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ORIGINAL ARTICLE

Molecular mapping of quantitative disease resistance loci for soybean partial resistance to *Phytophthora sansomeana*

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Abstract

Key message **Two soybean QDRL were identified with additive interaction to *P. sansomeana* isolate *MPS17-22*. Further analyses uncovered four interaction patterns between the two QDRL and seven additional *P. sansomeana* isolates.**

Abstract *Phytophthora sansomeana* is a recently recognized species that contributes to root rot in soybean. Previous studies indicated that *P. sansomeana* is widely distributed among soybean growing regions and has a much wider host range than *P. sojae*, a well-known pathogen of soybean. Unlike *P. sojae*, no known disease resistance genes have been documented that can effectively control *P. sansomeana*. Therefore, it is important to identify resistance that can be quickly integrated into future soybean varieties. E13901 is an improved soybean line that confers partial resistance to *P. sansomeana*. A mapping population of 228 F4:5 families was developed from a cross between E13901 and a susceptible improved soybean variety E13390. Using a composite interval mapping method, two quantitative disease resistance loci (QDRL) were identified on Chromosomes 5 (designated *qPsan5.1*) and 16 (designated *qPsan16.1*), respectively. *qPsan5.1* was mapped at 54.71 cM between Gm05_32565157_T_C and Gm05_32327497_T_C. *qPsan5.1* was contributed by E13390 and explained about 6% of the disease resistance variation. *qPsan16.1* was located at 39.01 cM between Gm16_35700223_G_T and Gm16_35933600/Gm16_35816475. *qPsan16.1* was from E13901 and could explain 5.5% of partial disease resistance. Further analysis indicated an additive interaction of *qPsan5.1* and *qPsan16.1* against *P. sansomeana* isolate *MPS17-22*. Marker assisted resistance spectrum analysis and progeny tests verified the two QDRL and their interaction patterns with other *P. sansomeana* isolates. Both QDRL can be quickly integrated into soybean varieties using marker assisted selection.

Introduction

Soybean (*Glycine max* (L.) Merr.) is an important crop for food and feed. However, advances in increasing soybean yield have been challenged by soilborne diseases. One of the most destructive diseases is Phytophthora root rot (PRR), which has been estimated to cause yield losses of up to 40 million bushels per year in the U.S. and Ontario, Canada (Allen et al. 2017). The disease has historically been attributed to *Phytophthora sojae*, which was first observed in 1948 in Indiana and all major soybean producing countries. *P. sojae* was once part of the *Phytophthora megasperma* species complex, a collection of morphologically similar species that are genetically distinct. In 2009, *Phytophthora sansomeana* E.M. Hansen & Reeser was also named and differentiated from the *P. megasperma* complex (Hansen et al. 2009).

Similar to *P. sojae*, *P. sansomeana* has wide geographic range and has been identified causing disease on soybean in nine states in the Midwest as well as other countries such as Canada, Iran, Japan, and China (Reeser et al. 1991; Malvick and Grunden 2004; Zelaya-Molina et al., 2010; Bienapfl et al. 2011; Rahman et al. 2015; Rojas et al. 2017a, b; Safaiefarahani et al. 2016; Rojas et al. 2019; Tande et al. 2020; Tang et al. 2010; Farr and Rossman, 2021). However, Unlike *P. sojae*, which has a narrow host range and primarily infects soybean, *P. sansomeana* infects a wide range of plant species including soybean, corn (*Zea mays* L.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), white clover (*Trifolium repens* L.), pea (*Pisum sativum*

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L.), gerbera, *Atractylodes macrocephala* Koidz, and several weed species such as wild carrot (*Daucus carota* L.) and white cockle (*Silene latifolia* Poir. Ssp. Alba (Miller) Greuter & Buerdet) (Hacker et al. 2005, Hansen et al. 2009; Hansen et al. 2012; Zelaya-Molina et al. 2009; Rahman et al. 2015; Rojas et al. 2017a, b; Chang et al. 2017; McCoy et al. 2018; An et al. 2019) *P. sansomeana* causes severe symptoms of seed and root rot (Dorrance 2018), and in an in-vitro comparative pathogenicity study, it was shown that *P. sansomeana* was more aggressive than *P. sojae* based on the measures of root area, root length, and dry weight (Rojas et al. 2017a).

Crop rotation is a common agronomic activity to aid in disease management (Schmitthenner 2000; Xiao et al. 2002; Dorrance et al. 2003; Dorrance et al. 2009). However, because *P. sansomeana* has a wide host range, traditional crop rotation may have little effect in managing this disease. Another way of disease control is the deployment of host resistance genes, which are effective, economic, and environmentally friendly (Dorrance et al. 2009; Zhang et al. 2009; Lin et al. 2013). Two types of host resistance are commonly deployed in plant breeding, including *R* gene mediated resistance, and partial resistance contributed by multiple small effect genes. *R* genes are race specific and typically non-durable, such as *Rps* genes that confer resistance to *P. sojae*, whereas partial resistance is often more durable and race non-specific (Dorrance et al. 2018). Currently more than 30 *Rps* genes and 40 QDRL (quantitative disease resistance loci) (Dorrance et al. 2018) have been reported to provide complete and partial resistance to *P. sojae*, respectively, however, little is known about soybean resistance against *P. sansomeana*, and no resistance genes have been reported before this study.

Given its high aggressiveness and wide host range, *P. sansomeana* can be a destructive soybean yield suppressor. Therefore, it is important to identify resistance sources and integrate the resistance genes into future soybean varieties. E13901 is a Michigan State University (MSU) improved soybean breeding line and contains *Rag1b* and *Rag3* gene for soybean aphid resistance (Bales et al. 2013). Preliminary screening of breeding lines identified that E13901 confers mediate level of partial resistance to *P. sansomeana* isolate *MPS17-22*, and therefore, the objectives of this study were to: 1) describe the inheritance pattern of resistance using F4:5 mapping population, 2) determine the genetic position of QDRL using molecular markers, and 3) Characterize the resistance spectrum of the QDRL using additional *P. sansomeana* isolates.

Materials and methods

Plant materials and isolates of *P. sansomeana*

A mapping population of 228 F4:5 families was made from a cross in 2015 between E13901 and another breeding line, E13390, developed at Michigan State University. The two parental lines shared a common ancestor line PI 567598B, from which two recessive aphid resistance genes were identified (Bales et al. 2013) (Figure S1). The F1 plant from this cross was self-pollinated in 2016 to create the F2 population which were advanced for two generations in the greenhouse using the single seed decent (SSD) method. The F4 seeds were then planted in the field in 2017 and self-pollinated to create the F4:5 mapping population (dubbed 'POP150029′). To validate the QDRL with progeny lines, all the F4:5 lines were advanced in the field in 2019 for F5:6 families.

For QDRL mapping, *P. sansomeana* isolate *MPS17-22* (McCoy unpublished data) was used to evaluate each of the 228 F4:5 families. To assess the effectiveness of QDRL against different isolates, an additional seven *P. sansomeana* isolates (McCoy unpublished data) were used, including *C-IASO2 6–15*, *V-KSSO23-6*, *MICO 3–28*, *MPS17-24*, *C-NESO2 5–12*, *V-NESO2 5–45*, and *KSSO 6–1*. *MPS17-22* was collected from Michigan in 2017, and the additional seven isolates were collected from Iowa, Kansas, Michigan, and Nebraska, during a continental scale survey of soybean oomycete seedling diseases (Rojas et al. 2017a). These isolates were then characterized for their aggressiveness, host range and geographic distributions (McCoy unpublished data). The above isolates were selected based on their diversity in aggressiveness, host range, and geographical distributions to best represent the screened *P. sansomeana* isolates (Rojas et al. 2017a; McCoy unpublished data). All the isolates were stored long term on potato carrot agar slants and hemp seed vials.

Disease evaluation

For QDRL mapping and validation experiments with F4:5 lines, *P. sansomeana* inoculum was prepared by transferring a 5-mm agar plug from an actively growing isolate to 60 mm × 15 mm petri dish plates containing lima bean agar (LBA) (Dorrance et al. 2008). To prepare inoculum for progeny test using F5:6 lines, 500 µl of macerated hyphae was pipette transferred to the same type of LBA cultural media. The plates were then incubated at the room temperature for 10–14 days before use.

A modified layer test assay (Dorrance et al. 2008) was used for disease evaluation at the Michigan State University greenhouse facilities, with the environmental conditions controlled at 22 ± 3 °C and 40 ± 5% humidity with 12-h photoperiod. Seed starting trays (each cell 3.96cmW × 5.99cmL × 7.95cmD, T.O. Plastics, Inc.) were filled with medium size vermiculite and soaked in tap water until the vermiculite was fully saturated. Two 2 cm-deep by 1 cm-wide holes were then made in each cell and one 4 mm × 4 mm piece of *P. sansomeana* inoculum was placed at the bottom of each hole. Two soybean seeds were placed in each hole and were softly pressed to make sure each seed adhered to the inoculum. For QDRL mapping and marker assisted resistance spectrum (MARS) experiments, 12 seeds were planted as one replicate for F4:5 families and the two parental lines with a total of three replicates for inoculation group and four replicates for non-inoculated control group, respectively. In the progeny test, each genotype (RR, RS, SR, and SS) contains 10 F5:6 lines, and only one replicate of 12 seeds were tested for each *P. sansomeana* isolate due to the limited number of seeds. A randomized block design was used for all the tests.

After planting, the seed starting trays were transferred to greenhouse benches covered with waterproof plastic. The benches were watered until the water reached over the level of the inoculum. After that, the benches were watered every other day to maintain a humid environment until the day before measurement. Fourteen days after planting, the number of germinated seeds was recorded, and the fresh root weight was measured using an electronic balance (Scout Pro, SP 4001; Ohaus Corp, Pine Brook, NJ). The response of each soybean line challenged with *P. sansomeana* was evaluated using the ratio of fresh root weight (RRW) (Lin et al. 2018) and the ratio of seedling emergence (RSE). To obtain RRW, the RWI (fresh root weight of inoculation) and the RWC (fresh root weight of non-inoculated control) were first calculated as

$$\mathrm{RWI} = \text{total fresh root weight of an inoculated replicate} / \mathrm{N},$$

where N represents the number of vigorous seeds of each inoculated replicate and is estimated using the mean of germinated seeds across all the non-inoculated replicates. To ensure the high quality of seeds, a cutoff of $\mathrm{N} \geq 10$ in each replicate was applied.

$$\mathrm{RWC} = \text{total fresh root weight of a non-inoculated replicate} / \text{number of germinated seeds of the replicate}$$

Then for each inoculated replicate, RRW = RWI / mean of RWC.

Also, RSE = number of germinated seeds of an inoculated replicate / N.

To comprehensively describe the soybean resistance against *P. sansomeana*, here we introduced another index, named disease resistance index (DRX), which considered both RSE and RRW:

$$\mathrm{DRX} = \sqrt{RSE \times RRW} \times 100$$

where, DRX ranges from 0 to 100, with 0 indicates soybean completely susceptible to the pathogen and 100 for complete immunity.

Sample collection and DNA isolation

The leaf samples of 12 F5 seedling plants were bulk collected one day before disease evaluation to represent the genotype of each F4 line. The genomic DNA was extracted using a standard Cetyl Trimethyl Ammonium Bromide (CTAB) method and the DNA pellet was dissolved in 200 µl 10 mM Tris–HCl buffer. The DNA samples were quantified using an ND-1000 Spectrophotometer (NanoDrop Technolgies, Inc., Wilmington, DE, USA) for chip analysis.

Construction of linkage map

All the samples used in this study were genotyped using Illumina Infinium BARCSoySNP6K iSelect BeadChip genotyping array (Illumina, San Diego, USA) (Song et al. 2013). Based on the parental genotypes, the SNP markers that were not polymorphic or had missing parental data were removed. After filtering, 978 polymorphic markers remained and were then imported into.

Joinmap software (v4.0) for linkage analysis (Van Ooijen 2006). The marker order was determined using maximum likelihood algorithm and mapping function was used to determine genetic distance. The linkage groups were then determined using an independence LOD = 4.0 and a max recombination frequency = 0.5. The markers that were not grouped with at least five other markers were excluded. The linkage maps were drawn using MapChart software (Voorrips 2002).

Statistics and QDRL mapping

The statistical descriptive analysis in this study was performed using the SPSS software (IBMSPSS Statistics, IBMCorporation,Chicago, IL).The broad-sense heritability was estimated according to the method described by Fehr (1987). The software QTL Cartographer V2.5 (Wang et al. 2007) was used for interval mapping (IM) and composite interval mapping (CIM). The window size was 5 cM and

the walking speed was 1 cM. The threshold of LOD score for statistical significance of QDRL effects was determined by 1,000 permutations, and the LOD value corresponding to an experiment-wise Type I error rate of 5% ($\alpha = 0.05$) was considered the threshold of significance (Churchill and Doerge 1994). The position of each QDRL was estimated as the point of maximum LOD score in the region under consideration.

Results

Response of soybean lines to *P. sansomeana* isolate *MPS17-22*

For the parental lines, E13901 appeared moderately resistant to *MPS17-22*, while E13390 was completely susceptible. The RSE of E13901 ranged from 0.089 to 0.536, while the RSE of E13390 ranged from 0 to 0.085. Statistically, the average RSE of E13901 (0.283 ± 0.071) was significantly higher than that of E13390 (0.028 ± 0.018) ($p < 0.001$). The RRW range of E13901 was from 0.047 to 0.326, and that of E13390 ranged from 0 to 0.101. The mean RRW of E13901 (0.146 ± 0.043) was also significantly higher than that of E13390 (0.021 ± 0.017) with $p < 0.01$. For DRX, E13901 ranged from 6.44 to 38.12 with a mean of 20.03 ± 5.18, which was significantly higher than E13390 (2.34 ± 1.59) (Table 1). The broad sense heritability of RSE, RRW, and DRX were 0.918, 0.861, and 0.906, respectively.

The range of the three traits within POP150029 mostly fell between the two parental lines, but there was some transgressive segregation, suggesting additive effects from the two parental lines (Table 1, Fig. 1). For example, the maximum values of RSE, RRW and DRX in POP150029 (0.551, 0.356, and 43.81, respectively) were all higher than the corresponding maximum values of the resistant parent E13901. Interestingly, the mean value of the three traits ($RSE = 0.251 \pm 0.007$, $RRW = 0.131 \pm 0.004$, and $DRX = 18.10 \pm 0.54$) of POP150029 were not significantly different than E13901, but all significantly higher than the susceptible parent E13390 (Table 1). Histograms of the frequency of RSE, RRW, and DRX of POP150029 were all right-skewed, suggesting that multiple genes may confer to soybean partial resistance against *P. sansomeana* isolate *MPS17-22*.

Table 1 Statistics of soybean response to *Phytophthora sansomeana* (isolate *MPS 17–22*)

	RSE		RRW		DRX	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
E13901	0.283 ± 0.071^a	0.089–0.536	0.146 ± 0.043^a	0.047–0.326	20.03 ± 5.18^a	6.44–38.12
E13390	0.028 ± 0.018^b	0–0.085	0.021 ± 0.017^b	0–0.101	2.34 ± 1.59^b	0–9.23
150,029 (E13901 x E13390)	0.251 ± 0.007^a	0.030–0.551	0.131 ± 0.004^a	0.027–0.356	18.10 ± 0.54^a	2.81–43.81
Broad sense heritability	0.918		0.861		0.906	

RSE ratio of seed emergence; *RRW* ratio of fresh root weight; *DRX* disease resistance index

Different letters indicate significant differences at $\alpha = 0.05$ level

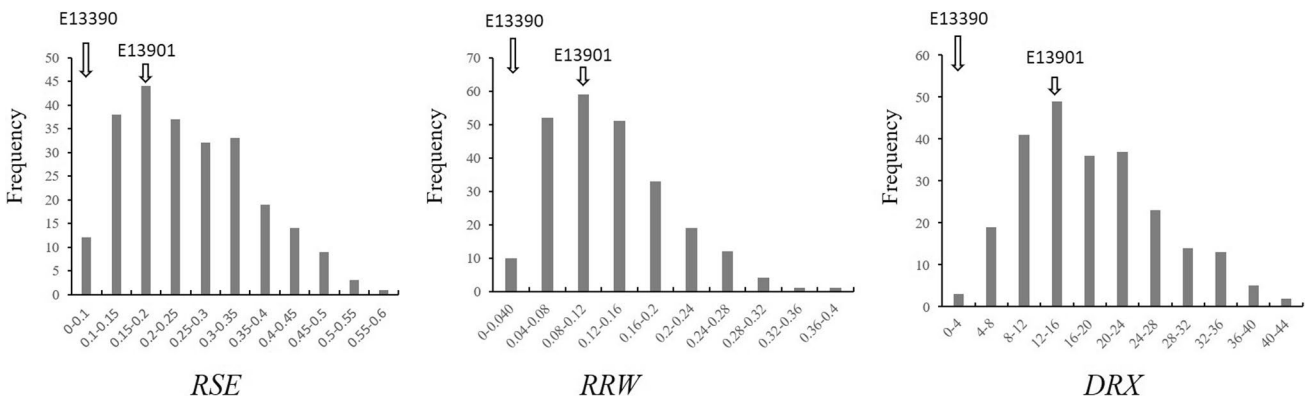

Fig. 1 Response of soybean F4:5 families and the parental lines (E13390 and E13901) to *P. sansomeana* isolate *MPS17-22*

Linkage analysis and QDRL mapping

Using the SoySNP6K bead chip, a total of 978 polymorphic SNP markers were identified from the parental lines, which was much fewer than the 1384 and 1373 polymorphic markers identified in POP1 and POP2 (Lin et al. 2018), probably due to the close relationship of E13901 and E13390 in their pedigree (Figure S1). The 978 polymorphic markers were classified into 23 linkage groups, with each linkage group containing 7—95 polymorphic markers (Table S1, Figure S2). The polymorphic markers were not evenly distributed on each chromosome and large monomorphic regions can be observed on many chromosomes (Figure S3), suggesting large fixed genomic regions between the two parental lines. This may partly be attributable to their common ancestor line 'PI 467598B' and the advanced selection for identical agronomic traits adaptive to Michigan environment. Because of the large monomorphic regions between the parental lines, the 978 polymorphic markers did not group perfectly as 20 chromosomes, but were grouped into 23 linkage groups (LG), with markers classified into two linkage groups for Chromosomes 2, 4, and 10, respectively (Table S1, Figure S2). All the polymorphic markers covered a total genetic distance of 1,245.89 cM and the average marker density ranged from 0.20 cM (LG8) to 3.56 cM (LG3).

Quantitative disease resistance loci (QDRL) were detected using all the 228 F4:5 lines of the POP150029 for partial resistance against the *P. sansomeana* isolate *MPS17-22* (Table 2). Composite interval mapping (CIM) using QTL cartographer identified two QDRL for RSE. The first QDRL (dubbed *qPsan5.1*) was located at 54.71 cM on

Table 2 QDRL detected in 150,029 (E13901 × E13390) for partial resistance to *Phytophthora sansomeana*

QDRL	Trait	Chr	Position (cM)	Flanking marker	LOD	LOD threshold [a]	R^2 (%)	Additive effect[b]
qPsan5.1	RSE	Gm05	54.71	Gm05_32565157_T_C and Gm05_32327497_T_C	3.38	3.26	5.49	0.027
	RRW	Gm05	54.71	Gm05_32565157_T_C and Gm05_32327497_T_C	3.37	3.30	5.53	0.016
	DRX	Gm05	54.71	Gm05_32565157_T_C and Gm05_32327497_T_C	3.67	3.10	5.99	2.106
qPsan16.1	RSE	Gm16	39.01	Gm16_35700223_G_T and Gm16_35933600_A_G / Gm16_35816475_T_C	3.53	3.26	5.72	− 0.027
	DRX	Gm16	39.01	Gm16_35700223_G_T and Gm16_35933600_A_G / Gm16_35816475_T_C	3.18	3.10	5.52	− 1.976

[a]LOD threshold of significance is determined by permutation tests of 1000 iterations ($P < 0.05$) (Churchill and Doerge, 1994)

[b]Positive value of additive effect indicates that the E13390 allele increases the trait value

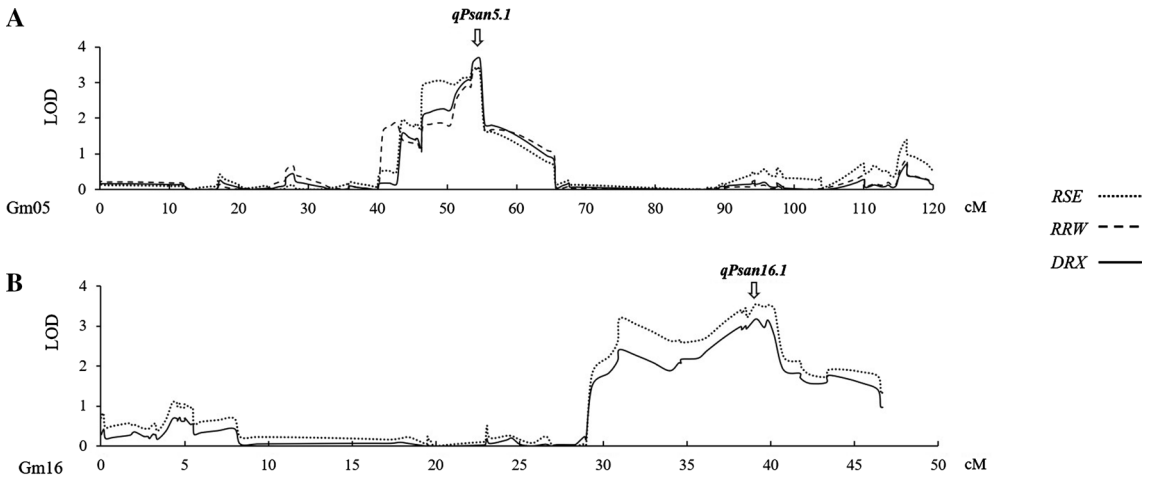

Fig. 2 Molecular mapping of *qPsan5.1,* **a** and *qPsan16.1,* **b** on soybean chromosomes 5 and 16, respectively. RSE: ratio of seedling emergence. RRW: ratio of root weight. DRX: disease resistance index

Chromosome 5, with a LOD score of 3.38 (higher than the LOD threshold of 3.26 from 1000 permutations) (Fig. 2a). The QDRL accounted for 5.49% of RSE variation and its additive effect was 0.027. The desirable allele of *qPsan5.1* was from the susceptible parent E13390 (Table 2). *qPsan5.1* was flanked by SNP markers Gm05_32565157_T_C (53.72 cM) and Gm05_32327497_T_C (55.41 cM) with Gm05_32327497_T_C being the nearest marker. The other QDRL (dubbed *qPsan16.1*) was detected on Chromosome 16 at a genetic distance of 39.01 cM with a LOD score of 3.53 (Fig. 2b). *qPsan16.1* explained 5.72% of the RSE variation and the desirable allele was from the partial resistant parent E13901 with an additive effect of -0.027. *Psan16.1* was located between Gm16_35700223_G_T (38.57 cM) and Gm16_35933600_A_G/ Gm16_35816475_T_C (39.04 cM).

qPsan5.1 was also detected using the RRW and DRX, with the LOD scores of 3.37 and 3.67, respectively (Table 2). It explained 5.53% and 5.99% of variations of the RRW and DRX, respectively. *qPsan16.1* could also be detected using the DRX, but not with RRW, with its LOD score of 3.18. *qPsan16.1* could explain 5.52% of the DRX variation.

Interaction of *qPsan5.1* and *qPsan16.1* on partial resistance to *P. sansomeana* isolate *MPS17-22*

To characterize the interaction of *qPsan5.1* and *qPsan16.1*, four groups of F4:5 lines were selected from the POP150029 containing two, one, or none of the QDRL (Table S2). The genotypic groups of the QDRL were selected according to the genotype of their flanking markers, for example, the *qPsan5.1* locus at the soybean line 150,029–001 was considered homozygous resistant, because the flanking markers Gm05_32565157_T_C and Gm05_32327497_T_C were both donor (E13390) genotype. Using this method, a total of 110 soybean F4:5 lines were identified, including 29 lines homozygous resistant at both *qPsan5.1* and *qPsan16.1* loci (dubbed the RR group); 31 lines homozygous resistant at the *qPsan5.1* locus but homozygous susceptible at the *qPsan16.1* locus (dubbed the RS group); 26 lines homozygous resistant for *qPsan16.1*, but homozygous susceptible for *qPsan5.1* (dubbed the SR group); and 24 lines with homozygous susceptible alleles at both loci (dubbed the SS group). The phenotypic value of a genotypic group was therefore calculated as the mean of all the soybean lines in the group.

One-way ANOVA was then performed to compare the four genotypic groups for the RSE, RRW, and DRX (Fig. 3). Not surprisingly, the SS group, which contains neither of the QDRL, was the most susceptible, with the means of RSE and DRX lower than the other three groups significantly. For the RRW, the mean of the SS group was significantly lower than the RR and the SR group, and was also lower than the RS group, although not significant ($p = 0.056$). The RS and the SR groups were both higher than the SS group, and contributed similar level of partial resistance to the *P. sansomeana* isolate *MPS17-22*. The RR group, which contains both QDRL, was the most resistant. Interestingly, the RSE, RRW, and DRX of the RR group were significantly higher than the RS and the SR groups which contain a single QDRL, respectively, suggesting an additive interaction of the *qPsan5.1* and *qPsan16.1* in partial resistant to *MPS17-22*.

The resistance of *qPsan5.1* and *qPsan16.1* to seven *P. sansomeana* isolates and progeny test

To validate *qPsan5.1* and *qPsan16.1*, and to characterize the resistance of each QDRL against different *P. sansomeana* isolates, a marker assisted resistance spectrum (MARS) analysis was performed against seven additional *P. sansomeana* isolates (Lin et al. 2013; Rojas et al. 2017a).

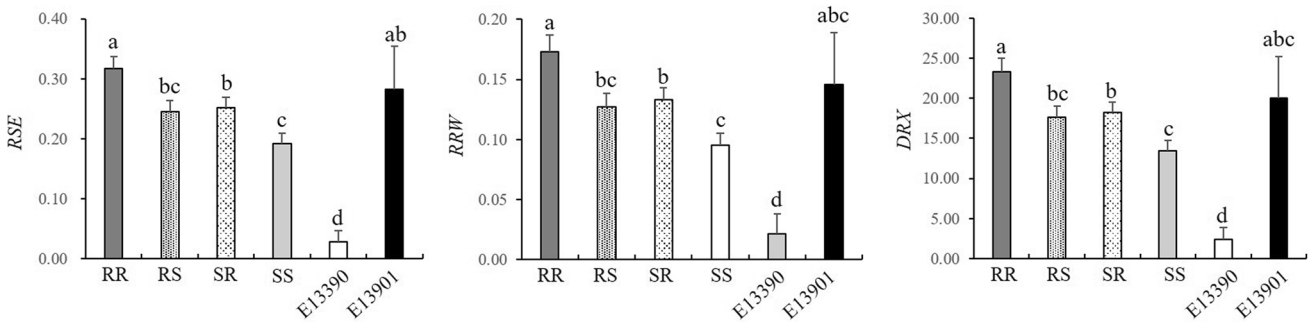

Fig. 3 Interaction of *qPsan5.1* and *qPsan16.1* for partial resistance to *P.sansomeana* isolate *MPS17-22*. RR group contains 29 F4:5 lines with both desirable alleles; RS group contains 31 F4:5 lines with desirable alleles for *qPsan5.1* only. SR group contains 26 F4:5 lines with desirable allele for *qPsan16.1* only. SS group contains 24 F4:5 lines with neither of the desirable alleles. Different letters on top of each bar indicated statistical significance of $p < 0.05$

Considering the limitation of seeds, a subset of 10 lines was selected from each genotypic group with abundant seeds and their mean values of the RSE, RRW, and DRX representing the mean of each group for *MPS17-22* (Table S3). The rest of the lines in each group were used as backup for the test.

Interestingly, the interaction of the two QDRL and the seven *P. sansomeana* isolates were different from each other and can be generally divided into four patterns using the DRX (Fig. 4, Table S3). In the first pattern, the *qPsan5.1* and *qPsan16.1* each contributed partial resistance separately to the *P. sansomeana* isolates (*V-NESO2 5–45* and *V-KSSO2 3–6*), and the combination of the *qPsan5.1* and *qPsan16.1* did not show additive effects against the isolates (Fig. 4a, b). In the second pattern, each single QDRL did not confer resistance to the *P. sansomeana* isolates (*MPS17-24* and *C-NESO2 5–12*), while the combination of both *qPsan5.1* and *qPsan16.1* conferred partial resistance to the isolates (Fig. 4c, d). In the third pattern (*C-IASO2 6–15* and *MICO3-28*), the partial resistance was only conferred by the *qPsan16.1*, whereas the *qPsan5.1* does not confer resistance to the *P. sansomeana* isolates (Fig. 4e, f). In the fourth pattern, neither of the QDRL conferred resistance to the *P. sansomeana* isolate *KSSO6-1* (Fig. 4g). Similar patterns can also be observed using the RSE and the RRW (Figure S4).

The progeny test confirmed the interaction patterns for some *P. sansomeana* isolates, but variations were also observed for the other isolates (Figure S5). For example, the partial resistance against the *MPS17-24* required the existence of the *qPsan5.1* and *qPsan16.1* in both MARS and progeny tests; Another example is that neither QDRL conferred resistance to the isolate *KSSO 6–1* in both tests. On the other hand, both QDRL were needed to confer resistance to the *P. sansomeana* isolates *V-NESO2 5–45*, *V-KSSO2 3–6*, and *MICO3-28*, while the resistance to *C-NESO2 5–12* was conferred only by the *qPsan16.1* in the progeny test. For *C-IASO2 6–15*, non-significant resistance was detected in the progeny test.

Discussion

P. sansomeana was differentiated from the *P. megasperma* complex as a causal agent of soybean root rot in 2009 (Hansen et al. 2009) and has been reported in various soybean growing regions in the US (Rojas et al. 2017a). However, *Rps* genes, which have been widely deployed for complete resistance against corresponding pathotypes of *P. sojae*, have been shown non-effective against *P. sansomeana* (unpublished data). This study is hence the first to report soybean QDRL conferring partial resistance to *P. sansomeana*. Using composite interval mapping, marker assisted resistance spectrum analysis, and progeny tests, two small effect QDRL were identified and verified.

The transgressive segregation of the mapping population could be explained by the interaction of *qPsan5.1* and *qPsan16.1* which were identified from E13390 and E13901, respectively. However, an interesting phenomenon is that although the desirable allele of *qPsan5.1* was mapped from E13390, E13390 was significantly more susceptible than the RS group which contains *qPsan5.1* but not *qPsan16.1*,

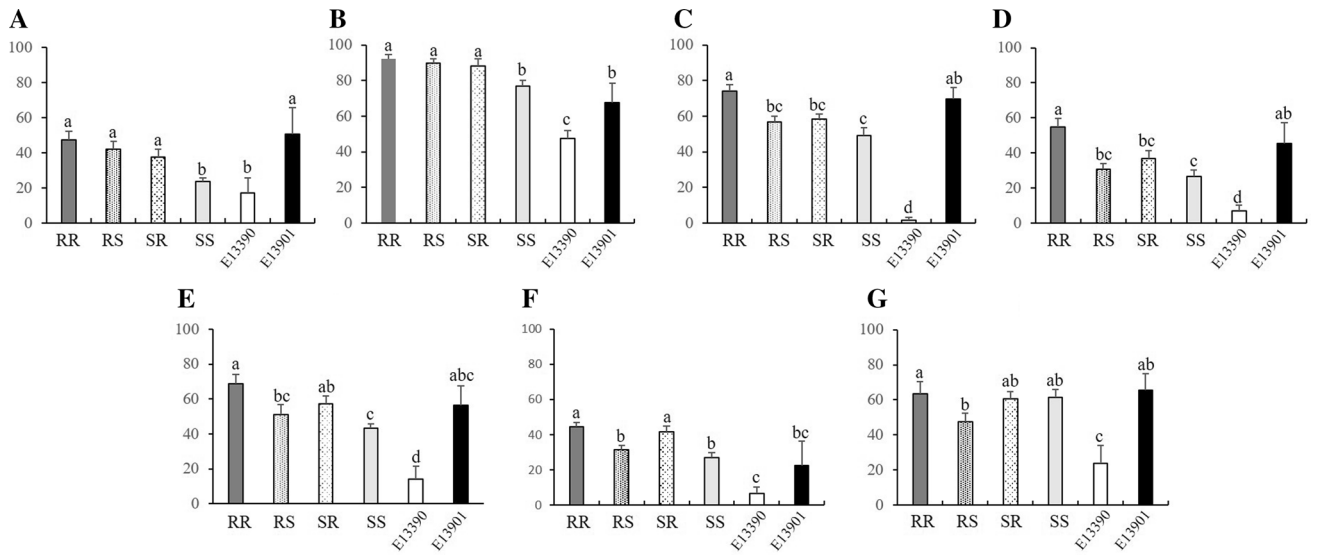

Fig. 4 Marker assisted resistance spectrum (MARS) analysis of soybean F4:5 lines to seven *P. sansomeana* isolates using disease resistance index (DRX). A: *V-NESO2 5–45*; B: *V-KSSO2 3–6*; C: *MPS17-24*; D: *C-NESO2 5–12*; E: *C-IASO2 6–15*; F: *MICO3-28*; G: *KSSO 6–1*. RR, RS, SR, and SS group each contained 10 selected F4:5 lines. Different letters on top of each bar indicated statistical significance of $p < 0.05$

for all the eight *P. sansomeana* isolates tested (Fig. 3 and 4). A possible explanation for this phenomenon is that the function of *qPsan5.1* in E13390 was suppressed in response to the infection of *P. sansomeana*. It is likely that the suppressor locus is unlinked with *qPsan5.1* and is recessively inherited. Hence the possibility of its co-segregation with *qPsan5.1* is low in the segregating population and may not significantly affect the average performance of RS group which consists of 31 progeny lines. What's more, the suppression of *qPsan5.1* in E13390 still could not explain why E13390 was also more susceptible than the SS group which contains neither *qPsan5.1* nor *qPsan16.1*, to seven of eight *P. sansomeana* isolates (except *V-NESO2 5–45*) (Fig. 3 and 4). This phenomenon may infer that more QDRL are functioning against *P. sansomeana* which were not yet detected in this study.

The progeny test confirmed the partial resistance conferred by *qPsan5.1* and *qPsan16.1* to several *P. sansomeana* isolates. However, the patterns of interaction between the QDRL and the pathogen were not always consistent in the MARS and progeny tests. This may partly be attributable to the variation of environments, for example, environmental influence of the gene expression (Gibson 2008). Also, notice that although 10 lines were used for each genotypic group, only one inoculated replicate was performed for each line in the progeny test due to the limitation of seeds, which allowed larger environmental variations. Another reason may be due to the variation of aggressiveness and growth rate of *P. sansomeana* in different tests, which can be attributable to environmental factors such as temperature (Rojas et al. 2017a). For example, the aggressiveness of isolates *V-NESO2 5–45* and *K-SSO2 3–6* were much higher in the progeny test than in the MARS test, which could be reflected by the values of phenotypic traits. It appears that in lower disease stress, each QDRL could confer partial resistance to the two isolates, while in higher stress, partial resistance was only conferred by the collaboration of both QDRL. Nevertheless, the MARS and Progeny tests indicated that the combination of *qPsan5.1* and *qPsan16.1* conferred higher level and more stable partial resistance to most of the *P. sansomeana* isolates than each single QDRL. Hence to obtain the best effect of controlling *P. sansomeana*, *qPsan5.1* and *qPsan16.1* should both be pyramided into a soybean variety.

Partial resistance or quantitative resistance has generally been considered broad spectrum and race non-specific (Dorrance et al. 2008; St.Clair 2010; Mundt 2014; Nelson et al. 2018; Karhoff et al. 2019). However, isolate specific QDRL have also been identified for partial resistance to *P. sojae* and other pathogens (Caranta et al. 1997; Li et al. 2006; Marcel et al. 2008; Poland et al. 2009; Lee et al. 2014; Stasko et al. 2016). In this study, the QDRL showed isolate specificity to different isolates of *P. sansomeana*. More interestingly, epistatic interactions have been observed for partial resistance to some of the isolates, suggesting the complexity of resistance mechanism to *P. sansomeana*.

Soybean is palaeopolyploid and has encountered two rounds of whole genome duplication events. We examined the loci of *qPsan5.1* and *qPsan16.1* and yet found that they were not in the duplicated regions (Shoemaker et al. 2006; Schmutz et al. 2010). The flanking markers of *qPsan5.1* corresponded to a 237.6 kb genomic region on soybean Chromosome 5, which contains 29 predicted gene loci based on Williams82 reference genome (Gmax2.0) including one gene encoding leucine-rich repeat receptor-like protein kinase (LRR-RLK) (Glyma.05g134800) and one F-box protein gene (Glyma.05g134000) (Table S5). The position of *qPsan16.1* spanned a genomic region of 233 kb and was closely linked to *RpsUN2*, a *R* gene identified from a soybean landrace PI 567139B, that confers complete resistance to a few isolates of *P. sojae* (Lin et al. 2013; Li et al. 2016). Twenty-five predicted genes were located within the *qPsan16.1* region, including 4 LRR-RLKs (Glyma.16g201200, Glyma.16g201500, Glyma.16g202200, and Glyma.16g202400) and 2 cysteine-rich RLK genes (Glyma.16g201900 and Glyma.16g202100) (Table S5). The RLK gene family has been shown to play a central role in signaling during pathogen recognition, the subsequent activation of plant defense mechanisms, and developmental control (Afzal et al. 2008, Srour et al. 2012; Schneider et al. 2016), and as such the LRR-RLK gene in *qPsan5.1* region and 6 RLK genes in *qPsan16.1* could be the strongest candidate genes. In addition, four F-box protein genes (Glyma.16g202600, Glyma.16g202800, Glyma.16g202900, and Glyma.16g203000) were also located in the *qPsan16.1* region. Soybean F-box protein genes GmCOI1 has been shown to mediate Jasmonate regulated plant defense response in Arabidopsis (Wang et al. 2005, Qiu et al. 2009), and hence the five F-box protein genes (1 for *qPsan5.1* and 4 for *qPsan16.1*) may also be considered candidate genes.

The markers identified in this study can be used in marker assisted selection directly for breeding resistant soybean lines against *P. sansomeana*. However, to improve the efficiency and accuracy of marker assisted selection, perfect markers that co-segregate with the QDRL will be needed. Current mapping results have delimited *qPsan5.1* and *qPsan16.1* to 237.6 kb and 233 kb regions, respectively. To fine map *qPsan16.1*, lines with homozygous susceptible allele at the locus *qPsan5.1* and heterozygous allele at the locus *qPsan16.1* will need to be identified and self-pollinated to establish large mapping populations. The fine mapping populations will then be advanced for one more generation to create enough progeny seeds for screening against the *P. sansomeana* isolate *MPS17-22*. More molecular markers will be identified from the soybean 50 K beadchip, or can be designed from the soybean reference genome (www.soybase.org).

More than thirty *Rps* genes have been identified conferring complete resistance to *P. sojae* (Dorrance et al. 2018). Unfortunately, none of the *Rps* genes identified thus far could confer resistance to *P. sansomeana* (unpublished data). This may not be too surprising because first, although both pathogens were isolated from the *P. megasperma* complex, phylogenetic analysis using ITS DNA sequences placed them in different clades (clade 7 for *P. sojae* and clade 8 for *P. sansomeana*, respectively) (Hansen et al. 2009). Moreover, unlike *P. sojae* that specifically hosts soybean, *P. sansomeana* has a much wider host range including soybean, Douglas-fir, and weeds (such as white clover, wild carrot, and white cockle) (Hansen et al. 2009). Therefore, more efforts may be needed to identify quantitative resistance for *P. sansomeana*.

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Declaration

Conflict of interest The authors declare that they have no conflict of interest.

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