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RESEARCH

Phenotypic Characterization of a Major Quantitative Disease Resistance Locus for Partial Resistance to *Phytophthora sojae*

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ABSTRACT

Major quantitative disease resistance loci (QDRLs) are rare in the *Phytophthora sojae* (Kaufmann and Gerdemann)–soybean [*Glycine max* (L). Merr.] pathosystem. A major QDRL on chromosome 18 (QDRL-18) was identified in PI 427105B and PI 427106. QDRL-18 represents a valuable resistance source for breeding programs. Thus, our objectives were to determine its isolate specificity and measure its effect on yield and resistance to both *P. sojae* and other soybean pathogens. We characterized near isogenic lines (NILs) developed from F_7 recombinant inbred lines heterozygous at QDRL-18; NILs represent introgressions from PI 427105B, PI 427106, and susceptible 'OX20-8'. The introgressions from PI 427105B and PI 427106 increased resistance to *P. sojae* by 11 to 20% and 35 to 40%, respectively, based on laboratory and greenhouse assays, and increased yield by 13 to 29% under disease conditions. The resistant introgression from PI 427105B was also effective against seven *P. sojae* isolates with no isolate specificity detected. Based on quantitative polymerase chain reaction assays, NILs with the susceptible introgression had significantly higher relative levels of *P. sojae* colonization 48 h after inoculation. No pleiotropic effects for resistance to either soybean cyst nematode or *Fusarium graminearum* were detected. This information improves soybean breeders' ability to make informed decisions regarding the deployment of QDRL-18 in their respective breeding programs.

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Abbreviations: hai, hours after inoculation; HG, *Heterodera glycines*; NIL, near isogenic line; qPCR, quantitative polymerase chain reaction; QDRL, quantitative disease resistance locus; RIL, recombinant inbred line; SSR, simple sequence repeat; SNP, single nucleotide polymorphism; SCN, soybean cyst nematode.

Phytophthora root and stem rot causes >816,466 metric tons (30 million bushels) of annual yield reductions in the United States alone (Koenning and Wrather, 2010; Allen et al., 2017). *Phytophthora sojae* (Kaufmann and Gerdemann) is the causal organism of Phytophthora root and stem rot of soybean [*Glycine max* (L). Merr.]. *Phytophthora sojae* is a soil-borne oomycete that thrives in warm, saturated soils, which promote zoospore movement and subsequent infection of soybean roots (Dorrance et al., 2012). Disease symptoms include seed decay, damping-off, and chocolate-brown root and stem lesions. Infection can occur throughout the entire growing season, reducing the benefits of seed treatment compounds that have limited effective periods (Dorrance et al., 2012). Cultural practices such as tillage or crop rotation are ineffective because *P. sojae* is also able to persist for

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multiple years in soil and plant debris as oospores (Schmitthenner, 1985). Thus, the development of resistant cultivars is necessary to manage this destructive disease.

Genetic resistance is the primary disease management strategy for Phytophthora root and stem rot (Schmitthenner, 1985). Single, dominant *Rps* genes have been used extensively by soybean breeders, and 27 *Rps* genes have been reported (Demirbas et al., 2001; Sandhu et al., 2004; Gordon et al., 2006; Sugimoto et al., 2011; Sun et al., 2011; Wu et al., 2011a; Lin et al., 2013; Zhang et al., 2013; Sun et al., 2014; Sahoo et al., 2017). However, the widespread use of race-specific *Rps* genes can lead to the adaptation of *P. sojae* populations to the deployed resistance (Dorrance et al., 2003, 2016; Stewart et al., 2016). *Phytophthora sojae* has a higher than expected level of diversity for a homothallic, soil-borne pathogen (Stewart et al., 2016), and in a recent survey, 213 unique virulence pathotypes were identified out of 873 isolates collected from the northern US soybean-producing area (Dorrance et al., 2016). This increase in pathotype complexity limits an *Rps* gene's lifespan to just 8 to 20 yr (Grau et al., 2004). In contrast, partial resistance to *P. sojae* is a quantitative trait that is generally race nonspecific and provides long-term yield stability in environments with various pathogen populations (Schmitthenner, 1985; Dorrance et al., 2003, 2009; Wang et al., 2012). The development of cultivars with increased levels of partial resistance requires the identification and characterization of sources of partial resistance.

More than 35 quantitative disease resistance loci (QDRLs) distributed across 17 chromosomes have been identified for partial resistance to *P. sojae* (Burnham et al., 2003; Weng et al., 2007; Han et al., 2008; Li et al., 2010; Tucker et al., 2010; Wang et al., 2010; Wu et al., 2011b; Lee et al., 2013a, 2013b; Abeysekara et al., 2016; Schneider et al., 2016; Stasko et al., 2016). However, the majority of these QDRLs explained <15% of the phenotypic variation. Previously, Lee et al. (2014) identified a major QDRL (QDRL-18) on chromosome 18 (8–16 cM) that explained up to 45% of the phenotypic variation and encompasses 222 predicted genes based on the Williams 82 reference genome (Grant et al., 2010). QDRL-18 was mapped in two $F_{7:8}$ recombinant populations derived from crosses between 'OX20-8' and PI 427105B and between OX20-8 and PI 427106. Both PI 427105B and PI 427106 originate from the Jilin Province in the People's Republic of China and have high levels of partial resistance to *P. sojae* (Dorrance and Schmitthenner, 2000). OX20-8 was developed in Ontario, Canada, and possesses *Rps1a* and a very low level of partial resistance (Mideros et al., 2007). Major QDRLs are rare in the *P. sojae*–soybean pathosystem, and QDRL-18 may serve as a valuable source of partial resistance for breeders.

Broad-spectrum resistance is highly desirable in a breeding program, and unlike *R* gene-mediated resistance, QDRLs are generally effective against a wider range of isolates (McDonald and Linde, 2002; Poland et al., 2009; St. Clair, 2010; Mundt, 2014; Nelson et al., 2018). However, isolate-specific QDRLs have been identified in multiple systems, including *Potato virus Y* in pepper (*Capsicum annuum* L.) (Caranta et al., 1997), leaf rust (*Puccinia hordei* Otth) in barley (*Hordeum vulgare* L.) (Qi et al., 1999), and Phytophthora root and stem rot in soybean (Lee et al., 2014; Stasko et al., 2016). QDRL-18 was mapped with two *P. sojae* isolates, 1.S.1.1 (vir 1a, 1b, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, and 8) and OH30 (vir 1a, 1b, 1k, 2, 3a, 3b, 3c, 4, 5, 6, and 7) (Lee et al., 2014), but lines carrying the resistant allele at QDRL-18 have yet to be screened with additional isolates. The evaluation of this resistance source across isolates is especially important due to the increase in pathotype complexity among *P. sojae* populations.

Quantitative disease resistance loci that confer resistance to multiple pathogens or pests are especially valuable from a breeding perspective. For example, the wheat (*Triticum aestivum* L.) gene *Lr34* encodes an ATP-binding cassette transporter and provides partial resistance to three leaf pathogens: leaf rust (*Puccinia triticina* Erikss.), stripe rust (*P. striiformis* Westend.), and powdery mildew [*Blumeria graminis* (DC) Speer] (Krattinger et al., 2009). QDRL-18 is genetically near resistance loci for other root pathogens, including soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe). The *Rhg1* locus, which mediates resistance to SCN via copy number variation (Cook et al., 2012), is located approximately in the same interval as QDRL-18 (Lee et al., 2015). It is currently unknown if QDRL-18 also confers quantitative resistance to SCN or other seedling diseases, including *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwien.) Petch], which has been shown to be pathogenic on soybean and is the most common *Fusarium* spp. in Ohio (Broders et al., 2007; Parikh et al., 2018). Neither PI 427105B or PI 427106 have been screened for *F. graminearum* Schwabe resistance (USDA-ARS, 2015), warranting further characterization of potential pleiotropic effects of QDRL-18.

The aims of this study were to describe the effect of QDRL-18 on (i) resistance to *P. sojae* with greenhouse and laboratory assays, (ii) yield under disease conditions, (iii) resistance to soybean pathogens SCN and *F. graminearum*, and (iv) effectiveness across *P. sojae* isolates of varying pathotype complexity. This information will facilitate the utilization of QDRL-18 in soybean cultivar development.

MATERIALS AND METHODS

Plant Materials

Three sets of near isogenic lines (NILs) were developed from F_7 recombinant inbred lines (RILs) heterozygous at the following markers flanking QDRL-18: BARC-020839-03962, BARC-025777-05064, and BARC-047665-10370 (Supplemental Table S1). The selected RIL-F_7 individuals were designated as 3064,

4060, and 4213 and are derived from the two RIL populations in which QDRL-18 was originally reported (Lee et al., 2014). The RIL 3064 originated from a cross between susceptible cultivar OX20-8 and PI 427106, and RILs 4060 and 4213 originated from a cross between OX20-8 and PI 427105B (Lee et al., 2014). For each RIL, 48 to 57 $F_{7:8}$ plants were genotyped with three simple sequence repeat (SSR) markers (Supplemental Table S1) that spanned the QDRL-18 region. Within each $F_{7:8}$ family, the marker genotypes segregated in a 1:2:1 ratio, demonstrated by a χ^2 test (data not shown). In total, 51 single F_8 plants that were homozygous for all three markers were selected and harvested. Selected lines were planted for two generations and bulk harvested to develop three sets of $F_{8:10}$ lines with contrasting alleles at QDRL-18. These NIL sets are hereafter referred to as 3064, 4060, and 4213. Three different introgressions are represented in these NIL sets, and they are designated R105B, R106, and SOX. R105B and R106 are named for introgressions originating from resistant lines PI 427105B, PI 427106, respectively, and SOX for introgression originating from susceptible OX20-8. Thus, each NIL set includes lines with either the susceptible (SOX) or one of the resistant (R105B/R106) introgressions, with introgression defined as the genetic region encompassing the resistant or susceptible marker allele haplotype from the QDRL-18 region from each parent. After line development, genotypes of all NILs were confirmed with nine single nucleotide polymorphism (SNP) markers (Supplemental Table S1).

Preparation of Zoospores from *P. sojae*

Zoospores were produced from *P. sojae* isolate 1.S.1.1. Briefly, *P. sojae* was cultured on nonclarified V8 agar for 4 d at 28°C and then flooded with 15 mL of sterile distilled water with an adjusted pH of 7.0 for 16 to 17 h. This treatment was followed by seven 30-min washes and a 3-h wash, with water decanted between each wash. The final zoospore suspension was obtained from a second 16- to 17-h wash. Zoospore concentration was calculated with a hemacytometer and adjusted to a final concentration of 1.0×10^4 zoospores mL^{-1} by adding sterile water as needed (Mideros et al., 2007).

Tray Test Assay for Resistance to *P. sojae*

All NIL sets were screened for resistance to *P. sojae* by means of both a tray test and layer test modified from Dorrance et al. (2008). For the tray test, plants were germinated on paper towels and incubated in the growth chamber for 7 d at 25°C. Ten plants per NIL were positioned on top of a polyester cloth and cotton wicking pad, placed on a plastic tray with one raised edge removed. A 1-cm sterilized paper towel strip was placed 2 cm below the tray edge underneath the plants. Then, 100 μL of zoospore suspension (1×10^4 zoospores mL^{-1}) was pipetted to the right of the main tap root of each seedling. A second 1-cm paper towel strip was placed on top of the seedling at the same location as the first. Another 100 μL of zoospore suspension was pipetted directly on top of the main tap root of each seedling. Trays were stacked and placed in a 25-L bucket containing 3 L of deionized water. Buckets containing seedlings were incubated for 7 d at 25°C with a 14-h photoperiod. After incubation, lesion length was measured from the inoculation site (2 cm below root crown) to the leading edge of the lesion margin, and mean lesion length was calculated for each line. The experimental design was a randomized complete block with three blocks separated in time. Each block included cultivars 'Conrad' (Fehr et al., 1989) and 'Sloan' (Bahrenfus and Fehr, 1980) as resistant and susceptible checks, respectively (Burnham et al., 2003; Stasko et al., 2016). Parental lines OX20-8, PI 427015B, and PI 427106 were also included in each block. The effect of QDRL-18 on lesion length was estimated with ANOVA in R version 3.5.0 (R Core Team, 2018) using the package 'lmerTest' (Kuznetsova et al., 2017). The linear mixed-effects model used was $Y_{ijk} = \mu + B_i + N_j + I(N)_{jk} + \varepsilon_{ijk}$ where μ is the overall mean, B_i is the effect of the ith block, N_j is the effect of the jth NIL set, $I(N)_{jk}$ is the effect of the kth introgression nested in the jth NIL set, and ε_{ijk} is the experimental error. Block was treated as a random effect and all other factors as fixed. Least squares means were calculated post hoc to compare introgressions within an NIL set. Additionally, variation in lesion length among lines within a specific NIL set and introgression was assessed with ANOVA in the 'stats' R package.

Layer Test Assay for Resistance to *P. sojae*

In the greenhouse-based layer test (Dorrance et al., 2008), 2-wk-old *P. sojae* (isolate 1.S.1.1) cultured on dilute lima bean agar was placed between two layers of vermiculite in a 1.2-L polystyrene container for the inoculated treatment. In the noninoculated treatment, there was no agar layer in the cup. Fifteen seeds per line were surface sterilized with chlorine gas, planted in each cup, and covered with additional vermiculite. Root rot score, root fresh weight, shoot fresh weight, root dry weight, and shoot dry weight were measured 3 wk after planting. Root rot score was on a scale of 1 to 9, with 1 = no disease, 3 = bottom third of root mass rotted, 5 = all root mass rotted and 10% seedling death, 7 = 75% seedling death and severe stunting, and 9 = 100% seedling death (Dorrance et al., 2008). Analysis was performed as in the tray test described above.

Real-Time Quantitative PCR Assay

The effect of QDRL-18 on *P. sojae* growth 3, 24, and 48 h after inoculation (hai) was evaluated in a real-time quantitative polymerase chain reaction (qPCR) assay. Five susceptible and five resistant NILs from set 4060 derived from PI 427105B were inoculated with the tray test method described above. Parental lines PI 427105B and OX20-8 were also included in the study. After inoculation, 1 cm of root tissue was collected at the inoculation point 2 cm below the root crown from 10 plants per line at 3, 24, and 48 hai. A noninoculated treatment consisting of sterile distilled water with an adjusted pH of 7.0 was included as a negative control for each line at each time point. A randomized complete block design with three biological replications was used. A set of lines was maintained for 7 d after inoculation and lesion length was measured to validate inoculation success. Average lesion length between resistant and susceptible NILs was compared with Welch's *t* test in R version 3.5.0 'stats' package (R Core Team, 2018). DNA was extracted from both infected and noninfected soybean root tissue with a Viogene DNA mini extraction kit according to the manufacturer's instructions. Quality and quantity of DNA extracts were determined by gel electrophoresis and Qubit fluorometer.

A primer pair based on the internal transcribed spacer region of ribosomal RNA gene sequence was used for the specific amplification of *P. sojae*. This primer pair (Psoj-1f/

Psoj-1r) was used by Catal et al. (2013) for relative quantification of *P. sojae* DNA and was shown to be specific to *P. sojae* and work across isolates. Another primer pair (Gmax-1f/Gmax-1r) specific to the 18S ribosomal DNA gene in soybean was used as a reference gene (Catal et al., 2013). Real-time qPCR assays with both primer pairs were performed in Bio-Rad Multiplate 96-well reaction plates using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad). A 20-μL reaction volume containing 10 μL of 1× SYBR Green PCR Master mix (Applied Biosystems) was used. Thermal cycling conditions consisted of 2 min at 95°C, followed by 40 cycles of 15 s at 95°C, 30 s at 57°C, and 15 s at 60°C. A melt curve analysis was completed for each run to verify amplification specificity.

Relative qPCR using the comparative quantification cycle method was used to quantify pathogen growth in infected soybean root tissue. This method requires amplification efficiency for both primer sets to be equal or close. To ensure this, standard curves were constructed from 10-fold dilution series of DNA from both a pure culture of *P. sojae* and uninfected soybean root tissue, for *P. sojae* and soybean specific primers, respectively. Samples were run in triplicate. Efficiency (Eff) of each primer pair was calculated with the formula $\text{Eff} = 10^{-1/\text{slope}} - 1$. For relative qPCR, samples were amplified with both target *P. sojae* and reference soybean primers. Two technical replications were completed with plates arranged in a split-plot design with time point as the main plot and treatment (inoculated or noninoculated) as the subplot. Technical replications were averaged and relative pathogen growth was calculated with the formula $2^{\text{Cq}_{(\text{Gmax})} - \text{Cq}_{(\text{Psoj})}}$, where Cq is the cycle number at which amplification exceeds the background fluorescence, $\text{Cq}_{(\text{Gmax})}$ is the average Cq value for the reference soybean primer, and $\text{Cq}_{(\text{Psoj})}$ is the average Cq value for the *P. sojae* primer (Park et al., 2012; Shi et al., 2018).

Differences in relative pathogen growth between resistant and susceptible NILs was compared with ANOVA in R version 3.5.0 (R Core Team, 2018) using package 'lmerTest' (Kuznetsova et al., 2017). The model was $Y_{ijkl} = \mu + R_i + T_j + I_k + T_j \times I_k + \varepsilon_{ijk}$, where μ is the overall mean pathogen growth, R_i is the effect of ith biological replication, T_j is the effect of jth time point, I_k is the effect of kth introgression, $T_j \times I_k$ is the effect of the interaction between jth time point and kth introgression, and ε_{ijk} is the experimental error. Biological replication was treated as a random effect, and time point and introgression were treated as fixed.

Field Evaluation

Field experiments were conducted over three consecutive seasons in separate fields located in Defiance County (in 2015) and Van Wert County (in 2016 and 2017), Ohio. All sites have a history of Phytophthora root and stem rot. Plots were arranged in a split-plot design with two randomized complete blocks per year. The main plot corresponded to NIL set, and the subplot corresponded to introgression from either the resistant PI or susceptible parent. All NIL sets and parents were included in the study. Experimental units consisted of 6.97-m^2 plots of four rows with 38.1-cm spacing. Plots were hand harvested in 2015 and combine harvested in 2016 and 2017. Seed was cleaned with a Clipper seed cleaner (A.T. Ferrell Company) and weighed to estimate yield.

The effect of QDRL-18 under field conditions was tested with ANOVA in R version 3.5.0 (R Core Team, 2018) using package 'lmerTest' (Kuznetsova et al., 2017). The following linear mixed-effects model was used to test the effect of QDRL-18 under field conditions across all three seasons: $Y_{ijkl} = \mu + Y_i + B(Y)_{ij} + N_k + I(N)_{kl} + B(Y)_{ij} \times I(N)_{kl} + \varepsilon_{ijkl}$ where μ is the overall mean yield, Y_i is the effect of the ith year, $B(Y)_{ij}$ is the effect of the jth block nested in the ith year, N_k is the effect of the kth NIL set, $I(N)_{kl}$ is the effect of the lth introgression nested in the kth NIL set, $B(Y)_{ij} \times I(N)_{jkl}$ is the effect of interaction between the jth block nested in the ith year and the lth introgression nested in the kth NIL set, and ε_{ijkl} is the experimental error. Year and block were treated as random effects, and NIL set and introgression were treated as fixed. Variability of yield among lines within a specific NIL set and introgression was also assessed with ANOVA.

Isolate Specificity

Seven different isolates of varying pathotype complexity were used: OH7 (vir 1a, 3a, 3c, 4, 5, 6, and 7), OH7-8 (vir 1a, 2, 3a, 3c, 4, 5, 6, 7, and 8), OH25 (vir 1a, 1b, 1c, 1k, and 7), OH12108 (vir 1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, and 8), C2.S1 (vir 1a, 1b, 1c, 1k, 2, 3a, 3c, 4, 5, 6, 7, and 8), OH2010.739 (vir 1a, 1b, 1c, 1k, 5, and 7), and OH2010.001 (vir 1a, 1b, 1c, 1k, 2, 3a, 3b, 5, 7, and 8). Pathotype refers to the set of *Rps*-gene differentials that have a susceptible response after inoculation of a given isolate. Isolates were selected to represent a range of virulence present in Ohio. For each isolate, a hypocotyl test including PI 427105B, PI 427106, OX20-8, and 14 differential lines was done to verify the pathotype (Dorrance et al., 2008). In the hypocotyl test, a 1-cm slit was made 1 cm below the cotyledon of 7-d-old etiolated seedlings with an 18-gauge needle prior to inoculation. Depending on the seed, 4 to 15 seedlings of each line were inoculated using methods described in Dorrance et al. (2008). Germination paper rolls containing inoculated plants were placed on a wire mesh in a 25-L bucket covered with a black plastic bag. Plants were incubated at room temperature (24–26°C) for 7 d. After incubation, individual plants were categorized as either resistant if a hypersensitive reaction was present, or susceptible if a lesion was present. Reactions for each line were scored as follows: ≤20% plant death as resistant, 21 to 79% plant death as intermediate, and ≥80% plant death as susceptible.

Near isogenic lines from sets 4060 and 4213 were phenotyped for resistance to the seven *P. sojae* isolates with the tray test as described above, with two modifications. First, a scratch was made on the main tap root ~2 cm below the root crown. Second, a mycelial slurry consisting of 7-d-old *P. sojae* grown on dilute lima bean agar was used for inoculum instead of a zoospore suspension. The mycelial slurry was placed directly on the wound created 2 cm below the root crown. A randomized incomplete block design was used in which each block contained four *P. sojae* isolates divided into four buckets, with one bucket per isolate to prevent contamination. Each bucket contained 19 NILs and four checks, Conrad, Sloan, OX20-8, and PI 427105B. One replication consisted of four blocks, and the experiment was replicated three times, so each NIL was evaluated three times for each isolate.

The interaction between introgression and *P. sojae* isolate was tested with ANOVA in R version 3.5.0 (R Core Team, 2018). R package 'lme4' (Bates et al., 2014) with options to allow for the fit of overparameterized models was used to perform ANOVA

of two linear mixed-effects models, referred to as Model A and Model B using the maximum likelihood method. Model A was as follows: $Y_{ijklm} = \mu + R_i + B(R)_{ij} + C_k + I(C)_{kl} + P_m + I(C) \times P_{klm} + \varepsilon_{ijklm,}$ where μ is the overall mean lesion length, R_i is the effect of the ith replication, $B(R)_{ij}$ is the effect of the jth block nested in the ith replication, C_k is the effect of the kth class, $I(C)_{kl}$ is the effect of the lth introgression nested in the kth class, P_m is the effect of the mth isolate, $I(C) \times P_{klm}$ is the effect of interaction between the lth introgression, nested in the kth class, and the mth isolate, and ε_{ijklm} is the experimental error. Model B did not include the interaction term between isolate and introgression nested in class and was $Y_{ijklm} = \mu + R_i + B(R)_{ij} + C_k + I(C)_{kl} + P_m + \varepsilon_{ijklm,}$ where each variable is as described in Model A. Replication, block, and isolate were treated as random effects, and class and introgression were treated as fixed effects for both Model A and B. The variable C_k consisted of the following class levels representing genetic background: NIL set 4060, NIL set 4213, Conrad, Sloan, OX20-8, PI 427105B. The influence of introgression and isolate on lesion length was tested by examining the differences between Model B with and without terms $I(C)_{kl}$ and P_m.

Resistance to Soybean Cyst Nematode Assay

All NIL sets were screened for resistance to SCN *Heterodera glycines* (HG) Type 0 using the HG type test described in Niblack et al. (2009). Parental lines PI 427105B, PI 427106, and OX20-8 were also screened. Seeds were surface sterilized with chlorine gas and germinated in sterilized germination paper for 3 d. Ten seedlings per NIL were then transplanted to polyvinyl chloride (PVC) containers containing a pasteurized sandy soil mix. Seven HG indicator type lines were included along with susceptible checks 'Lee 74' and 'Essex' (Niblack et al., 2009). Seedlings were inoculated with a prepared suspension of 20 eggs and second-stage juveniles (J2) per cubic centimeter of soil the same day of transplanting. Plants were incubated in the greenhouse for 30 d. Female nematodes were dislodged from soybean roots with water above nested sieves (850-μm pore sieve over 250-μm pore sieve). Female nematodes were counted under a compound microscope, and the female index was calculated by dividing the average egg count of each line by the average egg count of susceptible check Lee. Resistance levels were assigned according to female index value and were as follows: <10 as resistant, 10 to <30 as moderately resistant, 30 to <60 as moderately susceptible, and >60 as susceptible. Experimental design for this study was a randomized complete block with 10 single plant replications. An ANOVA in R version 3.5.0 (R Core Team, 2018) using the package 'lmerTest' (Kuznetsova et al., 2017) was used to determine the effect of QDRL-18 on SCN resistance. The linear mixed-effects model was $Y_{ijk} = \mu + B_i + N_j + I(N)_{jk} + \varepsilon_{ijk}$ where μ is the overall mean female index, R_i is the effect of the ith block, N_j is the effect of the jth NIL set, $I(N)_{jk}$ is the effect of the kth introgression nested in the jth NIL set, and ε_{ijk} is the experimental error. Block was treated as a random effect and all other factors as fixed. Least squares means was performed to compare introgressions within NIL set.

Resistance to *F. graminearum* Assay

All NIL sets and parents were also screened for resistance to *Fusarium graminearum* isolate Fay11 using a roll towel assay modified from Ellis et al. (2011). Isolate Fay11 was selected due to its high level of aggressiveness and use in previous studies (Ellis et al., 2011; Ellis et al., 2012; Stasko et al., 2016; Cheng et al., 2017). Only one isolate was included in the study, as little pathogenic variation exists among *F. graminearum* isolates in Ohio (Broders et al., 2007). The isolate *F. graminearum* (Fay11) was cultured on mung bean [*Vigna radiate* (L.) R. Wilczek] agar for 10 d at room temperature with a 12:12 h daylight schedule. Then, 5 mL of sterile water was added to plates and macroconidia were dislodged with a sterile glass rod and filtered through a cheesecloth to remove any mycelia. Macroconidia concentration was calculated using a hemacytometer (Bright-Line Hemacytometer, Hausser Scientific) and adjusted to a final concentration of 2.5×10^4 macroconidia mL^{-1} by adding sterile water as needed. Fifteen seeds per line were aligned on a sterilized germination towel previously wetted with deionized water. Each seed was then inoculated with 100 μL of prepared macroconidia suspension, covered with another germination towel, rolled, and placed on a wire mesh in a 25-L bucket covered with a black plastic bag. Seeds were incubated at room temperature in the dark. Disease severity was assessed 7 d after inoculation by assigning a disease rating of 1 to 5, with 1 = no sign of colonization, 2 = 1 to 19% of root colonized, 3 = 20 to 74% of root colonized, 4 = ≥75% of root colonized, and 5 = no germination and complete colonization (Ellis et al., 2011). Mean disease severity was calculated for each line. The assay was arranged in a randomized complete block design with three blocks. 'Wyandot' (Ohio Agricultural Research and Development Center, 2006) and PI 567301B were included in each block as susceptible and resistant checks, respectively (Acharya et al., 2015). Analysis was performed as in the SCN assay described above.

RESULTS

Near Isogenic Line Development

Three sets of NILs developed from RILs heterozygous at QDRL-18 were used for phenotypic characterization. Near isogenic line set 3064, derived from a cross between OX20-8 and PI427106, comprised 13 lines. Of these 13, six were homozygous for the R106 introgression and seven were homozygous for the SOX introgression. Near isogenic line set 4060, originating from a cross between OX20-8 and PI 427105B, also comprised 13 lines, with six homozygous for the R105B introgression and seven homozygous for the SOX introgression. Twenty-five lines were developed for NIL set 4213, which was derived from a cross between OX20-8 and PI 427105B, with 13 homozygous for the R105B introgression and 12 homozygous for the SOX introgression. Genotyping of RILs with three SSR markers and nine SNP markers revealed an introgression size of 3264 kb for NIL set 3064, and 7298 and 15,085 kb for sets 4060 and 4213, respectively.

Based on genotyping of the original RILs, we expect alleles to be fixed at 98.8, 97.8, and 98.6% of the loci differing between the two parents (OX20-8 and the respective PI) for NILs within sets 3064, 4060, and 4213, respectively. In

set 3064, NILs are expected to be segregating at 245,959 bp (BARC-059889-16199) on chromosome 1, in addition to the target area associated with QDRL-18. The NILs in set 4060 are expected to be segregating for the following nontarget areas: 52,319,789 bp (BARC-029125-06087) on chromosome 4, 6,621,540 (BARC-018889-03032) and 7,228,568 bp (BARC-028159-05778) on chromosome 16, and 4,989,210 (BARC-048043-10480) and 39,431,928 (BARC-011591-00299) on chromosome 17. Segregation among NILs in set 4213 is expected at 48,840,546 (BARC-010999-00814) and 50,198,454 bp (BARC-064441-18673) on chromosome 1 (Supplemental Fig. S1).

Effect of QDRL-18 on *P. sojae* Resistance

To characterize the effect of QDRL-18 on partial resistance to *P. sojae*, NILs were screened in both a tray and layer test. From the tray test, we observed a significant difference in lesion length between introgressions within NIL sets 4060 ($P < 0.001$, least squares means), 4213 ($P < 0.001$, least squares means), and 3064 ($P < 0.001$, least squares means) (Fig. 1). Within NIL set 4060, there was a 28% decrease in lesion length in lines with the R105B introgression compared with those with the SOX introgression. Similarly, lines with the R105B introgression in NIL set 4213 had 12% lower lesion length than those with the SOX introgression. The R106 introgression decreased lesion length as compared with the SOX introgression by 11% in NIL set 3064. Additionally, in the greenhouse-based layer test, we observed a significant reduction in overall disease development in NILs with either the R105B or R106 introgressions as compared with the SOX introgression in each set. Root rot score, root fresh weight, shoot fresh weight, root dry weight, and shoot dry weight were significantly different between lines with the susceptible and resistant introgressions within each of the three NIL sets (Fig. 2). The R105B introgression decreased root rot score by 47% in NIL set 4060, and 32% in NIL set 4213. The R106 introgression decreased root rot score by 35% in NIL set 3064. Furthermore, we evaluated the effect of lines within NIL set and introgression to determine the validity of grouping lines within an NIL set by introgression. There was no significant difference between lines within NIL set and introgression for either lesion length, root rot score, shoot fresh weight, root fresh weight, shoot dry weight, or root dry weight ($P > 0.05$, ANOVA), validating our grouping of lines.

Effect of QDRL-18 on *P. sojae* Growth

A qPCR assay was used to quantify *P. sojae* DNA relative to soybean DNA in colonized root tissues. From this, the relative amount of *P. sojae* growth at the point of inoculation (2 cm below root crown) was inferred for 10 plants per line 3, 24, and 48 hai, and differences in pathogen growth between introgressions R105B and SOX were evaluated. Near isogenic line set 4060 was selected since it exhibited the greatest difference in lesion length between lines in the previous tray test. A significant difference in lesion length 7 d after inoculation between introgressions ($P < 0.001$, Welch's *t* test) was observed, validating inoculation success. Amplification efficiencies of both primers were >0.91 and within 0.02 of each other, as determined by the slopes of the standard curves (Supplemental Table S2). There was a significant interaction between time point and introgression ($P < 0.05$, ANOVA), and NILs with the SOX introgression had significantly higher levels of pathogen growth than NILs with the resistant introgression 48 hai ($P < 0.01$, least squares means; Fig. 3).

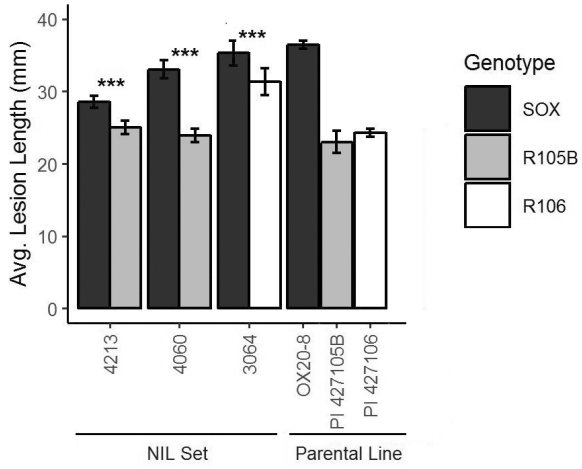

Fig. 1. Mean lesion length (± SE) after inoculation with *Phytophthora sojae* isolate 1.S.1.1 in tray test tray test of near isogenic lines (NILs) of each introgression type (genotype) within each NIL set and parental lines OX20-8 (susceptible), PI 427105B (resistant), and PI 427106 (resistant). Asterisks indicate significant differences between introgressions within each NIL set (***$P < 0.001$, least squares means).

Effect of QDRL-18 on Yield under Disease Conditions

At each of the three field locations, the susceptible parent OX20-8 developed Phytophthora root and stem rot throughout the season (data not shown). There was a significant interaction between NIL set and introgression and between NIL set and environment (block nested in year) (Table 1). Within NIL set 4060, a significant difference in yield between introgressions was also observed (Fig. 4). The R105B introgression increased yield under disease conditions by 7% in NIL set 4213 and by 18% in NIL set 4060. Likewise, the R106 introgression increased yield by 29% in NIL set 3064. Additionally, there was no significant difference in yield between lines within a specific NIL set and introgression ($P > 0.05$, ANOVA), confirming the validity of grouping of lines within NIL sets by introgression.

Isolate Specificity

To determine if the introgression R105B is isolate specific, NILs in sets 4060 and 4213 were inoculated

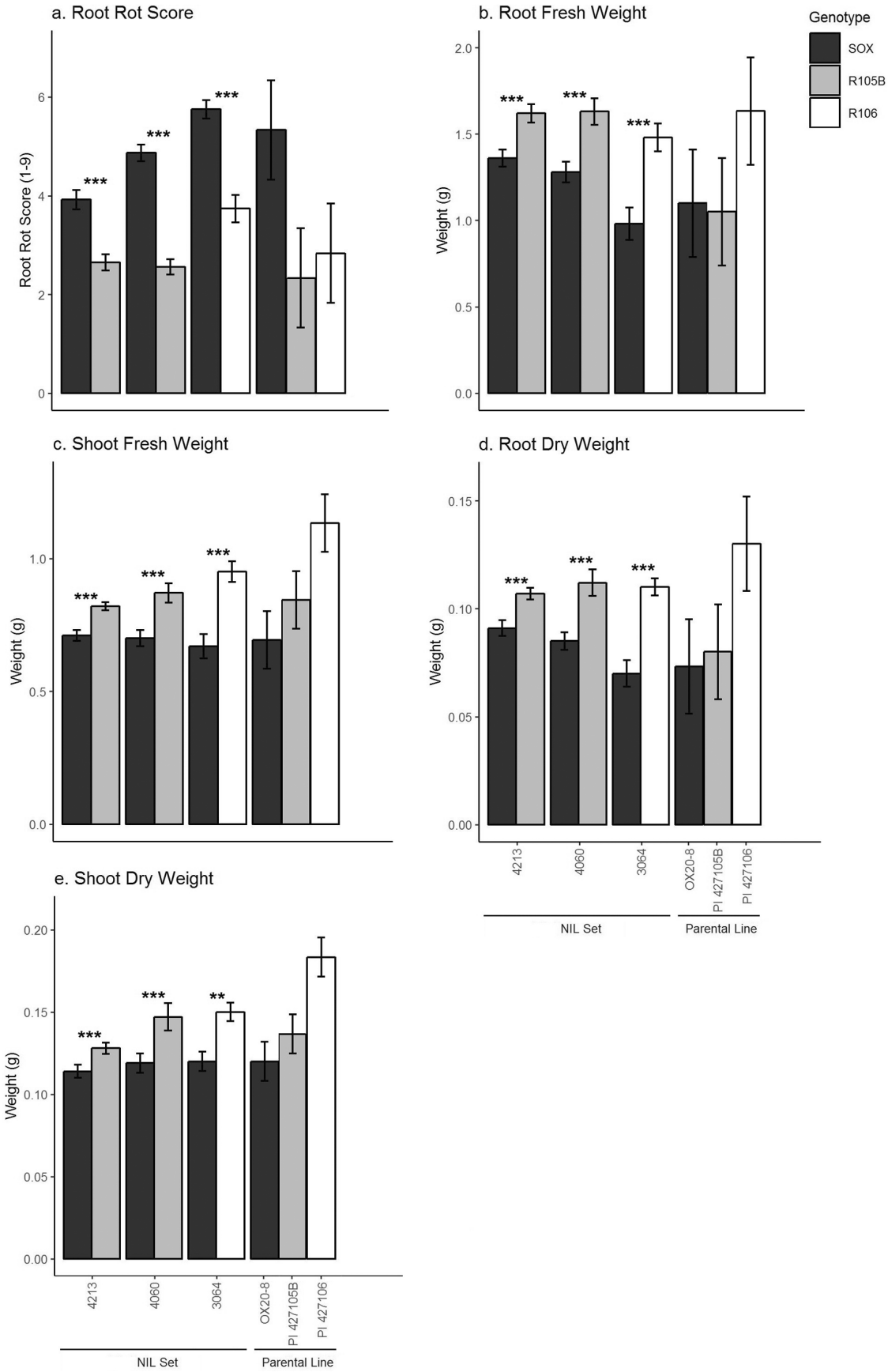

Fig. 2. Mean root rot score, root fresh weight, shoot fresh weight, root dry weight, and shoot dry weight (± SE) after inoculation with *Phytophthora sojae* isolate 1.S.1.1 in layer test tray test of near isogenic lines (NILs) of each introgression type (genotype) within each NIL set and parental lines OX20-8 (susceptible), PI 427105B (resistant), and PI 427106 (resistant). Asterisks indicate significant differences between introgressions within each NIL set (**$P < 0.01$, ***$P < 0.001$, least squares means).

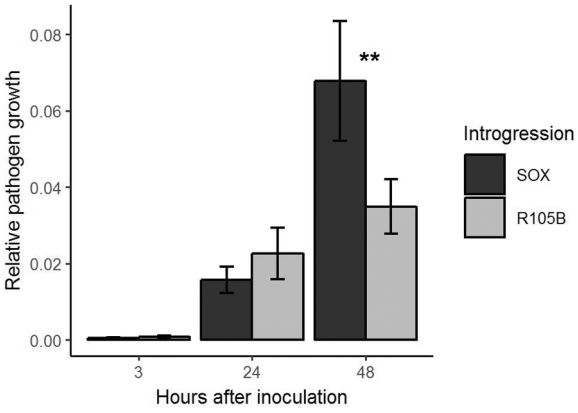

Fig. 3. Mean relative growth of *Phytophthora sojae* isolate 1.S.1.1 (± SE) 3, 24, and 48 h after inoculation in susceptible (SOX) and resistant (R105B) near isogenic lines in set 4060. Asterisks indicate significant differences between introgressions (**$P < 0.01$, least squares means).

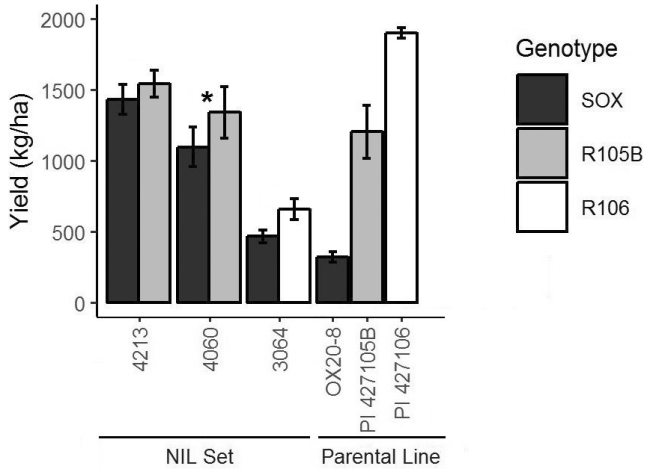

Fig. 4. Mean yield (± SE) of near isogenic lines (NILs) of each introgression type (genotype) within each NIL set and parental lines OX20-8 (susceptible), PI 427105B (resistant), and PI 427106 (resistant), grown under disease conditions from 2015 to 2017. Asterisks indicate significant differences between introgressions within each NIL set (*$P < 0.05$, least squares means).

with seven *P. sojae* isolates using the tray test. A hypocotyl test was first completed to ensure all seven isolates were virulent against the parental lines, and therefore also against the NILs. No hypersensitive response indicative of an *Rps* gene interaction was detected among parental lines OX20-8 and PI 427105B inoculated with the seven *P. sojae* isolates tested (Table 2). Thus, all seven isolates could be used for phenotyping of partial resistance in NIL sets 4060 and 4213.

Model comparisons showed no significant introgression × isolate interaction, indicating that the resistant introgression R105B is similarly effective across all tested *P. sojae* isolates (Table 3). Isolate had a significant effect on lesion length, revealing varying levels of aggressiveness among the seven isolates (Table 3). Furthermore, as described above, introgression had a significant effect on lesion length (Table 3). The R105B introgression increased resistance by 19%, averaged across all seven isolates (Fig. 5), very similar to the 20% increase in resistance when inoculated with isolate 1.S.1.1.

Evaluation for Pleiotropic Resistance Effects

Near isogenic lines were inoculated with SCN HG Type 0 and *F. graminearum* isolate Fay11 to test for pleiotropic effects. Both SCN and *F. graminearum* were selected for evaluation because they are also root pathogens of soybean. Furthermore, the SCN resistance locus *Rhg1* is genetically near QDRL-18 (Grant et al., 2010). Within each NIL set, there were no significant differences in female index values between resistant and susceptible introgressions after inoculation with SCN HG Type 0 (Fig. 6). All NILs were categorized as either susceptible or moderately susceptible based on their female index value. Parental lines PI 427105B and PI 427106 were classified as moderately susceptible and susceptible, respectively. All seven HG-type indicator lines showed a resistant response, confirming the Type 0 SCN population.

A rolled towel assay was performed to evaluate resistance to *F. graminearum*. Disease ratings did not significantly differ between resistant and susceptible

Table 1. Type III ANOVA results for yield under disease conditions from 2015 to 2017 using Satterthwaite's method to approximate df for fixed effects and likelihood ratio tests of model reductions to test significance of random effects.

Source of variation	Effect	Sum of squares	df	*F* value	*P* value
NIL† set	Fixed	1,124,274	2	6.8953	0.0061**
NIL set × introgression	Fixed	1,114,254	3	4.5559	0.0461*
Year	Random	–	1‡	–	0.0106*
Year × block	Random	–	1	–	1.0000
Year × NIL set × introgression	Random	–	1	–	0.1535
Year × block × NIL set	Random	–	1	–	<0.0001***
Year × block × NIL set × introgression	Random	–	1	–	1.0000

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† NIL, near isogenic line.

‡ Degrees of freedom for the likelihood ratio test equal the difference in the number of model parameters.

introgressions within NIL set (Fig. 7). On average, all NILs exhibited a susceptible response to *F. graminearum* isolate Fay11 and had a disease rating of 3.9 or greater, indicating that 75% of roots were colonized. Similarly, PI 427105B and PI 427106 had average disease ratings of 4.3 and 4.2, respectively. The resistant check PI 567301B did have significantly lower average disease rating than the susceptible check Wyandot ($P < 0.001$, Welch's *t* test), validating that the inoculation procedure was successful. Overall, there was no evidence of pleiotropic effects for resistance to either *F. graminearum* or SCN.

DISCUSSION

Host resistance is a vital component of disease management, and quantitative disease resistance, in general, provides more durable protection against a wider range of pathotypes than *R*-gene-mediated resistance (Poland et al., 2009; St. Clair, 2010; Nelson et al., 2018). Thus, much focus has been placed on identifying loci conferring quantitative disease resistance. However, incorporating multiple unlinked QDRLs of minor effect into cultivars is often an inefficient process (Bernardo, 2016). In contrast, many QDRLs with major effect have been widely adopted. The *Fhb1* QDRL for Fusarium head blight (*F. graminearum*) resistance in wheat (Anderson et al., 2001) and major QDRL for SCN resistance in soybean (Concibido et al., 2004) are two such examples. Major QDRLs for partial resistance to *P. sojae* are rare, making QDRL-18 a valuable resistance source. Further characterization of the locus is necessary to facilitate its use in cultivar development. In this study, NILs developed from the original mapping populations were used to validate QDRL-18, measure isolate specificity, and begin testing for pleiotropic effects of this region to other root pathogens.

Table 2. Hypocotyl test results of 14 differential lines and parental lines OX20-8, PI 427105B, and PI 427106 against seven *Phytophthora sojae* isolates.

		Phytophthora sojae isolate						
Line	*Rps* gene	OH7	OH7-8	OH25	OH12108	C2.S1	OH2010.739	OH2010.001
OX20-8	*Rps1a*	S†	S	S	S	S	S	S
PI 427105B		S	S	S	S	S	S	S
PI 427106		S	S	S	S	S	I‡	I
Williams		S	S	S	S	S	S	S
Harlon	*Rps1a*	S	S	S	S	S	S	S
Harosoy 13xx	*Rps1b*	S	S	S	S	S	S	S
Williams 79	*Rps1c*	S	S	S	S	S	I	R§
PI 103091	*Rps1d*	S	S	S	S	S	I	I
L76-1988	*Rps2*	S	S	S	S	S	R	R
L83-570	*Rps3a*	S	S	S	S	S	R	R
PRX-146-36	*Rps3b*	S	S	S	S	S	I	R
PRX-145-48	*Rps3c*	S	S	S	S	S	R	R
L85-2352	*Rps4*	S	S	S	S	S	R	R
L85-3059	*Rps5*	S	S	S	S	S	I	R
Harosoy 62xx	*Rps6*	S	S	S	I	R	R	R
Harosoy	*Rps7*	S	S	S	I	S	S	S
PI 399073	*Rps8*	S	S	S	S	S	S	I

† S, susceptible, ≥80% plant death.

‡ I, intermediate, 21–79% plant death.

§ R, resistant, ≤20% plant death.

Table 3. ANOVA comparing models including and excluding (A) introgression × isolate interaction, (B) isolate, and (C) introgression.

Section	Model†	AIC‡	Log likelihood	Deviance	χ^2	df	*P* value
A.	No interaction	5624.8	−2800.4	5600.8			
	Interaction	5626.8	−2800.4	5600.8	0	1	1 ns§
B.	No isolate	5931.2	−2954.6	5909.2			
	Isolate	5624.8	−2800.4	5600.8	308.4	1	<0.0001***
C.	No introgression	5882.8	−2931.4	5862.8			
	Introgression	5624.8	−2800.4	5600.8	262.0	1	<0.0001***

*** Significant at the 0.001 probability level.

† The full model for Section A is $Y_{ijklm} = \mu + R_i + B(R)_{ij} + C_k + I(C)_{kl} + P_m + I(C) \times P_{klm} + \varepsilon_{ijklm}$; the full model for Sections B and C is $Y_{ijklm} = \mu + R_i + B(R)_{ij} + C_k + I(C)_{kl} + P_m + \varepsilon_{ijklm}$.

‡ AIC, Akaike information criterion.

§ ns, nonsignificant at the 0.05 probability level.

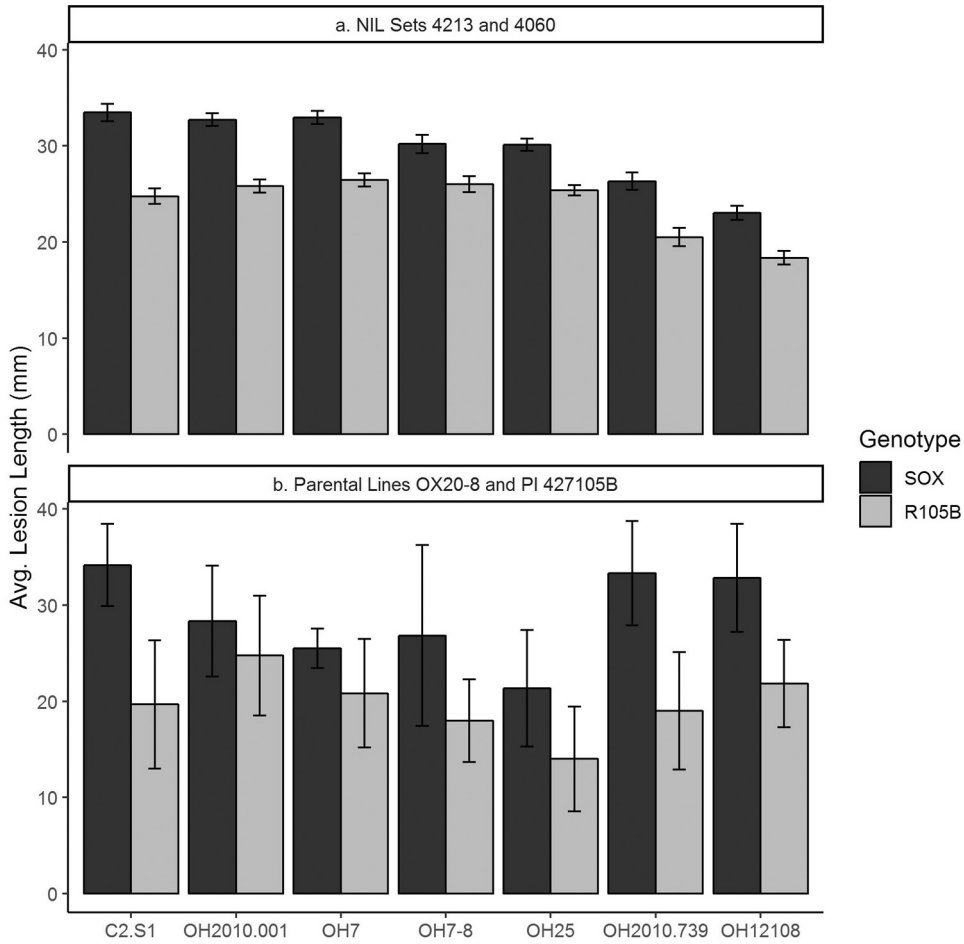

Fig. 5. Mean lesion length (± SE) of near isogenic lines (NILs) of each introgression type (genotype) within (a) each NIL set and (b) parental lines OX20-8 (susceptible) and PI 427105B (resistant) after inoculation with *Phytophthora sojae* isolates C2.S1, OH2010.001, OH7, OH7-8, OH25, OH2010.739, and OH12108 in tray test.

Overall, the tray test and layer test indicate that introgressions from PI 427105B (R105B) and PI 427106 (R106) significantly improve on the levels of partial resistance to *P. sojae*, validating QDRL-18. Both introgressions significantly reduced lesion length in the tray test and were effective in limiting overall disease development in the layer test. Furthermore, in the qPCR assay, the R105B introgression significantly reduced relative pathogen growth 48 hai. However, no differences in relative *P. sojae* levels were observed between lines at 3 and 24 hai. These time points may be too early to show differences in infection progress using relative qPCR. For instance, Ranathunge et al. (2008) observed no differences in zoospore germination at 4 hai between Conrad (strong partial resistance) and OX760-6 (low partial resistance), and though infection was delayed in Conrad, *P. sojae* had colonized the root steles of both cultivars at 24 hai. Future work, including sampling at later time points and sampling tissue directly above and below the inoculation point, may better elucidate differences in pathogen growth between resistant introgressions R105B and R106 and susceptible introgression SOX.

Many QDRLs associated with partial resistance to *P. sojae* have been shown to have an effect in the greenhouse or laboratory (Lee et al., 2014; Schneider et al., 2016; Stasko et al., 2016), but few have been tested in the field (Li et al., 2010; Wang et al., 2012) where more environmental variation exists. In NIL set 4060, the resistant introgression R105B significantly increased yield under disease conditions compared to the susceptible introgression derived from OX20-8 (SOX). Based on the present study, it is still unknown if resistant introgressions have a negative impact on yield under disease-free conditions. However, previous work has shown no yield differences between cultivars with partial resistance, single *Rps* genes, or *Rps* gene combinations when disease pressure is low (Wilcox and St. Martin, 1998; Dorrance et al., 2003).

Although quantitative disease resistance is generally assumed to be broad spectrum, isolate-specific QDRLs have been identified (Caranta et al., 1997; Qi et al., 1999; Stasko et al., 2016). In this study, no significant interaction between isolate and introgression was detected, and the R105B introgression significantly reduced lesion length by 19% across seven *P. sojae* isolates with complex pathotypes. Although a possible interaction between introgression and isolate cannot be completely ruled out without testing all extant isolates, the seven isolates used here represent a

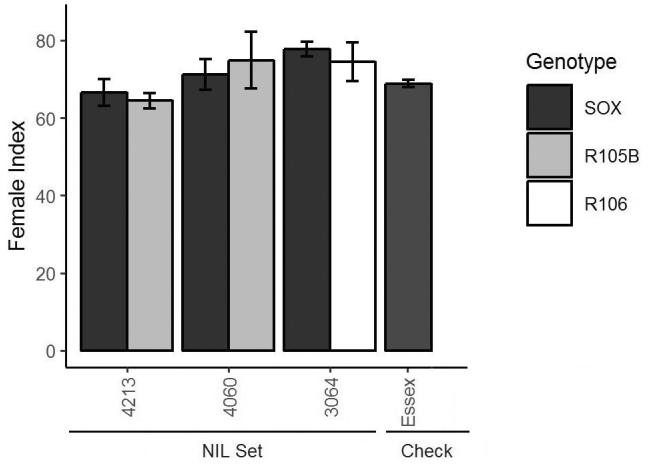

Fig. 6. Mean female index value (± SE) of each introgression type (genotype) within each near isogenic line (NIL) set and susceptible check Essex after inoculation with *Heterodera glycines* Type 0. No differences between introgression within each NIL set were significant ($P > 0.05$, least squares means).

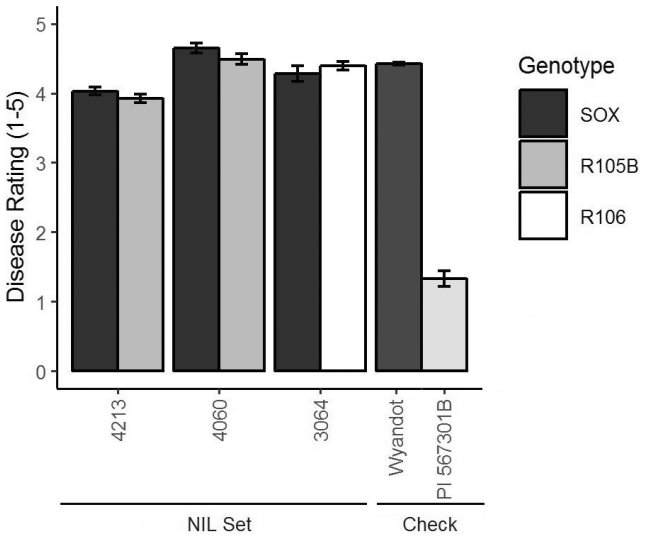

Fig. 7. Mean disease rating (± SE) of introgression type (genotype) within each near isogenic line (NIL) set and checks Wyandot (susceptible) and PI 57301B (resistant) after inoculation with *Fusarium graminearum* isolate Fay11 in rolled towel assay. No differences between introgression within each NIL set were significant ($P > 0.05$, least squares means).

range of virulence found in northeastern soybean growing regions. Thus, we do not expect isolate specificity to occur based on these results. This is especially important as pathotype complexity continues to increase and provides further rationale for the use of this resistance source in soybean cultivar development (Dorrance et al., 2016).

QDRL-18 colocalizes with quantitative loci associated with resistance to SCN (Lee et al., 2014), warranting further investigation into possible pleiotropic effects for other soybean diseases (Grant et al., 2010). In this study, no introgressions affected resistance to either SCN or *F. graminearum*. Furthermore, parental lines OX20-8, PI 427105B, and PI 427106 also showed susceptible responses to both SCN and *F. graminearum*. Lack of pleiotropy in these interactions is not unexpected due to differences in pathogen biology and infection type between *P. sojae* and both SCN and *F. graminearum*. Soybean cyst nematode infects soybean roots in the second juvenile stage (J2) by direct penetration followed by the development of feeding sites called syncytia in the root vascular tissue (Davis and Tylka, 2000). *Fusarium graminearum* is a necrotophic ascomycete that infects soybean seed and seedlings (Broders et al., 2007; Parikh et al., 2018). Areas for further investigation include testing for pleiotropic effects for other soybean oomycete pathogens, such as *Pythium* spp. and those with quantitative loci that colocalize with QDRL-18 such as *Fusarium virguliforme* O'Donnell & T. Aoki, the causal agent of sudden death syndrome.

The effectiveness of the R105B and R106 introgressions of QDRL-18 in field trials, the layer test, and across isolates in the tray test demonstrates its usefulness in breeding programs. To further facilitate its use in breeding programs, we are currently fine mapping QDRL-18 and identifying candidate gene(s) associated with it.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material is available online for this article.

Acknowledgments

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