**RESEARCH**

High-Density Mapping of Resistance QTL Toward *Phytophthora sojae*, *Pythium irregulare*, and *Fusarium graminearum* in the Same Soybean Population

Anna K. Stasko, Damitha Wickramasinghe, Brittany J. Nauth, Bhupendra Acharya, Margaret L. Ellis, Christopher G. Taylor, Leah K. McHale, and Anne E. Dorrance*

**ABSTRACT**

*Phytophthora sojae* Kaufm. and Gerd., *Pythium irregulare* Busiman, and *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwien.) Petch] are important pathogens of soybean [*Glycine max* (L.) Merr.] and are all capable of causing seed rot, damping-off, and root rot. The objective of this study was to identify quantitative trait loci (QTL) for resistance to *Py. irregulare* and to refine previously mapped QTL for resistance to *P. sojae* and *F. graminearum* in a larger, more advanced ‘Conrad’ × ‘Sloan’ F₉:₁¹ recombinant inbred line population. The population was mapped with 1032 single nucleotide polymorphisms from the SoySNP6K BeadChip and 31 polymerase chain reaction–based molecular markers. Families were evaluated for resistance response to three isolates of *P. sojae*, one isolate of *Py. irregulare*, and one isolate of *F. graminearum*. A total of 10, 2, and 3 QTL and suggestive QTL were found that confer resistance to *P. sojae*, *Py. irregulare*, and *F. graminearum*, respectively. Individual QTL explained 2 to 13.6% of the phenotypic variance. Quantitative trait loci for resistance toward both *Py. irregulare* and *F. graminearum* colocalized on chromosome 19. This resistance was contributed by Sloan and was juxtaposed to a QTL for *P. sojae* with resistance contributed from Conrad. Alleles for resistance to different pathogens contributed from different parents in the same region; the number of unique QTL for each pathogen and the lack of correlation of resistance suggest that different mechanisms are involved in resistance toward *P. sojae*, *Py. irregulare*, and *F. graminearum*.

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**Abbreviations:** BLUP, best linear unbiased predictor; CIM, composite interval mapping; dai, days after inoculation; DSI, disease severity index; kb, kilobase pairs; LOD, logarithm of odds; Mb, Megabase pairs; PAMSA, PCR amplification of multiple specific alleles; PCR, polymerase chain reaction; PI, plant introduction; PV, phenotypic variance; QTL, quantitative trait locus, loci; REML, restricted maximum likelihood; RIL, recombinant inbred line; SCN, soybean cyst nematode; SNP, single nucleotide polymorphism; SSR, simple sequence repeat.

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F. graminearum is best known for causing Fusarium head blight on wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), oat (Avena sativa L.) and Gibberella ear and stalk rot on maize (Zea mays L.). It has also been reported from several regions as a pathogen of soybean (Broders et al., 2007b; Díaz Arias et al., 2013a; Ellis et al., 2011; Pioli et al., 2004; Xue et al., 2007). Seed and seedling diseases caused by these pathogens have become more prevalent in Ohio and in several soybean growing areas of the North Central region as indicated by recent surveys in Iowa, Illinois, North Dakota, and Ontario, Canada (Broders et al., 2007a,b; Díaz Arias et al., 2013a,b; Ellis et al., 2011; Jiang et al., 2012; Marchand et al., 2014; Murillo-Williams and Pedersen, 2008; Rizvi and Yang, 1996; Zhang and Yang, 2000; Zitnick-Anderson and Nelson, 2014). This may be because of changes in management practices such as an earlier planting date, which delays germination due to cool soil conditions and increases the amount of time seeds are exposed to pathogens, and the long-term use of no-till and reduced-till systems, which increases the amount of inoculum in the seed bed (Broders et al., 2007a; Ellis et al., 2011, 2012, 2013; Workneh et al., 1998, 1999). Fungicides are often applied as seed treatments to manage these diseases. However, there have been changes in fungicide chemistries in recent years, and several of these fungicides have limited efficacy toward one or more of these pathogens (Broders et al., 2007a,b; Ellis et al., 2011).

Other management strategies for seed and seedling pathogens include tilling fields to improve drainage and rotating crops to prevent inoculum buildup. Most growers in Ohio use a corn–soybean or corn–soybean–wheat rotation (Broders et al., 2007b). This rotation strategy might be effective against P. sojae, which primarily infects soybean, but would not be effective against Py. irregulare and F. graminearum as these pathogens can infect both corn and wheat as well. Host resistance offers a more cost-effective management strategy for producers. It has been used effectively to manage P. sojae for more than fifty years (Bernard et al., 1957; Grau et al., 2004; Schmitthenner, 1985), but very little is known about resistance to Py. irregulare (Ellis et al., 2013) and F. graminearum (Acharya et al., 2015; Ellis et al., 2012).

The soybean cultivar ‘Conrad’ (Fehr et al., 1989) has been identified as a source of resistance to P. sojae and F. graminearum (Burnham et al., 2003; Li et al., 2010; Ellis et al., 2012; Wang et al., 2010, 2012a,b), while the cultivar ‘Sloan’ (Bahrenfuss and Fehr, 1980) is moderately to highly susceptible to these same pathogens (Ellis et al., 2012; Wang et al., 2010, 2012a,b). Both cultivars have similar levels of moderate susceptibility to Py. irregulare (Ellis et al., 2013). Quantitative trait loci (QTL) to P. sojae and F. graminearum were mapped in an earlier generation of a recombinant inbred line (RIL) population derived from a cross between Conrad and Sloan (Ellis et al., 2012; Wang et al., 2010, 2012a,b). Ten QTL were associated with resistance toward P. sojae and mapped to chromosomes 1, 12, 13, 14, 17, 18, and 19 in the F_{4,6} and F_{5,8} generations (Wang et al., 2010, 2012b). In the F_{5,8} generation, five QTL conferring resistance to F. graminearum were mapped to chromosomes 8, 13, 15, 16, and 19 (Ellis et al., 2012). Quantitative trait loci for both P. sojae and F. graminearum overlapped on chromosomes 13, 16, and 19 (Ellis et al., 2012). For both pathogens, only minor QTL, which contribute less than 20% phenotypic variance (PV; St. Clair, 2010), were detected (Ellis et al., 2012; Wang et al., 2010, 2012a,b).

In these earlier studies some of the QTL encompassed large regions of the chromosome. For example, the markers that flanked two QTL mapped to chromosome 19, conferring resistance to P. sojae, were ~0.5 Megabase pairs (Mb) and 1.5 Mb apart and explained 4.8 and 11.9% PV (Wang et al., 2012b). In a breeding program, it is impractical to introduce such large chromosomal regions into adapted germplasm. Additionally, it is expected that only a single or few genes underlying a resistance QTL contribute to the resistance response. As well as in some cases, genes underlying a QTL for a specific trait may contribute undesirable agronomic traits (Brouwer and St. Clair, 2004). Identifying key genes involved in defense and refining QTL to regions more narrowly defined by markers would greatly assist breeding efforts to incorporate quantitative resistance into adapted germplasm. It would also facilitate the targeting of candidate genes for cloning and functional analysis. Thus, from approximately 400 RILs derived from a cross between Conrad and Sloan, 186 to 262 lines were used for mapping in previous studies (Ellis et al., 2012; Wang et al., 2010, 2012a,b), and the whole population was advanced to the F_{9,11} generation. In the current study, this larger, advanced population of 316 remaining lines was used to map QTL conferring resistance to P. sojae, Py. irregulare, and F. graminearum. In addition to a larger population size, more markers were used to increase the mapping resolution compared with previous studies (Ellis et al., 2012; Wang et al., 2010, 2012a,b). Phytophthora sojae is a hemibiotroph, and Py. irregulare and F. graminearum have been regarded both as necrotrophs and, in more recent discussions, as hemibiotrophs (Adie et al., 2007; Brown et al., 2010; Trail, 2009). One difference that has been used to characterize necrotrophs from hemibiotrophs is the formation of specialized feeding structures within the host cell. Fusarium graminearum does not form haustoria within infected plant tissue on wheat (Brown et al., 2010) while P. sojae does on soybean (Enkerli et al., 1997). Pythium irregulare was reported to form haustoria-like structures in Arabidopsis thaliana (L.) Heynh. (Adie et al., 2007), but no studies have been reported to date of similar structures in soybean. Additionally, no RxLR effector type sequences were identified in Py. irregulare (Adhikari et al., 2013).
Thus, this RIL population, segregating for resistance toward three pathogens, affords a unique look into the inheritance of resistance toward a hemibiotroph and two necrotrophs within the same population. Therefore, the objectives of this work were to: (i) identify and compare QTL conferring resistance to one or more pathogens; (ii) confirm and more narrowly define the QTL identified in previous studies on a larger, more advance population; and (iii) identify candidate genes for further functional analysis to determine the mechanisms involved in quantitative resistance.

MATERIALS AND METHODS

Plant Materials
A RIL population derived from a cross between Conrad and Sloan was advanced from the F<sub>6:8</sub> population (Wang et al., 2012b) by single seed descent to the F<sub>9</sub> generation. Single rows of F<sub>9:10</sub> plants were bulk-harvested in 2012 to generate F<sub>9:11</sub> RILs used in this study. For P. sojae OH25 phenotypic assays, seed of single rows of F<sub>9:12</sub> were bulk-harvested in 2014.

Resistance to Phytophthora sojae Assay
A population of 316 F<sub>9:11</sub> RILs was phenotyped for resistance to P. sojae by means of the tray assay as described previously (Burnham et al., 2003; Lee et al., 2013a,b; Wang et al., 2010, 2012a,b). In brief, plants were grown in vermiculite (Perlite Vermiculite Packing Industries, Inc.) in the greenhouse for 7 d, and the roots were washed in tap water to remove vermiculite. Ten plants from each RIL were placed on a polyester cloth, which was on top of a cotton wicking pad on a plastic tray. A small scratch was made on the main tap root of the plants approximately 2 cm below the crown region. The wound was covered with a mycelial slurry of a 7–9-d-old isolate of P. sojae grown on dilute lima bean (Phaseolus lunatus L.) agar. Following inoculation, trays were placed in buckets and kept in a growth chamber at 25°C, 14 h light:10 h dark, and 20% relative humidity. Seven days after inoculation (dai), the lesion length was measured from the top of the inoculation site to the leading edge of the lesion margin.

Three different isolates of P. sojae were used to identify QTL in separate experiments, PT2004C2.S1 (hereafter C2.S1), 1.S.1.1. (both vir 1a, 1b, 1k, 2, 3a, 3c, 4, 5, 6, and 7), and OH25 (vir 1a, 1b, 1c, 1k, and 7). Each experiment used a randomized incomplete block design, and each block contained at least 110 RILs that were divided into six buckets for C2.S1, 40 to 116 RILs divided into two to ten buckets for 1.S.1.1, and at least 164 RILs divided into nine buckets for OH25. The parents were used as checks in each bucket. Each RIL was evaluated three separate times for both isolates. Best linear unbiased predictor (BLUP) values for each RIL were calculated from the mean of the lesion measurements within each bucket using PROC MIXED in SAS v.9.1 as previously described. The model was

\[
yijk = \mu + G_i + R(G)_{ij} + S(RG)_{ijk} + C_l + L(C)_{ln} + \varepsilon_{ijklm}
\]

where \(\mu\) is overall mean, \(G_i\) is the effect of the \(i\)th group, \(R(G)_{ij}\) is the effect of the \(j\)th replication in the \(i\)th group, \(S(RG)_{ijk}\) is the effect of the \(i\)th set in \(j\)th replication in the \(i\)th group, \(C_l\) is the effect of the \(l\)th class of entry (Conrad, Sloan, or experimental line), \(L(C)_{ln}\) is the effect of the \(n\)th genotype within class (genotypic variance, \(\sigma^2_e\)) and \(\varepsilon_{ijklm}\) is the experimental error (\(\sigma^2_{e}\)). (Acharya et al., 2015; Burnham et al., 2003; Ellis et al., 2012; Stroup, 1989; Wang et al., 2010, 2012a, 2012b). Class was treated as fixed, all other effects were treated as random. Variance components were estimated using the restricted maximum likelihood (REML) method (Patterson and Thompson, 1971). Broad-sense heritability was calculated as \(\sigma^2_{g}/(\sigma^2_{g} + \sigma^2_{e}/r)\), where \(\sigma^2_{g}\) is the genetic variance within the RILs, \(\sigma^2_{e}\) is the variance of error, and \(r\) is the number of replications per RIL. To examine possible relationships between resistance for a given isolate to resistance for another isolate, BLUP values were correlated using PROC CORR in SAS. In a separate analysis to examine possible effects due to an isolate × genotype interaction, the adjusted mean lesion lengths of a given RIL for all three isolates of P. sojae were analyzed using PROC GLM in SAS.

Resistance to Pythium irregularare Assay
Three hundred and sixteen RILs of the same population used for the resistance to P. sojae were screened for resistance to Py. irregularare using a sand-cornmeal cup assay as previously described (Broders et al., 2007a; Ellis et al., 2013; Kirkpatrick et al., 2006). In summary, each spawn bag (Myco Supply) was filled with 50 mL of cornmeal, 950 mL of sand, and 250 mL of deionized water, then sterilized on two successive days for 60 min. Eight 10-mm plugs of Py. irregularare isolate Brown 2–3–5, which was chosen because of its high pathogenicity on soybean (Ellis et al., 2012), from a 3–d-old culture grown on potato carrot agar (PCA) were added to the bags. The spawn bags were sealed, incubated at room temperature (approximately 24 to 26°C), and shaken every other day to ensure even colonization of the sand-cornmeal mixture by Py. irregularare. After 10 d, inoculum from each bag was mixed with fine vermiculite in a 1:4 (vol:vol) ratio of inoculum to vermiculite. Styrofoam cups (0.5 L) with holes in the bottom for drainage were filled with 100 mL of coarse vermiculite and 300 mL of inoculum-vermiculite mixture. Eight seeds were placed directly on top of the inoculum-vermiculite mixture in each cup and covered with 100 mL of coarse vermiculite.

Fresh root weight was collected 14 d after planting. To do this, plants were removed from the cups, and the inoculum-vermiculite mixture was washed from the roots. This experiment was arranged as a randomized complete block design with four replications over time. A total of 32 seedlings were evaluated from each F<sub>9:11</sub> family for resistance to Py. irregularare.

Best linear unbiased predictor values for each RIL were calculated from the fresh root weight of each RIL using PROC MIXED. The model applied was

\[
Y_{ijklm} = \mu + R_i + C_l + G(C)_{im} + \varepsilon_{ijklm}
\]

Variables are defined and effects are treated as described above for the P. sojae lesion length tray assay. Variance components were estimated using the REML method (Patterson and Thompson, 1971), and broad-sense heritability was calculated as \(\sigma^2_{g}/(\sigma^2_{g} + \sigma^2_{e}/r)\), where \(\sigma^2_{g}\) is the genetic variance, and \(r\) and \(\sigma^2_{e}\) are as described above.

Resistance to Fusarium graminearum Assay
A total of 316 F<sub>9:11</sub> RILs of the same population used for the P. sojae and Py. irregularare assays was also screened for resistance to F. graminearum by means of a roll towel assay modified from Ellis.
et al. (2011). *Fusarium graminearum* isolate Fay11, which was collected from soybean and is highly aggressive (Ellis et al., 2011), was cultured on mung bean [*Vigna radiate* (L.) R. Wilczek] agar for 10 d. Sterile water was added to the plates, and macroconidia were dislodged using a sterile glass rod. The suspension was filtered through cheesecloth to remove any mycelia in the solution. The concentration of macroconidia was calculated using a hemocytometer (Bright-Line Hemacytometer, Hauser Scientific) and was adjusted to 2.5 × 10^7 macroconidia mL^−1 by adding sterile water as needed. Fifteen seeds from each RIL were placed on a moistened germination towel and inoculated with 100 μL of macroconidia suspension. A second moistened towel was placed on top of inoculated seedlings. The towels were then rolled and placed on a wire mesh in a 25-L bucket, covered with a black plastic bag and kept in the dark at room temperature (approximately 24 to 26°C). Ten dai, the towels were removed from the bucket. Lesion length and total plant length were measured on each seedling. The disease severity index (DSI) was calculated by dividing the lesion length by the total plant length and multiplying by 100. The mean DSI of the seedlings in each F_9,1 family was used to obtain BLUP values as described previously (Ellis et al., 2012; Stroup, 1989). The population was evaluated in an incomplete block design with three replications. Each replication consisted of multiple subsets of the population and contained F_0,1 families; two parents; and plant introgression (PI) 567301B, a resistant line (Acharaya et al., 2015); ‘Wyandot’ (Ohio State University–Ohio Agricultural Research and Development Center), a moderately resistant cultivar (Acharaya et al., 2015); and Williams (Bernard and Lindahl, 1972), a susceptible cultivar (Ellis et al., 2012). The model applied was the same as for the calculation of BLUPs from the *P. sojae* lesion length tray assay described above with the exception that the C_i is the effect of the ith class of entry (Conrad, Sloan, PI 567301B, Wyandot, Williams, and experimental lines). Variance components were estimated using the REML method, and broad-sense heritability was calculated as σ^2_y/(σ^2_y + σ^2/μ).

**Single Nucleotide Polymorphism Genotyping and Genetic Mapping**

Fresh leaf tissue was collected from 316 RILs of the Conrad × Sloan population, and DNA was extracted using hexadecyltrimethylammonium bromide (CTAB) extraction modified from Doyle and Dickson (1987). DNA concentration and quality were checked with an ND-1000 Nanodrop (Nanodrop Technologies), with PicoGreen dsDNA quantification using a Beckman Coulter multimode detector (Beckman Coulter Inc.), and by electrophoresis on a 1% agarose gel. Samples were diluted to 50 ng/μL and sent for single nucleotide polymorphism (SNP) genotyping at the Genome Center, University of California, Davis, using the Illumina Infinium SoySNP6K BeadChip, a subset of the SoySNP50K BeadChip (Song et al., 2013). A total of 5403 SNPs out of 6000 SNPs passed manufacturing quality control and were used in the genotyping. The resulting marker genotypes were analyzed in GenomeStudio software v2011.1 (Illumina Inc.) with a GenCall threshold of 0.15. Recombinant inbred lines with a call rate less than 80% and SNPs that were monomorphic or displayed ambiguous clustering were removed from the analysis. The SNP data for the remaining samples was exported and used for genetic mapping. Individual lines and SNP markers with more than 10% missing genotypes or more than 10% heterozygous genotypes were removed from the data set. Markers with a significantly different segregation ratio from the expected ratio calculated by chi-square goodness-of-fit test were also removed. Genetic maps were created using Kosambi’s mapping function in JoinMap 4.0 (van Ooijen, 2006). The maximum likelihood mapping algorithm was used with logarithm of odds (LOD) threshold of 2 for grouping. Additional simple sequence repeat markers (SSR) and polymerase chain reaction (PCR) amplification of multiple specific alleles (PAMSA) markers that were localized to the previously known QTL were added to the map to fill in gaps as previously described (Song et al., 2010; Wang et al., 2012b). The graphical presentation of genetic maps was performed using custom script in Biopython (https://github.com/ajwije/chromosome_map; accessed 29 May 2016; Cock et al., 2009).

**QTL Analysis**

Best linear unbiased predictor values for the adjusted mean lesion length following inoculation with *P. sojae*, root weights for Py. irregulare, and for disease severity from *F. graminearum* were used for QTL analysis. Composite interval mapping (CIM) was performed using MapQTL 5 to identify putative QTL locations (van Ooijen, 2004). Permutation tests with 1000 iterations were performed on a genomewide and chromosomewide basis to identify significant LOD threshold levels (Churchill and Doerge, 1994). Genomewide thresholds were applied to identify significant QTL and chromosomewide thresholds were applied to identify suggestive QTL (Lander and Kruglyak, 1995; van Ooijen, 1999). Graphs were created with ggplot2 package (Wickham, 2009) in R (R Development Core Team, 2012).

**Gene Annotation and Categorization**

Genes underlying each QTL were identified from the soybean genome browser on SoyBase (Grant et al., 2010) by entering the physical map coordinates of the flanking markers. Genes were categorized based on Panther, GO, and PFAM terms from SoyBase. Only genes associated with QTL with suggestive or significant CIM LOD scores and overlapping for at least two isolates of *P. sojae* are reported here.

**RESULTS**

**Phenotypic Assays Phytophthora sojae**

For all three isolates of *P. sojae*, C2.S1, 1.S.1.1, and OH25, lesions developed on most seedlings in each of the F_9,11 RIL families. In some cases for 1.S.1.1 and OH25, 20 to 40% of seedlings on a tray for a given RIL did not have lesions in a given replication. For the final analysis, these few seedlings with no lesions were not included in the final means as lack of lesion development could be due to failed inoculation or infection efficiency. The lesion lengths were significantly different (*p* < 0.0001, Fisher’s protected LSD) between the parents for all three isolates (Table 1). The mean lesion length of the RILs ranged from 15.3 mm to 51.7 mm for C2.S1, 7.3 mm to 61.5 mm for...
were 8.5 and 29.1 for Conrad and Sloan, respectively. The BLUP values of checks PI 567301B, Wyandot, and Williams were 43.5, 11.0, and 9.0, respectively. Sixty-one RILs had BLUP values lower than Conrad, indicating transgressive segregation for resistance. The broad-sense heritability for the mean DSI was 0.73.

To investigate isolate-specific interactions to *P. sojae*, correlations between the BLUP values for each RIL for both isolates were examined. Pearson's correlation coefficient was 0.50 (*p* < 0.001) for resistance to C2.S1 and resistance to 1.S.1.1, 0.41 (*p* < 0.001) for resistance to C2.S1 and resistance to OH25, and 0.36 (*p* < 0.001) for resistance to 1.S.1.1 and to OH25. For all three isolates, BLUP values were calculated and had a normal distribution (Fig. 1), indicating that resistance is quantitative. Where a lower BLUP value indicates a higher level of resistance, the BLUP values for Conrad were estimated at −12.63, at −16.96, and at −14.65 for C2.S1, 1.S.1.1, and OH25, respectively. The BLUP values for Sloan were estimated at 0.00 for all three isolates. The broad-sense heritability was 0.54 for C2.S1, 0.53 for 1.S.1.1, and 0.67 for OH25.

**Pythium irregular**

Preemergence damping-off occurred in approximately 66 to 90% of the F<sub>9:11</sub> RIL families and the parents in the greenhouse assay. Root rot developed in all families and on both parents. There was a significant difference (*p* < 0.0001) among the RILs and the two parents for root weight (Table 1). The data were normally distributed, indicating a quantitatively inherited trait (Fig. 2A). Fifteen RILs had root weights significantly greater than Sloan, indicating transgressive segregation for resistance. The broad-sense heritability was 0.52.

**Fusarium graminearum**

The mean DSI was significantly different among the two parents and the checks, indicating that Conrad had moderate level of resistance and Sloan was susceptible to *F. graminearum* as previously reported (Ellis et al., 2012; Table 1). The means of parents and checks were separated by Fisher’s Protected LSD (*p* < 0.0001; Table 1). The mean DSI among the F<sub>9:11</sub> RILs ranged from 11.2 to 89.9%, and the overall mean DSI of all the RILs was 49.7%. The BLUP values calculated from the DSI were normally distributed in this population (Fig. 2B). The BLUP values were −8.5 and 29.1 for Conrad and Sloan, respectively. The BLUP values of checks PI 567301B, Wyandot, and Williams were −43.5, −11.0, and 9.0, respectively. Sixty-one RILs had BLUP values lower than Conrad, indicating transgressive segregation for resistance. The broad-sense heritability for the mean DSI was 0.73.

To investigate isolate-specific interactions to *P. sojae*, correlations between the BLUP values for each RIL for both isolates were examined. Pearson’s correlation coefficient was 0.50 (*p* < 0.001) for resistance to C2.S1 and resistance to 1.S.1.1, 0.41 (*p* < 0.001) for resistance to C2.S1 and resistance to OH25, and 0.36 (*p* < 0.001) for resistance to 1.S.1.1 and to OH25.

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Table 1. Means of parents, checks, and recombinant inbred line (RIL) populations derived from a cross of Conrad × Sloan following inoculations with three isolates of *Phytophthora sojae* (tray test), an isolate of *Pythium irregularare* (greenhouse cup assay), and an isolate of *Fusarium graminearum* (rolled towel assay) to measure levels of resistance.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th><em>P. sojae</em> C2.S1</th>
<th><em>P. sojae</em> 1.S.1.1</th>
<th><em>P. sojae</em> OH25</th>
<th><em>Py. irregularare</em></th>
<th><em>F. graminearum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Conrad</td>
<td>24.1 a</td>
<td>21.9 a</td>
<td>22.8 a</td>
<td>1.9 a</td>
<td>43.2 a</td>
</tr>
<tr>
<td>Sloan</td>
<td>36.8 b</td>
<td>38.7 b</td>
<td>34.3 b</td>
<td>3.3 b</td>
<td>80.7 b</td>
</tr>
<tr>
<td>PI 567301B</td>
<td>−#</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>8.1 c</td>
</tr>
<tr>
<td>Wyandot</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>40.6 a</td>
</tr>
<tr>
<td>Williams</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>60.6 d</td>
</tr>
<tr>
<td>RILs range</td>
<td>15.3–51.7</td>
<td>7.3–61.5</td>
<td>7.0–59.1</td>
<td>0.27–6.87</td>
<td>11.2–89.9</td>
</tr>
</tbody>
</table>

† mm, mean lesion length.
‡ g, means of root weight.
§ Disease severity index (DSI) = (lesion length/plant length) × 100.
¶ Values within columns followed by the same letter are not significantly different by Fisher’s protected LSD (0.05).
# Not tested.

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Fig. 1. Frequency distribution of best linear unbiased predictor (BLUP) values of mean lesion length of ‘Conrad’ × ‘Sloan’ F<sub>9:11</sub> recombinant inbred line families for resistance to *Phytophthora sojae* isolates (A) C2.S1, (B) 1.S.1.1, and (C) OH25 evaluated with tray phenotyping assay. Arrows indicate values of parents and/or checks. A smaller BLUP value indicates a higher level of resistance.
OH25, indicating a moderate correlation between resistance of the different isolates (Table 2). Additionally, no significant isolate × genotype interaction for the mean lesion length of each RIL was observed ($p = 0.1934$).

The correlation among the responses to the three pathogens from the RILs was also investigated. There was no significant correlation between two of the $P. sojae$ isolates (C2.S1 and 1.S.1.1) and the resistance response of the RILs toward $Py. irregulare$ or $F. graminearum$ (Table 2). However, there was a significant but very minor negative correlation between resistance to $P. sojae$ isolate OH25 and resistance to $Py. irregulare$ ($-0.13; p = 0.02$). There was also a moderate correlation ($-0.52; p < 0.0001$) between resistance to $Py. irregulare$ and $F. graminearum$ for BLUP values calculated from root weight and DSI, respectively.

### Table 2. Pearson’s correlation coefficients above the diagonal and significance below for the comparison of resistance response among recombinant inbred F$_{9:11}$ families derived from a cross of Conrad × Sloan between pathogens and between isolates of Phytophthora sojae.

<table>
<thead>
<tr>
<th>Pathogen isolate</th>
<th>$P. sojae$ C2.S1</th>
<th>$P. sojae$ 1.S.1.1</th>
<th>$P. sojae$ OH25</th>
<th>$Py. irregulare$ Br2-3-5</th>
<th>$F. graminearum$ Fay11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytophthora sojae C2.S1</td>
<td>–</td>
<td>0.50</td>
<td>0.41</td>
<td>–0.04</td>
<td>–0.00</td>
</tr>
<tr>
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<tr>
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<td>***</td>
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<tr>
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<td>ns</td>
<td>*</td>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
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</tbody>
</table>

* $P$ value < 0.05.

*** $P$ value < 0.0001.

† ns, indicates not significant.

OH25, indicating a moderate correlation between resistance of the different isolates (Table 2). Additionally, no significant isolate × genotype interaction for the mean lesion length of each RIL was observed ($p = 0.1934$).

### Linkage Map Construction

Among the 6000 SNP markers from the Soy6KSNP BeadChip, 5403 markers were scored in the array, but only 1133 markers were polymorphic between the two parents. From these, 1032 markers formed 23 linkage groups that matched with 20 chromosomes. Four chromosomes, 7, 13, 17, and 19, were represented by two linkage groups each (Supplemental Fig. S1). There were insufficient polymorphic SNP markers to form chromosome 10. An additional 30 PCR-based markers, and one phenotype marker, flower color, were used to fill gaps in the preliminary genetic map. Following the addition of these 31 markers, the total map length was 1908.7 cM. The average marker interval was 1.8 cM per marker. There were a total of 13 gaps that were larger than 20 cM in the map, and the largest was 43 cM located on chromosome 7. The highest number of gaps (>20 cM) per chromosome was four on chromosome 6 and three on chromosome 7. The marker order position on the genetic map was mostly aligned with the physical positions of markers annotated on Glyma.Wm82.a2.v1 (Grant et al., 2010; Supplemental Table S1).

### QTL Mapping Phytophthora sojae

Three QTL (including suggestive QTL that were significant at the chromosomeswide threshold) conferring resistance to all three $P. sojae$ isolates C2.S1, 1.S.1.1, and OH25 were mapped to chromosomes 1, 18, and 19-1 (Table 3). Two QTL conferring resistance to C2.S1 and 1.S.1.1 but not to OH25 were mapped to chromosomes 4 and 16. A suggestive QTL on chromosome 16 for OH25 mapped to a different location than the QTL for C2.S1 and 1.S.1.1. A second QTL on chromosome 19–2 and a QTL on chromosome 9 were detected for C2.S1 and OH25 but were not significant for 1.S.1.1 (Fig. 3; Table 3; Supplemental Fig. S6). Isolate-specific QTL on chromosome 15 and 19–3 were detected for C2.S1 and OH25,
Table 3. Quantitative trait loci (QTL) for quantitative resistance to *Phytophthora sojae*, *Pythium irregulare*, and *Fusarium graminearum* identified by composite interval mapping (CIM) using 316 F$_{2}$, recombinant inbred lines of ‘Conrad’ × ‘Sloan’.

<table>
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<tr>
<th>Chromosome</th>
<th>QTL name</th>
<th>Nearest marker†</th>
<th>Left marker</th>
<th>Right marker</th>
<th>Isolate</th>
<th>CIM‡</th>
<th>PV%§</th>
<th>Source¶</th>
<th>LOD thr#</th>
<th>GW LOD thr ††</th>
<th>Additive effect‡‡</th>
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<td>Sloan</td>
<td>2.0</td>
<td>3.2</td>
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</tbody>
</table>

† Name of single nucleotide polymorphism marker (*BARC* . Glyma.Wm82 assembly version chromosome_physical position), simple sequence repeat, or polymerase chain reaction amplification of multiple specific alleles (PAMSA) marker.
‡ LOD value for composite interval mapping calculated in MapQTL (van Ooijen, 2004).
§ Phenotypic variation explained by individual QTL as calculated in MapQTL (van Ooijen, 2004).
¶ Source of resistance.
# Chromosomewide LOD threshold based on permutation tests of 1000 iterations (Churchill and Doerge, 1994).
†† Genomewide LOD threshold based on permutation tests of 1000 iterations.
‡‡ Additive effects of nearest markers as calculated in MapQTL (van Ooijen, 2004).
§§ Suggestive QTL are italicized.
respectively. The resistant alleles at each QTL were primarily contributed by the resistant parent Conrad except for QTL on chromosomes 4, 16-1, and 16-2, which were contributed by the susceptible parent Sloan. The CIM LOD values ranged from 2.06 to 5.25, from 2.70 to 7.13, and from 2.06 to 10.01 for C2.S1, 1.S.1.1, and OH25, respectively. The QTL were all minor and individually explained approximately 2 to 13.6% of the PV across each isolate, with the highest contributed by the QTL on chromosome 18 of 13.6% for isolate OH25 (Table 3).

Pythium irregulare

Two QTL associated with resistance to *Py. irregulare* with significant LOD scores were identified through CIM. The first QTL was located on chromosome 14 and was flanked by markers BARC_2.0_Gm14_16152064 and BARC_2.0_Gm14_2131853 and was responsible for 6.6% of the PV. The second QTL on 19-2, flanked by PAMSA markers Glyma19g41390 (Wm82.a2.v1 name Glyma.19G230600) and Glyma19g41870 (Wm82.a2.v1 name Glyma.19G230600), was responsible for 5.5% of the PV (Fig. 4; Table 3). Resistance alleles for both QTL were contributed from Sloan.

Fusarium graminearum

Three QTL conferring resistance to *F. graminearum* were identified on chromosomes 13 (suggestive), 14, and 19-2 by CIM (Fig. 5; Table 3). The suggestive QTL on chromosome 13, flanked by markers BARC_2.0_Gm13_16811968 and BARC_2.0_Gm13_17047053, explained 3.1% of the PV, and alleles conferring resistance were contributed from Conrad (Table 3). The QTL on chromosome 14 was flanked...
by markers BARC_2.0_Gm14_2405177 and BARC_2.0_Gm14_2762413, and QTL on chromosome 19-2 was flanked by PAMSA markers Glyma19g41390 (Wm82.a2.v1 name Glyma.19G226100) and Glyma19g41870 (Wm82.a2.v1 name Glyma.19G230600), which explained 4.8 and 8.6% of the PV, respectively (Table 3). These overlap the QTL for *Py. irregulare*, and the resistance alleles were also contributed by Sloan (Table 3; Fig. 6).

All QTL detected in the current study can be classified minor QTL because they explained less than 15% of PV (St. Clair, 2010). Therefore, it seems more likely that there are several to many genes with small effects involved in these resistances, rather than few genes at a major QTL, as can be the case for other quantitative traits (Young, 1996). Additionally, Wang et al. (2012b) reported that several genes between the markers flanking QTL on chromosome 19 had sequence differences and/or expression differences between Conrad and Sloan. As multiple mechanisms are likely to be contributing to quantitative resistance (Poland et al., 2009), genes within the markers flanking QTL were sorted into categories based on Panther, GO, and PFAM information from SoyBase to begin to identify potential candidates and pathways that may be involved in resistance to each pathogen. Here, we report on genes within eight QTL regions at which at least two QTL were colocalized.

For *P. sojae*, many of the genes (91 genes) within the eight QTL regions have unknown functions. Of those with known annotations, 44 and 26 genes were involved in metabolism and signal transduction, respectively (Table 4). Interestingly, no genes encoding PR proteins were within the QTL of this particular study, and only 11 were annotated as playing a role in stress response.

On chromosome 1, 53 genes were associated with the QTL, including genes predicted to encode two leucine-rich repeats, an E2 ubiquitin conjugating enzyme, a thioredoxin, and several transcription factors. Forty-nine genes were associated with the QTL on chromosome 4, including an ubiquitin-like protease. There were 28 genes associated with the QTL on chromosome 16, which included genes predicted to encode a leucine-rich repeat containing, *Rps4* related protein. The QTL on chromosome 18 was associated with 21 genes, including an NB-ARC-encoding gene, an oxidoreductase-encoding gene, and several unknown genes (Supplemental Table S4). The QTL 19-1 was associated with 156 genes and 19-2 was associated with 32 genes (Table 4). In previous studies, QTL 19-1 was more narrowly delimited and found to be associated with 53 genes, while QTL 19-2 was broader and associated with 175 genes (Wang et al., 2012b).

The genes between the flanking markers of 19-1 include genes predicted to encode an alcohol dehydrogenase,
myosin, a GDSL-like lipase, a 26S protease subunit, a heat shock protein, a WKRY transcription factor, MYB-like transcription factors, several leucine-rich repeat proteins, as well as a Mlo family gene. The QTL 19-2 associated genes included genes encoding bHLH and MYB transcription factors, a nitrate transporter, a two-component sensor histidine kinase, and a pectinesterase (Supplemental Table S4).

For Py. irregulare, there were 65 genes associated with the QTL on chromosome 14, including genes predicted to encode TIR domain containing leucine-rich repeat
proteins, heat shock proteins, a WRKY transcription factor, and a sucrose synthase. On chromosome 19, there were 46 genes within the flanking markers of the QTL, including genes predicted to encode a clathrin coat assembly protein, a thioredoxin, and a stress response protein (Supplemental Table S5).

For the *F. graminearum* QTL, on chromosome 14, 37 genes were associated with the QTL, including genes predicted to encode a heat shock transcription factor, a leucine-rich repeat receptor-like kinase, a serine threonine kinase, and several chromatin remodeling genes (Supplemental Table S5). On chromosome 13a there were 23 genes within the flanking markers of the QTL. These included genes predicted to encode a serine threonine kinase, lipases, a cytochrome P450, and a peroxidase. The resistance QTL on chromosome 19 was associated with the same 46 genes as described above for *Py. irregulare* (Table 4; Fig. 6; Supplemental Table S5).

**DISCUSSION**

Host resistance is a key component of managing soil-borne seed, seedling, and root pathogens on many field crops, particularly soybean. Quantitative resistance is also effective against pathotypes (races) of biotrophic pathogens (Kou and Wang, 2010; Hulbert and Pumphrey, 2014; Pavelviet and Zadoks, 1977; Poland et al., 2009; St. Clair, 2010; Umarus, 1970). Additionally, it is often the only source of resistance for necrotrophic pathogens such as *Py. irregulare* and *F. graminearum* (Glazebrook, 2005; Kou and Wang, 2010; Kou and Wang, 2012). Prior to cloning genes from resistant genotypes associated with quantitative disease resistance in plants, characterizing the type of resistance, identifying and mapping the loci, and narrowing the locus requires a population that is both advanced in generation and large in size to capture the greatest number of recombination events (St. Clair, 2010; Xu et al., 2013). There have been several advancements in this arena where genes associated with quantitative resistance have been cloned and identified. In wheat, two genes for “slow rusting” in cereals were cloned, *Yr36* and *Li34*, which encode a kinase START lipid binding domain (Fu et al., 2009) and an ATP-binding cassette (ABC) transporter (Krattinger et al., 2009). Interestingly, *Li34* also contributes resistance to leaf rust, stripe rust, and powdery mildew as well as leaf-tip necrosis. Kou and Wang (2012) reviewed the mechanisms associated with characterized genes that contribute to QTL for disease resistance in rice and broke them into categories. The first and the largest number of characterized genes are those that were classified as defense responsive genes that lead to the expression of R gene mediated or basal resistance such as WRKY transcription factors and other defense responsive genes. One example was GH3-2, which differs in the promoter region, but not in the protein coding region between resistant and susceptible genotypes.

Following inoculation, this gene is activated in the resistant genotypes earlier than in the susceptible genotypes (Fu et al., 2011). The second category was types of NBS-LRR protein, such as *Ph1*, which contributes to a major QTL for *Magnaporthe oryzae* but has an atypical NBS domain. The third were “undetermined type of genes” such as *pi21* for resistance toward *Magnaporthe oryzae* first reported by Fukuoka et al. (2009), which encodes a metal transport detoxification protein, and different alleles of this gene are found in resistant and susceptible genotypes. Finally there are QTL with multiple physically clustered genes as well as colocated QTL that confer resistance toward different pathogens (Kou and Wang, 2012).

In a study more closely related to the present work, Cook et al. (2012) characterized a QTL for soybean cyst nematode (SCN; *Heterodera glycines* Riggs and Niblack), *rht1-b*, which contains genes for an amino acid transporter, an α-SNAP protein, and a wound inducible protein. Interestingly, in susceptible cultivars, there was only 1 copy of each of these genes, but in resistant lines there were 10 tandem copies. Overexpression of each gene individually in a susceptible background did not improve the resistance levels; only the simultaneous overexpression of all of the genes increased the expression of resistance to SCN.

Previously we had mapped earlier generations of this Conrad × Sloan population for resistance to *P. sojae* isolates 1.S.1.1, C2.S1, and OH25 (Wang et al., 2010, 2012a,b) and *F. graminearum* (Ellis et al., 2012). This is our first report of resistance to *Py. irregulare* for this population. Additionally, it was expected that the QTL would map to narrower regions of the chromosome because of the larger mapping population, as these tend to give better estimates of QTL location and genetic variance (Utz et al., 2000; Vales et al., 2005). In this study 316 lines were used, compared with previous studies in which 186 lines (Wang et al., 2010) and 246 lines (Wang et al., 2012b) for *P. sojae* resistance, and 262 lines were used to map for *F. graminearum* resistance (Ellis et al., 2012). The development of the SoySNP6K BeadChip (Song et al., 2013) also allowed for the generation of a denser genetic map, albeit there were still large gaps (Supplemental Figs. S1–S4) in this map because of the large number of monomorphic markers and potential identity by descent between Conrad and Sloan (Fehr et al., 1989; Bahrenfus and Fehr, 1980).

**Phytophthora sojae**

In a previous study by Wang et al. (2012b), five resistance QTL were contributed by Conrad: two on chromosome 19, two on chromosome 18, and one on chromosome 1. A QTL was confirmed on chromosome 1 in this study with all three isolates, though it was flanked by markers that mapped 1.2–1.6 Mb below the previous markers. This QTL is also near other QTL conferring resistance to *P. sojae* from populations derived from six plant introductions (Lee et al., 2010; Umaerus, 1970). Additionally, it is often the only source of resistance for necrotrophic pathogens such as *Py. irregulare* and *F. graminearum* (Glazebrook, 2005; Kou and Wang, 2010) and 246 lines (Wang et al., 2012b) for *P. sojae* resistance, and 262 lines were used to map for *F. graminearum* resistance (Ellis et al., 2012). The development of the SoySNP6K BeadChip (Song et al., 2013) also allowed for the generation of a denser genetic map, albeit there were still large gaps (Supplemental Figs. S1–S4) in this map because of the large number of monomorphic markers and potential identity by descent between Conrad and Sloan (Fehr et al., 1989; Bahrenfus and Fehr, 1980).
2013b, 2014). A QTL conferring resistance to SCN was also reported in this region (Wu et al., 2009). In the current study, only one QTL on chromosome 18 was confirmed. In the earlier study in the F_{4:6} population, this QTL was not detected with CIM but was detected with interval mapping (Wang et al., 2012a). Other QTL in the region include resistance to *P. sojae* (Lee et al., 2013a,b, 2014), SCN (Kabelka et al., 2005), southern root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood] (Tamulonis et al., 1997), and iron efficiency (Lin et al., 1997). Both QTL on chromosome 19 were confirmed with C2.S1 and OH25, but with 1.S.1.1, only one was detected. The second QTL on chromosome 19 may be environmentally sensitive or otherwise unstable, as Wang et al. (2012a) reported that this QTL was detected for 1.S.1.1 and OH25 but not for C2.S1. In the present study, QTL 19-1 was flanked by markers that mapped below the previous markers on the chromosome for 1.S.1.1 and OH25 and was broader than expected for C2.S1 on the basis of previous studies (Supplemental Table S2; Wang et al., 2012a). The QTL 19-2 was narrower in this study compared with the F_{6:8} generation, though as noted above it was detected with different isolates (Wang et al., 2012b). Additionally, a third, potentially novel QTL on chromosome 19 was detected with OH25. It is important to note, however, that QTL on the same chromosome can be difficult to resolve (Young, 1996) and may account for the varied QTL localization on chromosome 19 for the three isolates in this study compared with previous studies.

We observe a similar phenomenon on chromosome 16 for OH25, where the QTL mapped to a region approximately 2.6 Mb higher than the QTL 16-2 for C2.S1 and 1.S.1.1. Quantitative trait locus 16-2 was about 30 Mb away from a QTL for resistance to *P. sojae* from PI 427105B (Lee et al., 2014). A novel QTL on chromosome 4, with the resistant allele contributed from Conrad, was detected on chromosome 14; this QTL was not detected in the present study compared with the F_{6:8} generation but was reported from PI 398841 (Lee et al., 2013b). A QTL conferring resistance to *Fusarium virguliforme* is also in this region (Abdelmajid et al., 2012). An additional, novel suggestive QTL on chromosome 15 was detected with the isolate C2.S1 but not with the isolates 1.S.1.1 or OH25. Isolate-specific QTL have been previously observed (Lee et al., 2014; Wang et al., 2012a) and could indicate that a minor-gene-for-minor-gene interaction is involved in some of the resistance conferred by Conrad. However, in at least one instance (chromosome 19-2), our further study showed that a QTL identified as isolate specific in an earlier study was more likely environmentally influenced or unstable. Isolate-specific resistance QTL have been detected for other diseases including *Puccinia hordei* on barley (Gonzalez et al., 2012; Qi et al., 1999) and *Puccinia triticina* on wheat (Azzimonti et al., 2014). However, this study would need to be repeated and tested with several other isolates of *P. sojae* to confirm that the QTL are stable and isolate specific.

### Pythium irregulare

This is our first report of resistance to *Py. irregulare* in this population. While Conrad was previously identified as a possible source of resistance to *Py. irregulare* (Ellis et al., 2013), in this study the two QTL conferring resistance came from the second parent, Sloan. One of the QTL directly overlapped (QTL 19-2) or was near (273 kilobase pairs [kb]) the QTL on chromosome 14 for *F. graminearum*. The QTL on chromosome 14 is approximately 12 kb away from a previously reported QTL conferring resistance to *Py. irregulare* in a (Williams × PH424354) × OH303 population (Ellis et al., 2013).

### Fusarium graminearum

In a previous study by Ellis et al. (2012), four QTL for resistance toward *F. graminearum* on chromosomes 8, 13, 15, and 16 had resistance alleles contributed by Conrad, and one QTL on chromosome 19 had the resistant allele contributed from Sloan. In the present study, a QTL on chromosome 19 with resistance contributed from Sloan mapped to approximately the same region (about 26 kb below) as reported in the previous study by Ellis et al. (2012). The QTL on chromosome 13 was about 3.3 Mb away from a QTL previously reported on the same chromosome (Ellis et al., 2012). While the same assay methods and same isolates were used, the differences in the QTL locations in this study compared with the study by Ellis et al. (2012) may be due to the use of a more advanced population with higher number of RILs and denser genetic map. Quantitative trait loci were not detected on chromosomes 8, 15, and 16 in this study compared with the earlier study by Ellis et al. (2012; Supplemental Table S2).

A novel QTL with resistance contributed from Sloan was detected on chromosome 14; this QTL was not detected in the previous study by Ellis et al. (2012). Interestingly, the QTL on chromosome 13 conferring resistance to *F. graminearum* also overlapped with QTL conferring resistance to the necrotroph *Sclerotinia sclerotiorum* (Lib.) de Bary (Guo et al., 2008) and *F. virguliforme* as previously reported (Kassem et al., 2006).

Most QTL were unique to each pathogen. However, QTL 19-2 had resistant alleles contributed from Conrad for *P. sojae* and was immediately adjacent to a QTL from *Py. irregulare* and *F. graminearum* with resistant alleles contributed from Sloan. One QTL on chromosome 14 for resistance to *Py. irregulare* was adjacent to a QTL for resistance to *F. graminearum* (Table 3). In addition, little to no correlation in the RILs was found for resistance to *P. sojae* and resistance to either *Py. irregulare* or *F. graminearum* (Table 2). This indicates that different mechanisms contribute to the defense against *P. sojae* than *Py. irregulare* and *F. graminearum*. This is expected, as *P. sojae* is a host-specific hemibiotroph, while *Py. irregulare* and *F. graminearum* have broader host ranges and more of a necrotrophic lifestyle.
Additionally, comparative genomics between *P. sojae* and *Pythium* spp. indicates that the effectors released by the two pathogens differ, as *Py. irregulare* contains no predicted RxLR effectors unlike *P. sojae* (Adhikari et al., 2013; De Coninck et al., 2015; Jiang et al., 2008; Tyler et al., 2006).

Biotrophic and necrotrophic pathogens differ in the types of effectors that are released during infection, as biotrophs rely on maintaining living host cells while necrotrophs actively kill host cells (Glazebrook, 2005; McDowell, 2013; Oliver and Ipcho, 2004). Hemibiotrophic pathogens are characterized by an initial biotrophic phase during the early stages of infection, followed by a necrotrophic phase. This shift in lifestyle is likely accompanied by changes in the pathogen’s transcriptome (McDowell, 2013). Therefore, a more rapid response during the biotrophic phase of infection could improve host resistance to hemibiotrophs. A faster, stronger response to the pathogen is one hypothesis to explain mechanisms underlying quantitative resistance (Poland et al., 2009). While the initial host response to any of these types of pathogens appears to be similar, defenses are likely fine-tuned on the basis of the attacking pathogen and the portion of the root under attack (De Coninck et al., 2015; Glazebrook, 2005; Mengiste, 2012).

Defense responses to *Py. irregulare* and *F. graminearum* may have common mechanisms, as two QTL conferring resistance were either overlapping (QTL 19–2) or near each other, as on chromosome 14, with resistance contributed from the same parent at both QTL for both pathogens (Table 3). Overall trends differed for genes associated with QTL for the hemibiotrophic versus necrotrophic pathogens in the gene ontology categories of protein modification, vesicle trafficking, transporters, signal transduction, and metabolism (Table 4). Different types of candidate genes were found associated with QTL against *P. sojae* than those associated with QTL controlling resistance to *Py. irregulare* and *F. graminearum* (Supplemental Tables S4 and S5). For example, *Mlo* family genes were found within QTL 1 and QTL 19-1 conferring resistance to *P. sojae*. The recessive *mlo* allele confers resistance to powdery mildew in barley, *Arabidopsis*, tomato (*Solanum lycopersicum* L.), and pea (*Pisum sativum* L.; Bai et al., 2008; Humphry et al., 2006, 2011; Jørgensen, 1977). This same allele in barley is also associated with susceptibility to other fungal pathogens such as *Ramularia collo-cygni* B. Sutton and J.M. Waller (McGrann et al., 2014). However, none of these genes were found within QTL conferring resistance to *Py. irregulare* or *F. graminearum*.

Numerous genes that have been previously shown to contribute to the defense response in other host-pathogen systems were between the flanking markers of the QTL. Several genes containing domains known to occur in R-genes (Bent, 1996) were found under QTL conferring resistance to *P. sojae*, *Py. irregulare*, and *F. graminearum*. These included leucine-rich repeat encoding proteins, NB-ARC containing genes, and genes encoding serine threonine kinases. Defeated R-genes has been one of the many hypotheses for potential mechanisms of quantitative resistance (Poland et al., 2009).

Several transcription factors were between the markers flanking the QTL for *Py. irregulare* and *F. graminearum* compared with *P. sojae*. Many of these belonged to families that have previously been implicated in defense, such as WRKY transcription factors (Yin et al., 2013), which have previously been shown to be involved in the salicylic acid defense pathway and in salicylic acid–jasmonic acid cross talk both in *Arabidopsis* and in soybean (Euglem and Somssich, 2007; Liu et al., 2011). MYB transcription factors have also been shown to play a role in defense against the soybean rust pathogen *Phakopsora pachyrhizi* Syd. and P. Syd (Aoyagi et al., 2014). Two putative heat shock transcription factors were identified between the markers for the QTL on chromosome 14 conferring resistance to *Py. irregulare* and one was found between the markers for the QTL conferring resistance to *F. graminearum* on the same chromosome. Heat shock proteins are involved in response to both abiotic and biotic stress, including heat, cold, oxidative stress, and infection by bacteria, fungi, and nematodes (Lopes-Caitar et al., 2013).

There were also several genes involved in oxidation or host redox homeostasis within the flanking regions of QTL (Supplemental Tables S4 and S5). Oxidation and reduction are known to change the structure and function of proteins within the host cell (Chi et al., 2013). Additionally, reactive oxygen species are known to act both upstream and downstream of salicylic acid signaling pathways (Herrera-Vásquez et al., 2015). Therefore, changes in the redox state of the cell can directly affect the defense signaling and response. However, further studies that measure the redox state or characterize the genes that are potentially involved are needed to confirm their role in quantitative resistance.

Overall, the wide variety of putative gene functions associated with the resistant QTL to each of the three pathogens identified in this study suggests that there may be a variety of mechanisms that contribute to quantitative resistance, which is in agreement with previous hypotheses (Poland et al., 2009) as well as our earlier reports (Wang et al., 2012b). There are numerous pathways and candidate genes for further analysis to explore what appear to be multifaceted and potentially differentiated defense responses to these pathogens.

**Supplemental Information Available**

**Supplemental Fig. S1–S4.** Chromosome maps generated from 1032 single nucleotide polymorphism and 31 PCR based markers in JoinMap 4.0 (van Ooijen, 2006).

**Supplemental Table S1.** SNP and SSR marker names, SNP identifications (IDs), genetic position (cM), and physical position (bp) of the 1063 SNPs and SSR markers used in the genetic map of Conrad x Sloan F<sub>9</sub>RIL population.
Supplemental Table S2. Comparison of quantitative trait loci (QTL) conferring resistance to \textit{P. sojae} in three generations of Conrad x Sloan recombinant inbred lines (RILs).

Supplemental Table S3. Comparison of quantitative trait loci (QTL) conferring resistance to \textit{F. graminearum} in two generations of Conrad x Sloan recombinant inbred lines (RILs) using the rolled towel method.

Supplemental Table S4. Genes between flanking markers of quantitative trait loci (QTL) conferring resistance to \textit{Phytophthora sojae} in a Conrad x Sloan F\textsubscript{9:11} recombinant inbred line (RIL) population.

Supplemental Table S5. Genes between flanking markers of quantitative trait loci (QTL) conferring resistance to \textit{Pythium irregularum} and \textit{Fusarium graminearum} in a Conrad x Sloan F\textsubscript{9:11} recombinant inbred line (RIL) population.

Supplemental Table S6. Loci of interest for resistance at or near QTL for resistance to at least two isolates of \textit{Phytophthora sojae}.

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