
[c]http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?51.

2.3 | Genome-wide association analyses

All GWA analyses and establishments of significant thresholds were conducted in an R implementation (http://www.r-project.org). Associations between genotypic and phenotypic data were examined using three models, CMLM (Z. Zhang et al., 2010), MLMM (Segura et al., 2012) and FarmCPU (Liu, Huang, Fan, Buckler, & Zhang, 2016), in order to select the appropriate QQ-plots for identifying *Rps* loci. For CMLM and MLMM_cof (a modified version of MLMM), which allow principal components (PCs) to be used as covariates, all parameters and steps were followed as previously described by Lee et al. (2019). Thresholds for genome-wide (5%) and suggestive significance (10%) were calculated by the same procedure as Lee et al. (2019), with significant thresholds being dependent on the panel used in the GWA analysis (Supplemental Table S4). In addition to the default parameters for FarmCPU, the optimal number of PCs determined by the Bayesian information criterion in Genome Association Prediction Tool (Lipka et al., 2012) were used as covariates. Unlike CMLM and MLMM, the reduced computation time of FarmCPU allowed us to carry out permutations to better control for false positives and negatives (Liu et al., 2016). A total of 10,000 permutations were performed to identify genome-wide (5%) and suggestive (10%) significant thresholds for FarmCPU GWA analyses. Manhattan and quantile-quantile plots were used to visualize each association (Turner, 2014).

2.4 | Identification of nucleotide-binding site leucine-rich repeat proteins

Most *Resistance* genes (*R*-genes) identified to date in plants encode nucleotide-binding site leucine-rich repeat (NBS-LRR, NLR) proteins involved in the recognition of various pathogens (McHale, Tan, Koehl, & Michelmore, 2006). In order to examine the presence of a NB-ARC (nucleotide-binding adaptor shared by APAF-1, R proteins, and CED-4) domain, which is a conserved region found in NLR proteins (McHale et al., 2006), protein sequences of the second assembly of the Williams 82 reference genome (Wm.82.a2.v1) were downloaded from SoyBase (http://soybase.org). NB-ARC domains (PF00931) were identified by PfamScan, which uses HMMER (Finn, Clements, & Eddy, 2011) to search the Pfam protein database (Mistry, Bateman, & Finn, 2007) based on Hidden Markov Models (E-value < 0.1). Sequences with an NB-domain represent an initial list of putative NLR encoding genes.

The list of putative NLR encoding genes was further scrutinized by searching for the presence of conserved motifs within the NB-ARC domains. Common domains including P-loop, GLPL, Kinase, and RNBS motifs (Xue et al., 2012) were identified using the MEME Suite (http://meme-suite.org, Bailey et al., 2009), assuming one occurrence in each NLR domain, a maximum motif width of 20 amino acids, and an E-value < 0.01. Genes were considered as truncated NLR encoding if the amino acid sequences lacked these conserved motifs or were less than 200 amino acids in length. Genes predicted to encode proteins greater than 200 amino acids in length with at least seven of the ten MEME-predicted conserved motifs were identified as NLR encoding resistance gene analogs (Supplemental Table S5). In order to investigate associations among *Rps* loci identified by our study, previously identified *Rps* genes, NLR, and significant SNPs identified by previous GWA studies, a map representing physical distances was drawn with MapChart 2.32 (Voorrips, 2002).

3 | RESULTS AND DISCUSSION

Many known *Rps* genes are not effective towards all populations of *P. sojae* (Anderson et al., 2012; Costamilan et al., 2013; Dorrance et al., 2016; Kaitany et al., 2001; Ryley et al., 1998; Yan & Nelson, 2019; Yang et al., 2019), yet a wealth of data exits for the resistance reaction of PIs to various isolates with complex pathotypes (Matthiesen et al., 2016; Rolling et al., 2020; USDA GRIN database). Thus, the purpose of our study was to identify novel *P. sojae* resistance loci via GWA analyses from panels of soybean PIs with available *P. sojae* reaction data, especially with several isolates of complex pathotypes. In this study, a novel *Rps* locus is defined as an LD block containing a SNP significantly associated with resistance where the LD block was not located nearby (< 5 kb) previously identified *Rps* genes nor *Rps*-mediated QTL. However, if identified *Rps* loci were positioned nearby partial resistance QTL, these *Rps* loci were considered novel because *Rps* loci and partial resistance differ in phenotypic output (Dorrance, 2018b; Mideros et al., 2007; Tooley & Grau, 1984). We detected novel *Rps* loci associated with *P. sojae* resistance on all chromosomes except Chrs 3 and 13 (Tables 2–4; Supplemental Figure S1; Supplemental Table S6). The lack of loci identified on these two chromosomes is most likely due to many of the isolates, alone or in combination, having virulence to *Rps* loci previously identified on Chrs 3 and 13. Overall, a total of 75 out of 111 *Rps* loci significant at genome-wide (5%) and suggestive (10%) thresholds are novel and only novel *Rps* loci will be discussed.

TABLE 2 SNPs associated with resistance to *P. sojae* in 448 *Glycine max* (Panel 1) or 520 *G. soja* (Panel 2) accessions

QTL[a]	Chr	SNP	Position (bp)	LD block[b]	Panel/Method	*P*-value	Previously identified QTL[c,f]/*Rps* gene nearby[d]/marker by GWA[f]/NLR within LD block[d]
qHM6-1[g]	6	ss715594898	48,466,050	48,451,005..48,468,113	1/FarmCPU	1.07×10^{-8}###	SDS 1-3, 2-6, 16-5, SCN 17-3, 20-2 (QTL)
qHM8-1[g]	8	ss715602853	9,332,121	9,323,205..9,472,553	2/FarmCPU 2/CMLM	1.81×10^{-12}### 1.30×10^{-6}###	Fusarium lesion length 1-1, Phytoph 11-2, SDS 5-2, 15-3, 16-3 (QTL)
qHM12-1[g]	12	ss715613620	8,854,648	8,680,704..8,904,446	2/FarmCPU	4.47×10^{-8}###	SDS disease incidence 20-3 (QTL)
qHM13-1	13	ss715615005	30,628,076	30,575,285..30,646,059	1/CMLM	3.60×10^{-6}##	Phytoph 8-4, 9-3, 11-21, 12-1, Sclero 5-7, 6-6, SDS 15-1, 16-8, SDS disease incidence 21-1 (QTL) *Glyma.13g193000*, *Glyma.13g193100* (NLR)
qHM16-1	16	ss715623655	20,565,775	20,386,869..21,087,178	1/FarmCPU	3.99×10^{-6}##	Phytoph 7-1, 8-1, SCN 40-3, SDS 14-12, 15-7 (QTL) *Rps2*
qHM18-1	18	ss715632346	56,486,375	56,483,164..56,497,952[e]	2/ FarmCPU	4.17×10^{-8}###	Phytoph 13-5, SCN 29-3 (QTL) *Rps4*, *Rps5*, *Rps12*, *RpsJS*
qHM19-1[g]	19	ss715635571	46,329,244	46,249,047..46,397,235	1/CMLM	1.19×10^{-6}###	Phytoph 13-2, 15-2, SCN 29-7, SDS 9-2, 18-2 (QTL)

[a] QTL for hypocotyl test with a 1:1:1 mixture of three *Phytophthora sojae* isolates (PT2004 C2.S1, R7-2a and 1005-2.9).
[b] Linkage disequilibrium (LD) blocks were constructed based on four-gamete method. Blocks were merged if adjacent blocks were separated by < 10 kb.
[c] Previously identified QTL (http://soybase.org) were listed if QTL was located within 5 cM apart from each end of significant marker.
[d] *Rps* gene/NLR nearby was listed, if *Rps*/NLR was located within 150 kb apart from a LD block. References for *Rps* genes were listed in Supplemental Table S1.
[e] SNP was not in LD with any other SNPs, thus LD block was defined by positions of adjacent markers.
[f] All references are listed in Supplemental Table S7.
[g] Novel *Rps* locus was determined based on the information of previously known Phytophthora QTL and locations of *Rps* genes.
suggestive threshold (10%).
genome-wide significance threshold (5%).

TABLE 3 SNPs associated with resistance to *P. sojae* OH.121 (Panel 3) and C2.S1 (Panel 4)

QTL[a]	Chr	SNP	Position (bp)	LD block[b]	Panel/Method	*P*-value	Previously identified QTL[c,f]/*Rps* gene nearby[d]/marker by GWA[f]/NLR within LD block[d]
qHC2-1[g]	2	ss715584058	8,923,676	8,827,345..9,029,909	4/FarmCPU	7.94×10^{-8}###	Phytoph 14-4, SDS 13-5 (QTL)
qHC3-1	3	ss715585067	3,036,784	3,022,388..3,111,469	4/MLMM_cof 4/CMLM	3.64×10^{-10}### 2.70×10^{-6}###	Phytoph 14-5, Sclero 2-21, 3-15, 4-9, 5-13, 6-10 (QTL) *Rps1a, Rps1k, Rps9/Q, RpsX, RpsWY*
qHO3-1		ss715585371	3,607,392	3,582,559..3,617,955[e]	3/CMLM	4.73×10^{-6}##	Phytoph 14-5, Sclero 2-21, 3-15, 4-9, 5-13, 6-10 (QTL) *Rps1d, RpsYu25*
qHO3-2		ss715586333	4,289,618	4,277,380..4,296,322	3/FarmCPU 3/MLMM_cof 3/CMLM	1.75×10^{-15}### 6.90×10^{-12}### 4.94×10^{-10}###	Phytoph 14-5, SCN 4-3, 44-15, Sclero 2-21, 3-15, 4-9, 5-13, 6-10 (QTL) *Rps7*, *Rps* gene in E00003, *RpsGZ*, *RpsHC18*, *RpsUN1*, *Rps* gene in Waseshiroge ss715586321, ss715586333, ss715586336, ss715586346 (GWA)
qHC5-1[g]	5	ss715590933 ss715590944 ss715590958	33,948,991 34,007,017 34,066,088	33,998,134..34,070,006	4/CMLM 4/CMLM 4/FarmCPU	1.99×10^{-6}### 4.59×10^{-6}## 6.72×10^{-8}###	SCN 18-1 (QTL)
qHO6-1	6	ss715594028	26,103,041	25,640,707..26,528,747	3/FarmCPU	2.13×10^{-9}###	Phytoph 5-1, 6-7, 6-8, 6-9, Sclero 7-1, 7-2, SDS 11-1, 16-6 (QTL)
qHO7-1[g]	7	ss715598441	6,147,344	6,108,248..6,187,284	3/FarmCPU	3.19×10^{-7}###	Phytoph 14-8 (QTL) *Glyma.07g067900* (NLR)
qHO8-1[g]	8	ss715599604	1,476,014	1,430,415..1,506,737	3/FarmCPU	2.01×10^{-6}###	Fusarium lesion length 1-1, SDS 13-7 (QTL)
qHO9-1[g]	9	ss715605368	6,936,878	6,929,310..7,033,037	3/FarmCPU	1.76×10^{-6}###	Sclero 1-1, 1-3, 1-6, 8-3, SDS 14-7, 16-1, 18-3 (QTL)

(Continues)

TABLE 3 (Continued)

QTL[a]	Chr	SNP	Position (bp)	LD block[b]	Panel/Method	*P*-value	Previously identified QTL[c,f]/*Rps* gene nearby[d]/marker by GWA[f]/NLR within LD block[d]
qHC10-1[g]	10	ss715607061	41,316,525	41,180,154..41,423,143	4/FarmCPU	1.12×10^{-7}###	Phytoph 5-3, Sclero 2-23, 3-18, 4-10, 5-15, 6-12 (QTL)
qHC12-1[g]	12	ss715613311	6,694,566	6,679,988..6,772,514	4/MLMM_cof 4/CMLM	1.89×10^{-29}### 1.58×10^{-6}###	Phytoph 9-1, SDS disease incidence 20-3 (QTL)
qHC13-1	13	ss715616768	16,421,869	16,417,884..16,493,900	4/MLMM_cof	3.86×10^{-6}###	Phytoph 1-1, 2-1, 3-1, 4-1, 9-2, Sclero 9-5, SDS 17-2 (QTL) *RpsSN10*
qHC13-2		ss715614840	29,698,315	29,671,496..29,741,893	4/FarmCPU	1.67×10^{-7}###	Phytoph 8-4, 9-3, 11-1, 12-1, Sclero 5-7, 6-6, SDS 15-1, 16-8, SDS disease incidence 21-1 (QTL)
qHO14-1[g]	14	ss715619417	47,590,507	47,541,804..47,621,296	3/FarmCPU	3.44×10^{-8}###	
qHC15-1[g]	15	ss715620190	1,017,643	966,358..1,092,662	4/FarmCPU	1.33×10^{-8}###	Fusarium lesion length 1-3, Phytoph 14-9 (QTL)
qHC17-1	17	ss715626781	33,549,403	33,331,824..33,549,403	4/FarmCPU	9.18×10^{-10}###	Phytoph 9-5, 11-20, 12-2, SCN 16-1, 38-6, Sclero 2-9, 4-2, SDS 11-2 (QTL)
qHC18-1[g]	18	ss715630895	44,368,782	44,353,367..44,405,371	4/FarmCPU	7.86×10^{-10}###	Phytoph 14-3 (QTL)
qHO19-1[g]	19	ss715634100	3,415,217	3,377,050..3,447,202	3/FarmCPU	6.15×10^{-7}###	Sclero 10-10 (QTL)
qHC20-1[g]	20	ss715637465	34,663,053	34,622,286..34,699,144	4/MLMM_cof 4/CMLM	1.77×10^{-30}### 6.82×10^{-6}###	Phytoph 8-2, 14-10, SDS 7-6, 15-9 (QTL)
qHO20-1[g]		ss715638856	47,074,681	47,044,176..47,111,315	3/FarmCPU	1.33×10^{-7}###	SCN 44-8, 44-12 (QTL)

[a]QTL for hypocotyl test inoculated with *P. sojae* isolates, OH.121 or C2.S1.
[b]Linkage disequilibrium (LD) blocks were constructed based on four-gamete method. Blocks were merged if adjacent blocks were separated by < 10 kb.
[c]Previously identified QTL (http://soybase.org) were listed if QTL was located within 5 cM apart from each end of significant marker.
[d]*Rps* gene/NLR nearby was listed, if *Rps*/NLR was located within 150 kb apart from a LD block. References for *Rps* genes were listed in Supplemental Table S1.
[e]SNP was not in LD with any other SNPS, thus LD block was defined by positions of adjacent markers.
[f]All references are listed in Supplemental Table S7.
[g]Novel *Rps* locus was determined based on the information of previously known Phytophthora QTL and locations of *Rps* genes.
[##]suggestive threshold (10%); [###], genome-wide significance threshold (5%).

TABLE 4 QTL identified with GRIN data (Panels 5–16) by an MLMM_cof or FarmCPU model. Detailed information for each QTL is listed in Supplemental Table S6

Chr	Panel 5	Panel 6	Panel 7	Panel 8	Panel 9	Panel 10	Panel 11	Panel 12	Panel 13	Panel 14	Panel 15	Panel 16
1						qGF1-2[a] (P)[b]	qGF1-1[a] (P)					qGF1-3[a] (P)
2		qGF2-1[a] (P)	qGM2-1[a] (O)	qGF2-4[a] (N)				qGF2-2[a] (P), qGF2-3[a] (N)				
3	qGM3-4				qGM3-1, qGM3-2, qGM3-3							
4				qGF4-3[a] (O)			qGF4-1[a] (P)				qGF4-2[a] (O)	
5	qGF5-3[a] (N)		qGF5-1[a] (O), qGM5-1[a] (O)									qGF5-2[a] (O)
6								qGF6-3[a] (O)		qGF6-2		qGF6-1[a] (P)
7	qGF7-2[a] (P)			qGF7-3[a] (O)								qGF7-1[a] (N)
8		qGF8-4[a] (O)		qGF8-2[a] (O)				qGF8-1[a] (P)				qGF8-3
9	qGF9-7[a] (O)	qGF9-3[a] (O)			qGF9-5[a] (N)				qGF9-6[a] (O)	qGF9-2[a] (N)	qGF9-1[a] (N)	qGF9-4[a] (O)
10		qGF10-6			qGF10-5			qGF10-1, qGF10-3		qGF10-4		qGF10-2, qGF10-7
11		qGF11-2[a] (O)	qGF11-1[a] (O)							qGF11-2[a]		
12	qGF12-2[a] (O), qGF12-3[a] (N)			qGF12-5[a] (N)							qGF12-4[a] (N)	qGF12-1[a] (O)
13	qGM13-1	qGF13-2, qGF13-4, qGF13-6		qGF13-3		qGF13-5						qGF13-1
14	qGM14-1[a] (P)					qGF14-1[a] (O), qGF14-4[a] (N)		qGF14-3[a] (O)				qGF14-2[a] (P)
15			qGM15-1[a] (O)								qGF15-1[a] (P)	qGF15-2[a] (O), qGF15-3[a] (O)
16	qGF16-1[a] (N)				qGF16-5						qGF16-3, qGF16-4	qGF16-2, qGF16-6
17		qGF17-2[a] (N)							qGF17-1[a] (O)			
18		qGF18-1[a] (P), qGF18-2[a] (O), qGF18-4									qGF18-3[a] (P)	
19		qGF19-1[a] (N), qGF19-3[a] (O), qGF19-4[a] (O)	qGF19-2[a] (N)		qGF19-5[a] (O)							
20					qGF20-1[a] (O)							

[a]Novel *Rps* locus was determined based on the information of previously known Phytophthora QTL and locations of *Rps* genes.

[b]Parenthesis indicated novel *Rps* locus located previously identified QTL related to partial resistance to *P. soaje* (P), other soybean diseases (O), or no disease (N). Information of previously identified QTL were obtained from SoyBase (http://soybase.org), if QTL was located within 5 cM apart from each end of significant marker. 'F' or 'M' in the QTL name refers to the GWAS model of MLMM_cof or Farm CPU, respectively.

FIGURE 1 Manhattan plot for GWA of 448 *Glycine max* accessions (Panel 1) for *Phytophthora sojae* resistance. 39,860 SNPs were plotted for CMLM, MLMM_cof, and FarmCPU. Green (CMLM) and pink (FarmCPU) dots represent the SNPs significantly associated at suggestive significance thresholds (10%) (blue and red horizontal lines for CMLM & MLMM_cof, and FarmCPU, respectively)

3.1 | Novel *Rps* loci in Panels 1 and 2

The 1:1:1 mixture of three *P. sojae* isolates, PT2004 C2.S1, R7-2a, and 1005-2.9, was used to screen 448 *G. max* (Panel 1) and 520 *G. soja* (Panel 2) accessions for novel sources of resistance. The combination of these three isolates was selected for disease assays because when combined, they are virulent against most known *Rps* genes (Matthiesen et al., 2016). Of the *G. max* accessions, 17.9% (80 lines) were resistant (≤30% dead plants), 64.5% (289 lines) were susceptible (>70% dead plants), with the remaining lines possessing indeterminate phenotypes. Among *G. soja* accessions, 60 lines (11.5%) were resistant and 377 lines (72.5%) were susceptible, with the remaining lines possessing indeterminate phenotypes (Table 1; Supplemental Figure S2; Supplemental Table S3). GWA analyses using percentage of dead plants as the phenotype and implementing CMLM and FarmCPU (Supplemental Figure S3) identified a total of seven SNPs with genome-wide or suggestive associations to resistance against the combination of *P. sojae* isolates (Table 2). No suggestive or significant *Rps* loci were identified with MLMM_cof.

In Panel 1, two SNPs each for CMLM and FarmCPU models were associated with resistance to the mixture of *P. sojae* isolates at genome-wide or suggestive threshold (Figure 1; Table 2). qHM13-1 (see Table 2 footnote for designation) identified by CMLM was positioned near four previously known *Rps*-mediated (Nguyen et al., 2012) and qHM16-1 identified by FarmCPU was positioned nearby *Rps2* (Demirbas et al., 2001). While qHM19-1 identified by CMLM was not located near any previously reported *Rps* genes, it was co-localized with QTL for partial resistance to *P. sojae*, Phytoph 13-2 and Phytoph 15-2 (Wang, St. Martin, & Dorrance, 2012). The fourth *Rps* locus, qHM6-1, represents a putative novel locus for resistance to *P. sojae*. Thus, of the four suggestive loci identified from analysis of Panel 1, qHM6-1 and qHM19-1 were both reported as novel *Rps* loci (Supplemental Figure S1).

In Panel 2, three SNPs, ss715602853 on Chr 8 (qHM8-1) identified by both CMLM and FarmCPU, ss715613620 on Chr 12 (qHM12-1) by FarmCPU, and ss715632346 on Chr 18 (qHM18-1) by FarmCPU, were associated at genome-wide threshold of 5% (Figure 2; Table 2). Each of these three regions have been previously associated with soybean disease resistance. While the partial resistance QTL Phytoph 11-2 (Wang et al., 2012) was located nearby, qHM8-1 represents a novel *Rps* locus. Also, qHM12-1, which is within 5 cM of a QTL for SDS incidence, had not been previously reported for resistance to *P. sojae*. Thus, of these three loci identified in analysis of Panel 2, qHM8-1 and qHM12-1 represent suggestive novel *Rps* loci (Supplemental Figure S1).

3.2 | Novel *Rps* loci in Panels 3 and 4

A total of 429 (Panel 3) and 460 (Panel 4) *G. max* accessions were phenotyped for resistance against the complex *P. sojae* isolates, OH.121 and C2.S1, respectively (Rolling et al., 2020). In Panel 3 and Panel 4, 46 and 22 *G. max* lines showed resistance to OH.121 (10.7%) and C2.S1 (4.8%), respectively (Table 1). A total of 20 *Rps* loci represented 22 SNPs ($-\log_{10}(P) > 5.06$ and 5.09 for OH.121 and C2.S1 for both CMLM and MLMM_cof models and $-\log_{10}(P) > 5.55$ and 6.20 for OH.121 and C2.S1 in FarmCPU) (Supplemental Figure S4) with a suggestive association to resistance against *P. sojae* and were identified across 16 chromosomes,

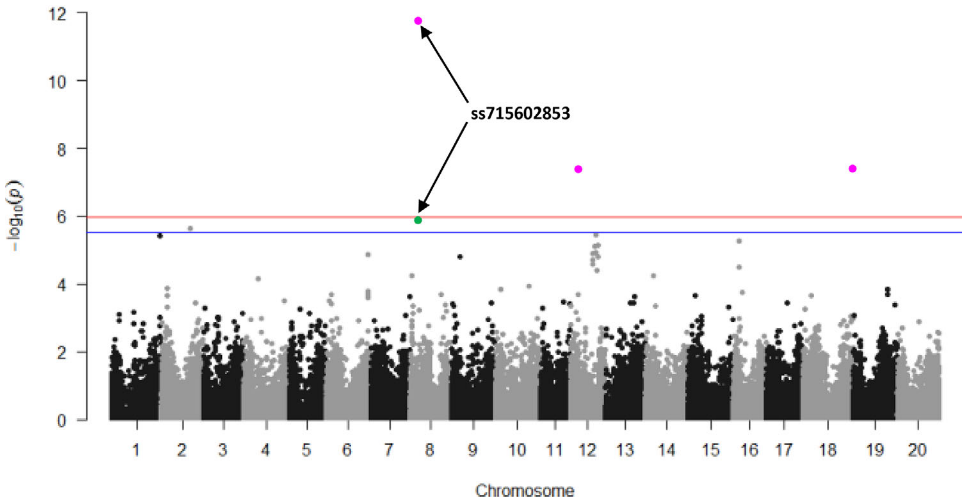

FIGURE 2 Manhattan plot for GWA of 520 *G. soja* accessions (Panel 2) for *P. sojae* resistance. 40,954 SNPs were plotted for CMLM, MLMM_cof, and FarmCPU. Green (CMLM) and pink (FarmCPU) dots represent the SNPs significantly associated at suggestive significance thresholds (10%) (blue and red horizontal lines for CMLM & MLMM_cof, and FarmCPU, respectively)

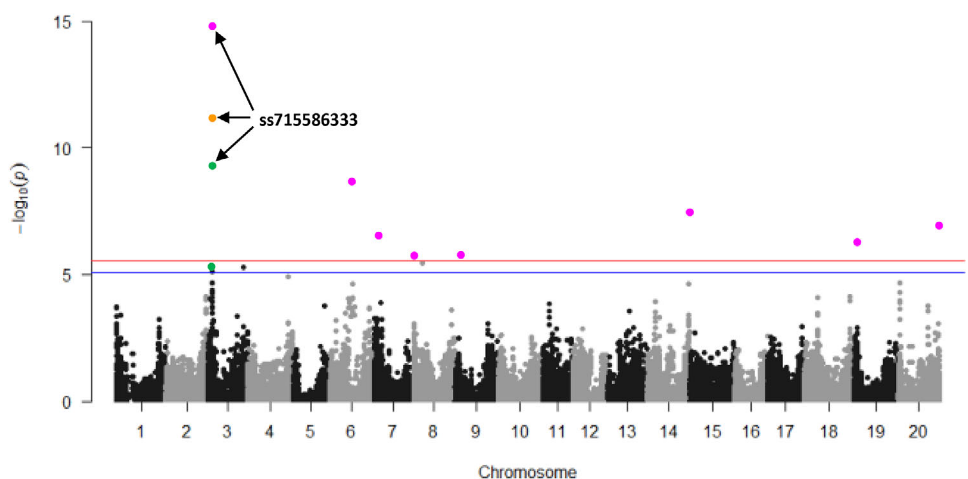

FIGURE 3 Manhattan plot for GWA of 429 *G. max* accessions (Panel 3) against OH.121 for *P. sojae* resistance. 34,247 SNPs were plotted for CMLM, MLMM_cof, and FarmCPU. Green (CMLM), orange (MLMM_cof), and pink (FarmCPU) dots represent the SNPs significantly associated at suggestive significance thresholds (10%) (blue and red horizontal lines for CMLM & MLMM_cof, and FarmCPU, respectively)

excluding Chrs 1, 4, 11, and 16 (Figures 3 and 4; Table 3). Multiple *Rps* loci were identified on Chrs 3 (qHC3-1, qHO3-1, and qHO3-2, see Table 3 footnote for designation), 13 (qHC13-1 and qHC13-2), and 20 (qHC20-1 and qHO20-1), whereas the remaining 13 chromosomes each possessed a single *Rps* locus for *P. sojae* resistance.

Thirteen *Rps* loci identified from Panels 3 and 4 represented potential novel *Rps* loci. Seven of these 13 loci (qHC2-1, qHO7-1, qHC10-1, qHC12-1, qHC15-1, qHC18-1, and qHC20-1) were co-localized with or nearby (<5 cM) previously identified QTL for partial resistance to *P. sojae*. qHC2-1 and qHO7-1 were co-localized with the QTL Phytoph 14-4 and Phytoph 14-8, respectively, against *P. sojae* isolate C2.S1 (Lee et al., 2013). The locus qHO7-1 was overlapped with both *Glyma.07g067900* (6,106,491 bp–6,110,846 bp) encoding leucine-rich repeat-containing protein and *FJ014879.1* (6,106,491 bp–6,110,714 bp) for the soybean *cw18* resistance gene. Cw18 is a lipid transfer protein shown to be induced by pathogen inoculation (Molina & Garcia-Olmedo, 1993) and suggested as a growth inhibitor of bacterial and fungal pathogens (Molina, Segura, & Garcia-Olmedo, 1993). qHC10-1 by FarmCPU and qHC12-1 by both CMLM and MLMM_cof models were located nearby partial resistance QTL, Phytoph 5-3 (Wu et al., 2011b) and Phytoph 9-1 (Wang et al., 2010), respectively. Lee et al. (2013) identified Phytoph 14-9 and

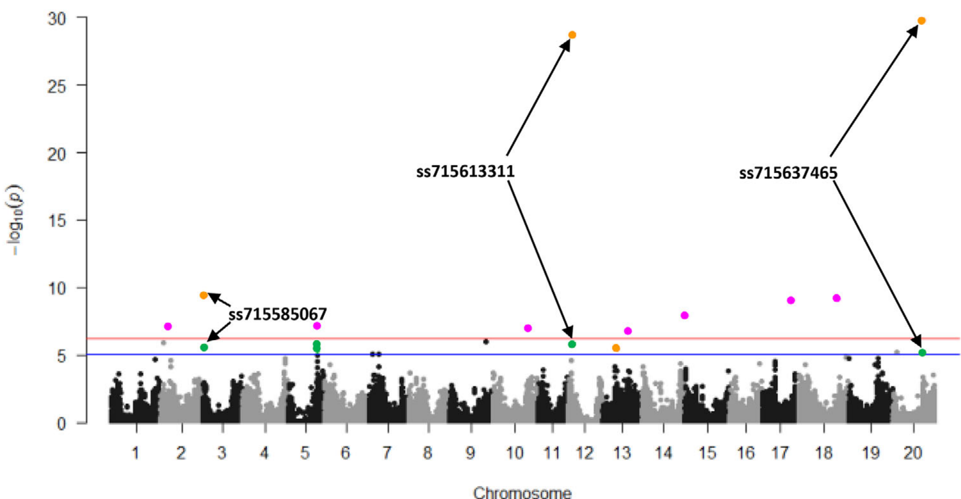

FIGURE 4 Manhattan plot for GWA of 460 *G. max* accessions (Panel 4) against C2.S1 for *P. sojae* resistance. 33,641 SNPs were plotted for CMLM, MLMM_cof, and FarmCPU. Green (CMLM), orange (MLMM_cof), and pink (FarmCPU) dots represent the SNPs significantly associated at suggestive significance thresholds (10%) (Blue and red horizontal lines for CMLM & MLMM_cof, and FarmCPU, respectively

Phytoph 14-3 for partial resistance against C2.S1, and these QTL were also positioned nearby qHC15-1 and qHC18-1, respectively. qHC20-1 was identified by both MLMM_cof and FarmCPU and two partial resistance QTL, Phytoph 8-2 (Tucker et al., 2010) and Phytoph 14-10 (Lee et al., 2013), were located nearby this *Rps* locus (Table 3). Our study detected novel *Rps* loci on Chr 20 against each *P. sojae* isolate, qHO20-1 for OH.121 resistance and qHC20-1 for C2.S1 resistance (Table 3; Supplemental Figure S1).

The locus qHO6-1 was not considered as a novel *Rps* locus because Phytoph 6-7, 6-8, and 6-9 identified with *P. sojae* isolates collected from field and greenhouse environments were placed nearby this QTL (Li et al., 2010). Phytoph 12-1 and 12-2, positioned near qHC13-2 and qHC17-1, were both identified by hypocotyl test with *P. sojae* OH2 and OH17 isolates (Nguyen et al., 2012). Consequently, these two loci were not considered novel *Rps* loci. Thus, six and seven novel *Rps* loci were identified for reaction to *P. sojae* OH.121 (Panel 3) and C2.S1 (Panel 4) resistance, respectively (Supplemental Figure S1).

3.3 | Novel *Rps* loci in Panels 5 to 16

Phenotypic data for twelve *P. sojae* races (1, 3, 4, 5, 7, 10, 12, 17, 20, 25, 30T, and 31; Panels 5–16, respectively) were obtained from USDA GRIN. While publicly available historical data represent a great resource, there are also associated limitations. For example, there are no quality metrics, experimental design, or heritability associated with the phenotypic data which has been collected on a potentially biased selection of PIs (Table 1). However, there remains value in the ready availability of phenotypic data of PIs from various origins. Unlike Panels 1 through 4, a larger proportion of PI lines showed resistance in each of Panels 5–16, ranging from 20.7% to 90.5% (Table 1). After all analytic methods were tested to identify *Rps* loci, MLMM_cof and FarmCPU were chosen because these two methods showed acceptable deviation from the expected *P*-value distribution at the tail in the QQ-plots (Supplemental Figures S5–S16). A total of 84 *Rps* loci represented by 87 SNPs (Table 4; Supplemental Table S6) were identified for genome-wide or suggestive associations (Supplemental Table S4) with resistance to *P. sojae* by MLMM_cof and FarmCPU models. These *Rps* loci were distributed on all 20 chromosomes ranging from one (Chr 20) to seven (Chrs 9, 10, and 13) *Rps* loci (Table 4; Supplemental Table S6). Overall, a total of 58 novel *Rps* loci were identified in Panel 5 through 16, ranging from 2 (Panels 11, 13, and 14) to 10 novel *Rps* loci (Panel 6) per Panel (Table 4; Supplemental Table S6).

3.3.1 | Novel *Rps* loci identified by MLMM_cof in Panels 5 to 16

MLMM_cof detected nine significant SNPs on Chrs 2, 3, 5, 13, 14, and 15, one SNP each on Chrs 2, 5, 13, 14, and 15 and four SNPs on Chr 3. These nine *Rps* loci were associated with *P. sojae* races 1 (Panel 5), 4 (Panel 7), and 7 (Panel 9) at suggestive thresholds of 10% ($-\log_{10}(P) > 5.23$, 5.19 and 5.20, respectively) (Table 4; Supplemental Figures S5b, S7b and S9b; Supplemental Table S6). All *Rps* loci on Chr 3 were located near many previously identified *Rps* genes (Table 4; Supplemental Figure S1; Supplemental Table S6). On Chr 13, qGM13-1 was co-located with Phytoph 12-1

identified by hypocotyl test (Nguyen et al., 2012). However, the remaining four *Rps* loci (qGM2-1, qGM5-1, qGM14-1, and qGM15-1) identified by MLMM_cof were novel *Rps* loci. Three of these loci, qGM2-1, qGM5-1, and qGM15-1, all associated with *P. sojae* race 4 resistance (Panel 7) and were located nearby QTL previously associated with other diseases (Table 4; Supplemental Table S6). qGM14-1 against race 1 (Panel 5) was positioned nearby a QTL for partial resistance to *P. sojae*, Phytoph 9-4 identified by Wang et al. (2010), using *P. sojae* isolate 1.S.1.1 (vir. 1a, 1b, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8). Thus, a total of 4 novel *Rps* loci were identified by MLMM_cof in Panels 5 (one *Rps* locus) and 7 (3 *Rps* loci).

3.3.2 | Novel *Rps* loci identified by FarmCPU in Panels 5 to 16

FarmCPU identified 75 *Rps* loci (78 significant SNPs) against *P. sojae* resistance and at least one SNP marker was significant on all chromosomes except Chr 3 (Table 4; Supplemental Table S6). Out of 78 significant markers, about a half were associated with *P. sojae* resistance against race 3 (vir. 1a, 1c, 7) (Panel 6; 15 SNPs on Chrs 2, 8, 9, 10, 11, 13, 17, 18, and 19) and race 31 (Panel 16; 17 SNPs on Chrs 1, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, and 16); the remaining 10 races had an association to *P. sojae* resistance with an average of 4.6 significant SNPs. Most of the *Rps* loci were located near previously identified QTL for reactions to Phytophthora and other diseases. Overall, a total of 54 novel *Rps* loci were identified by FarmCPU; 15 loci were not associated with any soybean disease, while 26 loci were related to soybean diseases other than partial resistance to *P. sojae* and 13 loci were involved in Phytophthora partial resistance.

Novel putative mechanisms for new Rps *loci identified by both Panels 4 and 16*

Interestingly, qGF5-2 (race 31, Panel 16) and qHC5-1 (C2.S1, Panel 4) share the same LD block (33,998,134 bp–34,070,006 bp), although GWA analyses were conducted with different panels (Tables 3 and 4; Supplemental Figure S1). Therefore, this 71.9 kb LD block may be a valuable region for studying resistance to *P. sojae*. For example, within this LD block, *Glyma.05g146400* and *Glym.05g146500*, which putatively encode Mannosyl-oligosaccharide glucosidases, may have a role in digesting β-glucans. β-linked glucose polysaccharides are the most abundant component of Phytophthora cell walls, and *P. sojae* generated β-glucan is one of the major polymers composing oomycete cell walls at the host-pathogen interface during infection and modulate plant innate immunity (Robinson & Bostock, 2015). *Glyma.05g146600*, putatively encoding ER Metallopeptidase I in the 71.9 kb LD block provides another interesting candidate gene for resistance to *P. sojae*. Liu, Dammann, and Bhattacharyya (2001) showed that transcript level of *GmMMP2* (a novel soybean metalloproteinase gene) was increased in soybean tissue with *P. sojae*, suggesting this gene might be involved in a novel defense response against pathogenic infections. Additionally, under stress conditions, Arabidopsis ER membrane-associated transcription factor, AtbZIP28, travels from the ER to the Golgi, where it is processed by ER Metallopeptidase I (Srivastava, Chen, Deng, Brandizzi, & Howell, 2012) and involved in programmed cell death (Eichmann & Schafer, 2012). A fourth candidate gene, *Glyma.05g146900*, putatively encoding an exostosin (heparan sulfate glycosyltransferase-related), may be function in resistance to *P. sojae*. An Arabidopsis gene encoding exostosin family protein showed significant genetic association with stress (Coolen, Van Pelt, Van Wees, & Pieterse, 2019).

Novel Rps *loci mapped nearby QTL associated with no soybean diseases or soybean diseases other than partial resistance to* P. sojae

Fifteen detected *Rps* loci were not mapped near (within 5 cM) any previously identified QTL associated with soybean diseases. These LD blocks were significant for *P. sojae* resistance against race 1 (Panel 5; qGF5-3, qGF12-3, qGF16-1), race 3 (Panel 6; qGF17-2, qGF19-1), race 4 (Panel 7; qGF19-2), race 5 (Panel 8; qGF2-4, qGF12-5), race 7 (Panel 9; qGF9-5), race 10 (Panel 10; qGF14-4), race 17 (Panel 12; qGF2-3), race 25 (Panel 14; qGF9-2), race 30T (Panel 15; qGF9-1, qGF12-4), and race 31 (Panel 16; qGF7-1), indicating a number of novel *Rps* loci as well as novel loci for resistance in general (Table 4; Supplemental Figure S1; Supplemental Table S6). Also, one (Chrs 6, 7, 17, 18, and 20), two (Chrs 4, 5, 8, 11, 12, 14, and 15), three (Chr 9), and four (Chr 9) *Rps* loci, for a total of 26 novel *Rps* loci, were positioned nearby (within 5 cM) any previously identified QTL associated with soybean diseases other than partial resistance to *P. sojae*, such as Fusarium root rot, Rhizoctonia root and hypocotyl rot, Sclerotinia stem rot, soybean cyst nematode, soybean sudden death syndrome, etc. Thus, 41 novel *Rps* loci associated with none (15 loci) or soybean diseases other than partial resistance to *P. sojae* (26 loci) were identified (Table 4; Supplemental Figure S1; Supplemental Table S6).

Novel Rps *loci mapped nearby QTL associated with partial resistance to* P. sojae

Rps loci and partial resistance QTL potentially might share a complex defense network with multiple mechanisms or have various phenotypic output depending on the isolate (St. Clair, 2010; Wang et al., 2012). So, our identified *Rps* loci could be novel, although they were located nearby

QTL previously associated with partial resistance towards *P. sojae*. Among the *Rps* loci identified by FarmCPU, 13 from Panels 5–16 were nearby partial resistance QTL and therefore were considered as novel *Rps* loci (Table 4; Supplemental Table S6).

On Chr 1, three *Rps* loci, qGF1-1 to race 12 (Panel 11), qGF1-2 to race 10 (Panel 10), and qGF1-3 to race 31 (Panel 16) were identified (Table 4; Supplemental Table S6). The LD block of each *Rps* locus was relatively narrow, 103 kb for qGF1-1, 56 kb for qGF1-2, and 38 kb for qGF1-3. Each of these loci were co-localized with Phytoph 13-3 related to partial resistance to *P. sojae* isolate 1.S.1.1 (Wang et al., 2012). *Glyma.01g171000,* annotated as Arabidopsis disease resistance protein (CC-NBS-LRR class) family, was located only 118 kb away from qGF1-1. Two more NLRs (*Glyma.01g183300* and *Glyma.01g183400*), annotated as Arabidopsis disease resistance protein (CC-NBS-LRR class) (AT5G35450, Benschop et al., 2007), were located ∼400 kb upstream of qGF1-2. Like qHC2-1, qGF2-1 (race 3, Panel 6) and qGF2-2 (race 17, Panel 12) were co-localized with Phytoph 14-4 associated with partial resistance to *P. sojae* isolate C2.S1 (Lee et al., 2013). Our *Rps* loci on Chr 2 identified by this study were novel QTL for *P. sojae* resistance (Supplemental Figure S1).

Only the GRIN data were able to detect *Rps* loci associated with *P. sojae* resistance on Chr 4, qGF4-1 for race 12 (Panel 11), qGF4-2 for race 30T (Panel 15), and qGF4-3 for race 5 (Panel 8) (Supplemental Figure S1). A previously identified QTL related to *P. sojae* partial resistance (Phytoph 14-7, Lee et al., 2013) was located near qGF4-1. Among three *Rps* loci (qGF6-1, qGF6-2, and qGF6-3) identified with GRIN data, the previously identified QTL for *P. sojae* resistance were positioned near qGF6-1 and qGF6-2. qGF6-1 (race 31, Panel 16) is the novel *Rps* locus because Phytoph 5-1, with partial resistance against race 2, was located nearby (Wu et al., 2011b). However, qGF6-2 for race 25 resistance was not considered to be a novel *Rps* locus since Phytoph 6-6 and 6-8, conferring *Rps*-mediated or partial resistance to *P. sojae* conducted in field and greenhouse, are positioned nearby (Li et al., 2010).

Like qHO7-1, qGF7-2 (race 1, Panel 5) was co-localized with Phytoph 14-8 associated with partial resistance to *P. sojae* isolate C2.S1 (Lee et al., 2013). qGF8-1 for race 17 resistance was novel, as a QTL for partial resistance to *P. sojae*, Phytoph 11-2 (Wang et al., 2012), was located nearby this *Rps* locus. qGF8-3 for race 31 resistance is positioned near previously known QTL for *P. sojae* resistance (Phytoph 6-4, Li et al., 2010) and not a novel *Rps* locus. None of the known *Rps* genes, SNP markers by GWA studies, or NLRs were located within LD block of these *Rps* loci on Chrs 4, 6, 7, and 8 (Supplemental Figure S1). qGF14-2 (race 30T resistance) was also positioned nearby a partial resistance QTL, Phytoph 9-4 identified by Wang et al. (2010). Lee et al. (2013) detected Phytoph 14-9 for partial resistance against C2.S1, and this QTL was located nearby qGF15-1 (race 30T resistance).

Among the four *Rps* loci on Chr 18 (Table 4; Supplemental Table S6), three (qGF18-1, qGF18-3, and qGF18-4) were located near previously reported *P. sojae* partial resistance QTL. qGF18-1 (associated with race 3 resistance) and qGF18-3 (race 30T resistance) were positioned near Phtyoph 8-3 (Tucker et al., 2010) and Phytoph 14-3 (Lee et al., 2013), respectively. qGF18-4 (race 3 resistance) is located not only near Phytoph 14-3 but also *Rps6*. Thus, qGF18-1 and qGF18-3 out of these three *Rps* loci identified by our study are novel QTL (Table 4; Supplemental Table S6). In summary, a total of 13 *Rps* loci positioned nearby partial resistance QTL to *P. sojae* are novel.

4 | CONCLUSIONS

Numerous studies have identified genes and genetic markers contributing to both *R*-gene and quantitative resistance towards *P. sojae* for management applications as well as study of the pathogen diversity. Since *P. sojae* populations continue to increase in complexity of virulence and rapidly adapt to *Rps* genes (Anderson et al., 2012; Costamilan et al., 2013; Dorrance et al., 2016; Grau et al., 2004; Kaitany et al., 2001; Ryley et al., 1998; Schmitthenner et al., 1994; Stewart et al., 2014; Yan & Nelson, 2019), studies for identifying novel *Rps* genes are needed for development of new commercial soybean cultivars. Our study identified a total of 75 novel *Rps* loci for *P. sojae* resistance using 16 panels, with novel loci distributed across all chromosomes except Chrs 3 and 13. Three GWA models identified novel *Rps* loci, one by CMLM, four by MLMM_cof, and 66 by FarmCPU and two novel loci each were detected by two models, namely CMLM & MLMM_cof and CMLM & FarmCPU. Evaluations using diverse pathogen panels are needed to verify resistance to *P. sojae*, while continuous monitoring of pathogen virulence towards these novel *Rps* loci is necessary to determine their utility. Each of these practices, evaluations of diverse pathogen panel and continuous monitoring, are essential in the development of new soybean cultivars with resistances that are effective against numerous *P. sojae* populations. Panel 1 and Panels 5–16 shared 335 PIs and eight and five PIs were overlapped in Panels 1 and 3 and Panels 1 and 4, respectively. Also, Panels 1, 3, and 5–16 and Panels 1, 4, and 5–16 shared 18 and 15 PIs, respectively. Panel 2, comprised entirely of *G. soja* PIs, had no overlap with the other panels. A total of 77 PIs from Panel 1 showed less than 30% dead plants after screening with pooled inoculum. The same set were also resistant when tested with 12 *P. sojae* races in Panels 5–16. Therefore, the set of 77 resistant PIs may be good candidates

for developing new soybean cultivars with *P. sojae* resistance through breeding programs using the *Rps* resistance-associated DNA markers of this study. The comprehensive tables and figures of this report containing linkage maps, candidate/known function genes, identified NLRs, small LD blocks and previously observed QTL, provide a useful resource for soybean disease workers.

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CONFLICT OF INTEREST

None of the authors have competing interests.

AUTHOR CONTRIBUTIONS

K. Van carried out association analyses, wrote draft/edited the manuscript.

W. Rolling performed disease assays and NLR identification and revised manuscript.

R.M. Biyashev conducted field operations and managed data.

R.L. Matthiesen, N.S. Abeysekara, and D.J. Veney performed disease assays.

A.E. Robertson supervised disease assays and revised manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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