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ORIGINAL PAPER

Joint linkage QTL analyses for partial resistance to *Phytophthora sojae* in soybean using six nested inbred populations with heterogeneous conditions

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Abstract Partial resistance to *Phytophthora sojae* in soybean is controlled by multiple quantitative trait loci (QTL). With traditional QTL mapping approaches, power to detect such QTL, frequently of small effect, can be limited by population size. Joint linkage QTL analysis of nested recombinant inbred line (RIL) populations provides improved power to detect QTL through increased population size, recombination, and allelic diversity. However, uniform development and phenotyping of multiple RIL populations can prove difficult. In this study, the effectiveness of joint linkage QTL analysis was evaluated on combinations of two to six nested RIL populations differing in inbreeding generation, phenotypic assay method, and/or marker set used in genotyping. In comparison to linkage analysis in a single population, identification of QTL by joint linkage analysis was only minimally affected by different phenotypic methods used among populations once phenotypic data were standardized. In contrast, genotyping of populations with only partially overlapping sets of markers had a marked negative effect on QTL detection by joint linkage analysis. In total, 16 genetic regions with QTL for partial resistance against *P. sojae* were identified, including four novel QTL on chromosomes 4, 9, 12, and 16, as well as significant genotype-by-isolate interactions. Resistance alleles from PI 427106 or PI 427105B contributed to a major QTL on chromosome 18, explaining 10–45 % of the phenotypic variance. This case study provides guidance on the application of joint linkage QTL analysis of data collected from populations with heterogeneous assay conditions and a genetic framework for partial resistance to *P. sojae*.

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Introduction

Caused by the oomycete *Phytophthora sojae* (Kaufmann and Gerdemann), Phytophthora root and stem rot is a major disease of soybean in many countries, including the United States (Schmitthenner 1985). Single-gene (*Rps*) mediated resistance has been introgressed into many soybean cultivars to manage this disease. However, as the diversity of the pathotypes of *P. sojae* has increased over the past three decades, the importance of partial resistance for long-term management of Phytophthora root and stem rot has also increased (Schmitthenner 1985; Grau et al. 2004). In many host-pathogen systems, partial resistance, conferred by multiple genes or quantitative trait loci (QTL), is more durable and effective to a broad spectrum of strains of a pathogen (Kou and Wang 2010; St Clair 2010). Previous studies on QTL mapping dissected the genetic basis of partial resistance to *P. sojae* in several recombinant inbred line (RIL) populations (Burnham et al. 2003; Weng et al. 2007; Han et al. 2008; Li et al. 2010; Tucker et al. 2010; Wang et al. 2010, 2012; Wu et al. 2011; Nguyen et al. 2012; Lee et al. 2013a, b). In these studies, most of the QTL identified had small to medium effects, each of which individually explained less than 20 % of phenotypic variance in a population.

It is often difficult to identify QTL with small effects due to the limited size of a mapping population (Beavis 1998; Schon et al. 2004; Vales et al. 2005; Holland 2007). Joint linkage QTL analysis of multiple populations represents an alternative approach which diminishes this limitation, allowing the dissection of genetic variation for complex traits among diverse germplasm (Lander and Kruglyak 1995). This approach can increase power to detect QTL with small to medium effects and can provide a more precise estimation of QTL effects and genomic positions in comparison to linkage QTL analysis with individual bi-parental populations (Lander and Kruglyak 1995). The power and resolution of joint linkage QTL analysis with a nested association mapping (NAM) population comprised of 25 RIL populations nested by a common parent was recently demonstrated for several important traits in maize (*Zea mays* L.) (Buckler et al. 2009; Tian et al. 2011; Cook et al. 2012b; Chandler et al. 2013). Moreover, joint linkage QTL analysis can be effective even in a small-scale NAM population. For example, joint linkage QTL analysis with three *Arabidopsis* RIL populations nested by a common parent could detect more QTL than those identified by linkage analyses in each of the three populations (Li et al. 2011).

In maize NAM population studies, traits of interest were evaluated with the same phenotypic assays and genotyped with common markers for all population. With advances in genotyping, the phenotyping and population development have increasingly become the primary bottlenecks in genetic analyses. Bi-parental populations are commonly developed from parents segregating for traits of interest, with multiple populations being developed and phenotyped over a period of years and sometimes comprised of varying generations (Kump et al. 2010). Phenotypic assays for populations of large sizes may be limited by labor and cost (White et al. 2012). In addition, phenotyping methods tend to be modified, improved, or otherwise changed over experiments (Walling et al. 2000; Wang et al. 2012). Even in the absence of these changes in methods, it is also often difficult to control environmental conditions of experiments. For example, availability/virulence of pathogen isolates for disease assays may not be the same across multiple populations. Thus, confounding factors may become common when multiple populations are combined for the joint linkage QTL analysis.

In the present study, a NAM design was used in the development of six RIL populations derived from crosses of six plant introductions with *P. sojae* resistance from the Republic of Korea, the People's Republic of China, and Japan (Dorrance and Schmitthenner 2000) to a common inbred line OX20-8, which is susceptible to *P. sojae*. These six populations were heterogeneous for the breeding generation, the markers used for genotyping, and the phenotypic assay methods employed to evaluate partial resistance to *P. sojae* (Table 1). The primary objective for this study was to evaluate the benefits and drawbacks of joint linkage QTL analysis using different combinations of RIL populations with differing confounding conditions. To achieve this objective, four scenarios were tested: (1) joint linkage QTL analysis limited to combinations of two populations with the least confounding conditions, (2) joint linkage QTL analysis with four RIL populations in which the generations of inbreeding for individual populations differed and sets of SNP markers only partially overlapped, (3) joint linkage QTL analysis with four RIL populations for which two different phenotypic assay methods were used to evaluate the resistance to *P. sojae*, and (4) joint linkage QTL analysis in six RIL populations with non-homogeneous phenotypic assays, differing inbreeding generations, and partially overlapping marker sets. The secondary objective was to identify QTL associated with partial resistance to *P. sojae* in four RIL populations first described in this manuscript.

Materials and methods

Mapping populations, sampling, and DNA extraction

Two recombinant inbred populations (OP1 and OP2) derived from the cross of OX20-8 × PI 398841(PI1) or PI

Table 1 Heterogeneous conditions in assays of six OP populations and four scenarios for joint linkage QTL analysis

Population				Conditions in assays			Scenario[d]			
Name	Resistance source[a]	Generation	Size (lines)	SNP set[b]	Phenotypic assay (traits measured[c])	*P. sojae* isolates	1	2	3	4
OP1	PI 398841 (K)	$F_{7:8}$	305	OPA-B	Tray test (Lesion length)	C.2.S.1	+	+	+	+
OP2	PI 407861A (K)	$F_{7:8}$	157	OPA-B	Tray test (Lesion length)	OH25	+	+	+	+
OP3	PI 427106 (C)	$F_{7:8}$	367	OPA-B	Layer test (RR, PW, RFW, FDW)	1.S.1.1 and OH30	+	−	+	+
OP4	PI 427105B (C)	$F_{7:8}$	338	OPA-B	Layer test (RR, PW, RFW, FDW)	1.S.1.1 and OH30	+	−	+	+
OP5	PI 398297 (K)	$F_{4:6}$	111	OPA-A	Tray test (Lesion length)	OH7	+	+	−	+
OP6	PI 417178 (J)	$F_{4:6}$	128	OPA-A	Tray test (Lesion length)	OH7	+	+	−	+

[a] Resistance source of each population. The origin of plant introductions is indicated in the parentheses: *K* Republic of Korea, *C* People's Republic of China, *J* Japan

[b] Two sets of SNP markers were used to genotype populations using Illumina BeadXpress ® SNP genotyping

[c] Traits were measured to evaluate levels of partial resistance to *P. sojae* in the corresponding phenotypic assays. *RR* root rot score, *PW* plant weight, *RFW* root fresh weight, *RDW* root dry weight. RR was based on a scale of 1–9; 1 indicates no disease, and 9 indicates all seedlings were dead (Dorrance et al. 2008)

[d] Populations included in each joint linkage QTL analysis indicated by the "+" sign. Scenario 1 is the joint linkage QTL analysis of two populations (OP1 and OP2, OP3 and OP4, OP5 and OP6) with least confounding conditions. Scenario 2 is the joint linkage QTL analysis of four populations (OP1, OP2, OP5, and OP6) with the same phenotypic assay method but different SNP sets were used, as well as different breeding generations. Scenario 3 is the joint linkage QTL analysis of four populations (OP1, OP2, OP3, and OP4) with the same composite generation, the same SNP set, but different phenotypic assays. Scenario 4 is joint linkage QTL analysis of all six populations

407861A (PI2) have been previously described (Lee et al. 2013a, b). Four additional populations (OP3 to OP6) were developed from crosses between OX20-8 and PI 427106 (PI3), PI 427105B (PI4), PI 398297(PI5), and PI 417178 (PI6). OX20-8 is a breeding line developed in Ontario, Canada, which possesses *Rps1a* and a very low level of partial resistance to *P. sojae* (Buzzell and Anderson 1982). Both PI3 and PI4 were originally collected from Jinlin, China. PI5 and PI6 were originally collected from Korea and Japan, respectively. These PIs all have high levels of partial resistance to *P. sojae* (Dorrance and Schmitthenner 2000). Twenty-two F_1 seeds from crosses between OX20-8 and PI3 and nine F_1 seeds from crosses between OX20-8 and PI4 were planted and self-pollinated in a field near the Ohio Agricultural Research and Development Center (OARDC), Wooster, OH, in 2005. In the following growing season, 1241 F_2 seeds from OP3 crosses and 1273 F_2 seeds from OP4 crosses were planted and advanced to F_7 generation by single-seed descent (SSD). The F_7 plants were harvested and threshed individually to obtain $F_{7:8}$ seeds in the fall of 2009. The final populations for OP3 and OP4 consisted of 367 and 338 RILs, respectively, all of which were assayed in the present study. Young leaf tissue was collected from each parent and single F_7 individuals at V_1 or V_2 stage and lyophilized. DNA was extracted from tissue using a CTAB buffer method (Lee et al. 2013a).

OP5 and OP6 were developed from crosses of OX20-8 × PI 398297 (PI5) and OX20-8 × PI 417178 (PI6). The first crosses were made in the summer of 2004. In 2005, six F_1 seeds of OP5 crosses and five F_1 seeds of OP6 crosses were planted and self-fertilized in a nursery near the Ohio State University, Columbus, Ohio. In the following years, F_2 seeds of each population were planted and advanced to F_4 generation by SSD. In 2008, 111 $F_{4:6}$ RILs of OP5 and 128 $F_{4:6}$ RILs of OP6 were harvested and threshed. Using a CTAB buffer method (Lee et al. 2013a), DNA was extracted from the bulked leaf tissues collected from ten F_6 seedlings per RIL.

Experimental design and phenotypic assay for partial resistance

Phytophthora sojae isolate C.2.S.1 and OH25 were used in inoculations of OP1 and OP2, respectively (Lee et al. 2013a, b). For OP5 and OP6, *P. sojae* isolate OH7 (vir 1a, 3a, 6, 7) was used for inoculations. These isolates were chosen because the parental lines did not exhibit *R*-gene mediated response to each of the selected isolates following hypocotyl inoculation. Tray tests were used to evaluate levels of partial resistance to *P. sojae* in OP1, OP2, OP5 and OP6 based on lesion development following inoculation, as described in the previous reports (Lee et al. 2013a, b). In brief, ten 7-day-old seedlings for each RIL were placed on trays, and wounded 20 mm below the crown using a scalpel. Macerated *P. sojae* culture-grown agar was applied to the wound using a syringe. The length of lesion developed on the root and stem was measured 7 days after inoculation.

OP3 and OP4 were evaluated in a greenhouse in OARDC, Wooster, Ohio between January and July, 2012 for resistance to *P. sojae* using the layer test, which was described in Wang et al. (2012) and Dorrance et al. (2008). In brief, 15 seeds per RIL were planted in a Styrofoam cup

filled with vermiculite containing a *P. sojae* culture-grown agar layer. Root rot score (RR), plant weight (PW), root fresh weight (RFW), root dry weight (RDW) were measured 2 weeks after planting. RILs in each population were randomly assigned to eight blocks in an augmented randomized complete block design with blocks established according to the temperature gradient in the greenhouse. The cultivars Conrad and Sloan, OX20-8, and the corresponding PI were included as checks in each block. These checks were used to normalize raw data to obtain the best linear unbiased predictor (BLUP). The experiment was repeated four times (two replications by two isolates) per population. Each RIL was evaluated for partial resistance to 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). These isolates were chosen because the parents did not show *R*-gene mediated response following hypocotyl inoculations using these isolates.

Phenotypic data analysis

Lesion lengths of RILs of OP1 and OP2 were analyzed as described in Lee et al. (2013a, b). For OP3 and OP4, traits were analyzed separately by isolates for each population. The BLUP (Stroup 1989) was estimated using PROC MIXED in SAS 9.3 (SAS Institute Inc. 2011) per RIL for RR, PW, RFW, and RDW. The estimated BLUP value represents a relative genetic value of an individual RIL within a population, obtained by excluding environmental effects from a given trait. The model was

$$Y_{ijkl} = \mu + R_i + B(R)_{ij} + C_k + G(C)_{kl} + \varepsilon_{ijkl},$$

where μ = overall mean, R_i = effect of ith replication, $B(R)_{ij}$ = effect of jth block in ith replication, C_k = effect of kth class of entry ($k = 1$, 2, 3, 4, and 5 for OX20-8, either PI3 or PI4, Conrad, Sloan, and RIL, respectively), $G(C)_{kl}$ = effect of lth genotype within class for RIL only (genotypic variance, σ^2_G), ε_{ijkl} = experimental error (σ^2). Class of entry was treated as a fixed effect and all other terms were treated as random effects. Variance components were estimated using the restricted maximum likelihood (REML) method (Patterson and Thompson 1971). The broad-sense heritability on a line-mean basis was calculated as $\sigma^2_G/(\sigma^2_G + \sigma^2/r)$, where r = the number of replications per RIL.

BLUP values of RILs in OP5 and OP6 were also calculated using the same mixed model as above with the exception of class of entry, where PI3 and PI4 were substituted for PI5 and PI6.

Marker genotyping and genetic map construction

The parental genotypes, OX20-8 and PI1–6 were genotyped with 1,536 SNPs (Universal Soybean Linkage Panel 1.0) at Dr. Perry Cregan's laboratory at the United States Department of Agriculture, Agricultural Research Service, Beltsville, MD. Based on the initial SNP genotyping, two sets of 384 SNPs were chosen to develop two custom Illumina Oligo Pool Alls (OPAs). OPA-A was used to genotype OP5 and OP6, which included 198 polymorphic SNPs for each population. OP1–4 were genotyped using OPA-B. There were 230–250 polymorphic SNPs between OX20-8 and each individual PI. All populations were genotyped using Illumina GoldenGate® BeadXpress® SNP genotyping (Illumina Inc., San Diego, CA, USA) at the Molecular and Cellular Imaging Center (MCIC) at OARDC. SNP data were analyzed, and alleles were called using the GenomeStudio™ Genotyping Module v1.0. To increase the number of markers on OP5 and OP6, additional SNPs were genotyped using PCR amplification of multiple specific alleles (PAMSA), which converted SNPs to length polymorphisms resolvable via electrophoresis on a high-resolution agarose gel (Gaudet et al. 2007).

Selected simple sequence repeat (SSR) markers (Song et al. 2010) were additionally genotyped to construct genetic maps with higher genome coverage. The PCR reactions for SSRs had a 20 µl final volume containing 50 ng of template DNA, 1× PCR buffer, 1.0 mM of $MgCl_2$, 50 µM of each of the dNTPs, 0.1 µM of each of forward and reverse primers (IDT Inc., Coralville, IA, USA), and 1.0 U of *Taq* polymerase (GeneScript Corp., Piscataway, NJ, USA). The PCR program was: 95 °C for 5 min, followed by 32 cycles of denaturing at 95 °C for 30 s, annealing at 48–61 °C for 30 s, and extension at 72 °C for 45 s. An additional 10 min of extension at 72 °C followed at the end of the last cycle. The PCR product was resolved on a 4 % high-resolution agarose gel (Research Products International Corp., Mt. Prospect, IL, USA) by gel electrophoresis.

A genetic map of each population was constructed using the software JoinMap4® (Van Ooijen 2006) with the Kosambi's mapping function. Linkage was determined at the LOD threshold of 3.0 with a maximum map distance of 50 centiMorgan (cM). Genetic maps of individual populations were integrated to build joint genetic maps for combinations of two to six populations using JoinMap4®, according to the four scenarios described earlier. Genetic maps were graphically presented using MapChart 2.2 (Voorrips 2002).

Evaluation of effects of standardization of phenotypic data

In this study, standardization of phenotypic data is required for joint linkage QTL analysis because the differing isolates and methods of phenotypic assay used for individual populations exhibited unequal variances. In conjunction with the BLUP values calculated from the raw data in a single population with no standardization, standardization

Table 2 Description of five standardization methods, Levene's test, and the effect on QTL detection using ICIM

Abbreviation	Description	Equation	Levene's test[a]	Change in QTL detection	
			(*P* value)	False positive	False negative
BR	BLUP calculated from raw data	NA	<0.0001	NA	NA
BCS	BLUP calculated from raw data which was standardized by common checks	$Z_{BCS} = (\chi - \mu_C)/\sigma_C$	<0.0001	2 QTL in OP4	0
BPS	BLUP calculated from raw data which was standardized by the population	$Z_{BPS} = (\chi - \mu_P)/\sigma_P$	<0.0001	2 QTL in OP4	0
CSB	Check standardization of BR	$Z_{CSB} = (BR - \mu_{BC})/\sigma_{BC}$	<0.0001	0	0
PSB	Population standardization of BR	$Z_{PSB} = (BR - \mu_{BP})/\sigma_{BP}$	0.9999	0	0

Z standardized data, χ raw data (RD), μ_C mean of the common checks' RD, σ_C standard deviation of the common checks' RD, μ_P mean of RD in each population, σ_P standard deviation of RD in each population, μ_{BC} mean of the common checks' estimated values, σ_{BC} standard deviation of the common checks' estimated values, μ_{BP} mean of BLUP in each population, σ_{BP} standard deviation of BLUP in each population

[a] The null hypothesis of the test is that there is no difference in variance among populations

of phenotypic data between individual populations was carried out by four additional methods as described in Table 2. The five sets of data consisted of BLUP values calculated from raw data (BR), BLUP values calculated from raw data which were standardized by common checks in each population (BCS), BLUP values calculated from raw data which were standardized by the population (BPS), check-standardization of BR (CSB), and population-standardization of BR (PSB). BR was calculated within each population using the raw measurements of lesion length for OP1, 2, 5, and 6 or RDW for OP3 and OP4. For other standardization methods, raw phenotypic data (χ) or BR were transformed based on the mean (μ) and standard deviation (σ) of common checks or each population (Table 2). Since levels of resistance in RDW are negatively correlated with lesion length, the sign of the residual between χ and μ for RDW was reversed in the standardization equations; so that the direction of additive effects would be consistent across populations. The standardized data were tested for the equality of variances among populations by Levene's test (Levene 1960) using SAS 9.3. Using inclusive composite interval mapping (CIM) (Li et al. 2007), linkage analysis was conducted for the five sets of BLUP values in each population to evaluate the effect of standardization on QTL analysis.

Inclusive composite interval mapping (ICIM) and joint ICIM (JICIM)

Marker genotype data were formatted as follows; homozygous OX20-8 and PI allele were designated as "0" and "2", respectively, and missing and heterozygous alleles were designated as "−1" and "1", respectively. Missing and heterozygous alleles were imputed via QTL IciMapping v3.2. ICIM was conducted with the BR phenotypic data set to detect QTL in single populations using QTL IciMapping v3.2. Total phenotypic variance explained by all QTL identified for each trait and/or isolate was calculated using PROC REG in SAS 9.3. ICIM was conducted with BCS, BPS, CSB and PSB data sets to evaluate the effects of standardization on QTL detection. JICIM (Li et al. 2011) was performed with the PSB data for joint linkage QTL analysis based on the four scenarios. A selection threshold of *P* value of 10^{-4}, determined by a 1,000-permutation test, was used to determine entry and removal of markers in stepwise regression. With the selected markers as cofactors, genome-wide scans were conducted with a scan interval of 1 cM. LOD thresholds were determined at Type I error $\alpha = 0.05$ based on a 1,000-permutation test (Churchill and Doerge 1994). For JICIM, the phenotypic variance (%) explained (PVE) by each QTL was calculated as described in Li et al. (2011).

Results

Phenotypic variation within individual population

RILs of OP1, OP2, OP5, and OP6 were each evaluated for partial resistance to *P. sojae* by measurement of lesion length in the tray test. Results of phenotypic assays for OP1 and OP2 were described in Lee et al. (2013a, b). Partial resistance to *P. sojae* in RILs of OP5 and OP6 were evaluated against isolate OH7 by tray test. Mean lesion length was significantly different among parents and checks (supplementary Table 1). Lesion length was significantly shorter in PIs than OX20-8 for both populations (supplementary Table 1). Estimated BLUP values for lesion length were −17.2 for Conrad, −12.1 for PI5, −0.02 for Sloan, and 3.9 for OX20-8 in OP5; and −18.3 for Conrad, −15.6 for PI6, −1.1 for Sloan, and 1.5 for OX20-8 in OP6. BLUP values of RILs were normally distributed in each population (Fig. 1). Broad-sense heritability of lesion length was 0.67 and 0.88 in OP5 and OP6, respectively.

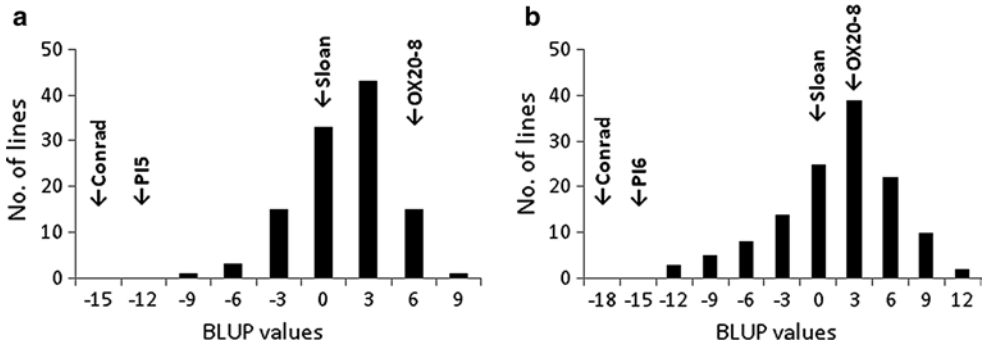

Fig. 1 Frequency distribution of BLUP values for lesion length (mm) of 111 RILs in OP5 and 128 RILs in OP6 following inoculation with *Phytophthora sojae* isolate OH7 in tray tests. *Y*- and *X*-axis indicate the number of RILs and BLUP values, respectively. The estimated values of parents, Conrad, and Sloan are indicated by *arrows*

RILs of OP3 and OP4 were assayed for partial resistance to two isolates of *P. sojae* using layer tests. Levels of partial resistance were evaluated based on RR, PW, RFW, and RDW.

In OP3, the susceptible parent OX20-8 resulted in more root colonization following inoculation of *P. sojae*, with RR scores of 5.4 and 6.6 which were significantly higher than the RR scores of 2.6 and 2.9 for the resistant parent PI3 for the two isolates (supplementary Table 2). Similarly, PW, RFW, and RDW values of OX20-8 were significantly lower than the respective values of PI3 for each isolate, indicating that OX20-8 was more susceptible than PI3 (supplementary Table 2). The values of all traits for PI3 were generally very similar in comparison to those for Conrad, a check with high level of partial resistance for both isolates (supplementary Table 2).

In OP4, RR scores were 1.9 and 2.9 for PI4, significantly lower than the RR score of 4.7 and 6.6 for OX20-8 for the two isolates ($P < 0.05$). There was also significant difference between OX20-8 and PI4 in PW for both isolates and RFW and RDW for isolate OH30 only (supplementary Table 2).

BLUP values of each RIL population were estimated for each isolate separately, as well as with the two isolates combined (data not shown), using the mixed model. In both populations, the BLUP values for each trait were normally distributed, and values for the four parents and checks were well separated in all traits (Figs. 2, 3). In OP3, broad-sense heritability of the four traits was moderate to high for both isolates: RR 0.72, PW 0.70, RFW 0.68, RDW 0.58 for isolate 1.S.1.1, and RR 0.62, PW 0.69, RFW 0.64, RDW 0.67 for isolate OH30. In OP4, broad-sense heritability of the four traits was also moderate to high for both isolates: RR 0.68, PW 0.72, RFW 0.57, RDW 0.52 for isolate 1.S.1.1, and RR 0.69, PW 0.72, FRW 0.55, DRW 0.69 for isolate OH30.

Genetic maps of individual populations and joint populations

Genetic maps were constructed for six single populations, and these were integrated to construct genetic maps of joint populations based on the four scenarios (supplementary Figs. 1–12). Joint linkage maps had 306–607 markers with approximate genome coverage of 68–90 % (Table 3).

Linkage QTL analysis in single populations using ICIM

QTL analysis using ICIM was conducted on individual families using BR. In OP1, three significant QTL for lesion length were identified on chromosomes 1, 3, and 13, which explained approximately 9, 4, and 20 % of phenotypic variance (PV), respectively (Fig. 4; supplementary Table 3). Total PV explained by three QTL was 31 % by regression. PI1 provided resistance alleles for all three loci. In OP2, a single QTL for lesion length was detected on chromosome 3, accounting for 13 % of PV (Fig. 4; supplementary Table 3). The resistance allele at this locus was contributed by PI2. In OP5, a single QTL on chromosome 8 was detected for lesion length and explained 13 % of PV (Fig. 4; supplementary Table 3). The resistance allele was contributed by PI5. In OP6, no significant QTL was identified by ICIM.

Two *P. sojae* isolates were used to evaluate levels of partial resistance for OP3 and OP4. QTL analysis was conducted for RR, PW, RFW, and RDW separately by isolates, as well as with combined data. The genotype × isolate interaction was significant for RR, PW, and RFW in OP3 (supplementary Table 4). Although no significant genotype × isolate interaction was identified for traits measured in OP4 (supplementary Table 4), isolate-specific QTL were identified in both OP3 and OP4. These isolate-specific QTL tended to be of small effect and were significant for only a subset of traits (supplementary Table 5, 6).

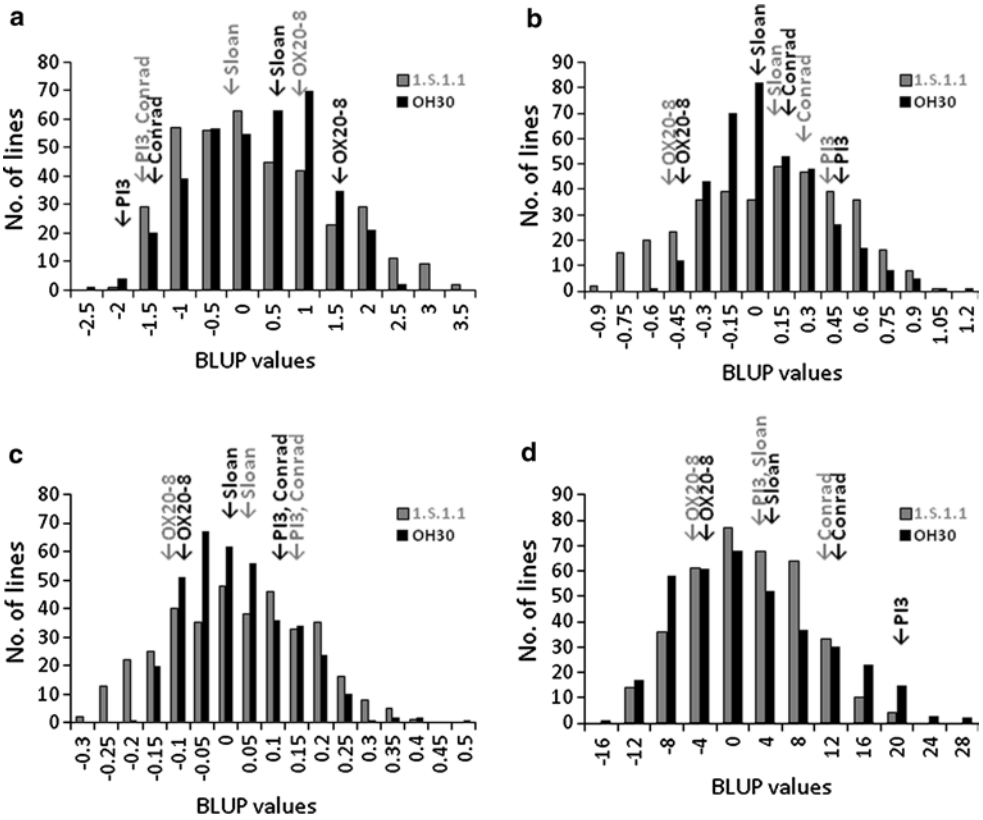

Fig. 2 Frequency distribution of BLUP values for root rot score (**a**), whole plant weight (**b**), fresh root weight (**c**), and dry root weight (**d**) from layer tests of 367 RIL of OP3 following inoculation with *Phytophthora sojae* isolate 1.S.1.1 and OH30. *Y*- and *X*-axis indicate the number of RILs and BLUP values, respectively. The estimated values of parents, Conrad, and Sloan are indicated by *gray* and *black arrows* for 1.S.1.1 and OH30

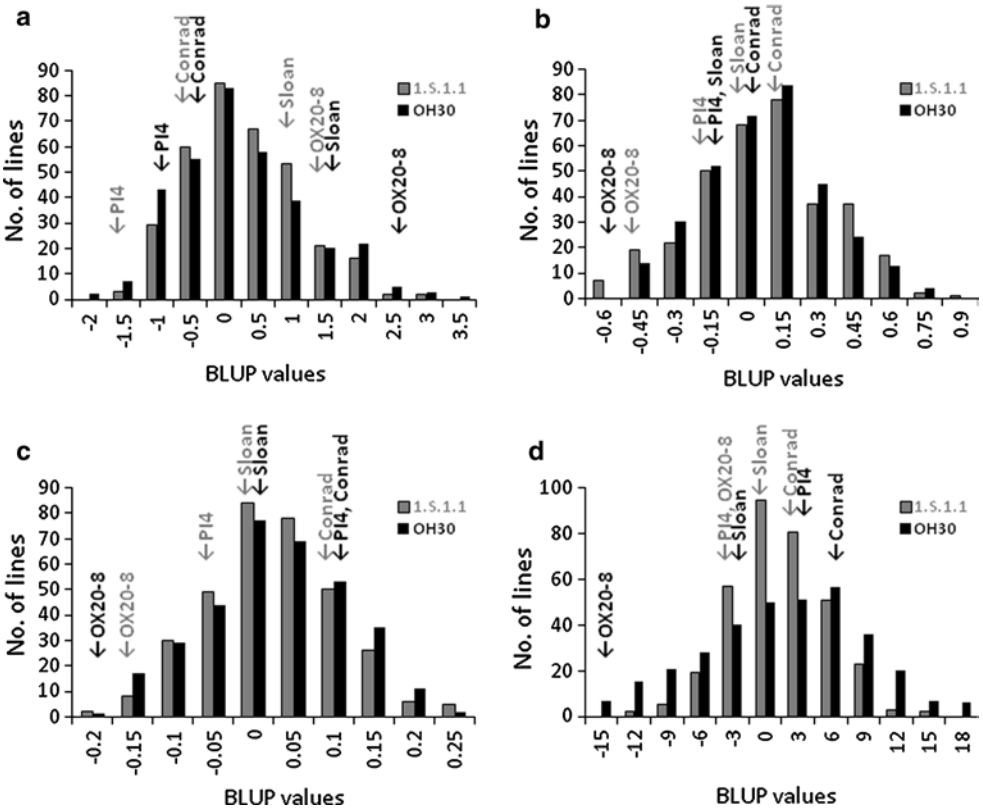

Fig. 3 Frequency distribution of BLUP values for root rot score (**a**), whole plant weight (**b**), fresh root weight (**c**), and dry root weight (**d**) from layer tests of 338 RIL of OP4 following inoculation with *Phytophthora sojae* isolate 1.S.1.1 and OH30. *Y*- and *X*-axis indicate the number of RILs and BLUP values, respectively. The estimated values of parents, Conrad, and Sloan are indicated by *gray* and *black arrows* for 1.S.1.1 and OH30

Table 3 Description of the genetic maps for individual populations and combined populations

Population	No. of markers	No. of linkage groups	Average marker interval (cM)	Genome coverage (%)
OP1	275	31	8.1	70
OP2	287	33	7.5	75
OP3	225	32	9.8	67
OP4	216	32	8.8	63
OP5	221	46	5.2	51
OP6	213	44	5.7	50
OP12	378	28	6.6	81
OP34	321	31	7.8	83
OP56	306	48	5.3	68
OP1256	511	31	4.8	85
OP1234	484	25	5.6	90
OP123456	607	26	4.5	90

In OP3, QTL which were consistently identified from assays with both isolates also tended to be significant for all traits. QTL on chromosomes 12 and 18 were significant for both isolates and all traits, with the exception of RFW against isolate OH30, which lacked significance at the chromosome 12 locus (Fig. 4; supplementary Table 5). The QTL on chromosome 12 explained 3.1–7.0 % of PV (supplementary Table 5). The QTL on chromosome 18 had the largest effect, explaining 19–38 % PV (supplementary Table 5). Total PV explained by QTL ranged between 30 and 43 % for each trait to isolate 1.S.1.1., and between 19 and 27 % for each trait to isolate OH30. PI3 provided resistance alleles for the QTL on chromosome 10, 16, and 18, while OX20-8 provided resistance alleles at the other loci.

For OP4, over all traits, two to five QTL were identified against either 1.S.1.1 or OH30, and three to six QTL were detected in the two-isolate combined analysis (supplementary Table 6). QTL common across both isolates were located on chromosomes 16, 18, and 19 (supplementary Table 6). The QTL on chromosome 18 (8–16 cM) had a major effect, explaining up to 45 % of PV, and were significant across all traits and isolates (Fig. 4; supplementary Table 6). With the exception of this QTL, all other QTL individually explained between 2.9 and 9.3 % PV and were significant for only subsets of traits and/or isolates (supplementary Table 6). All the QTL for isolate 1.S.1.1 explained 13–32 % of the total PV for each trait. All QTL for isolate OH30 explained 27–35 % of the total PV for each trait. Similar to OP3, QTL identified in OP4 with the combined data from both isolates corresponded closely with the QTL detected against each isolate separately (supplementary

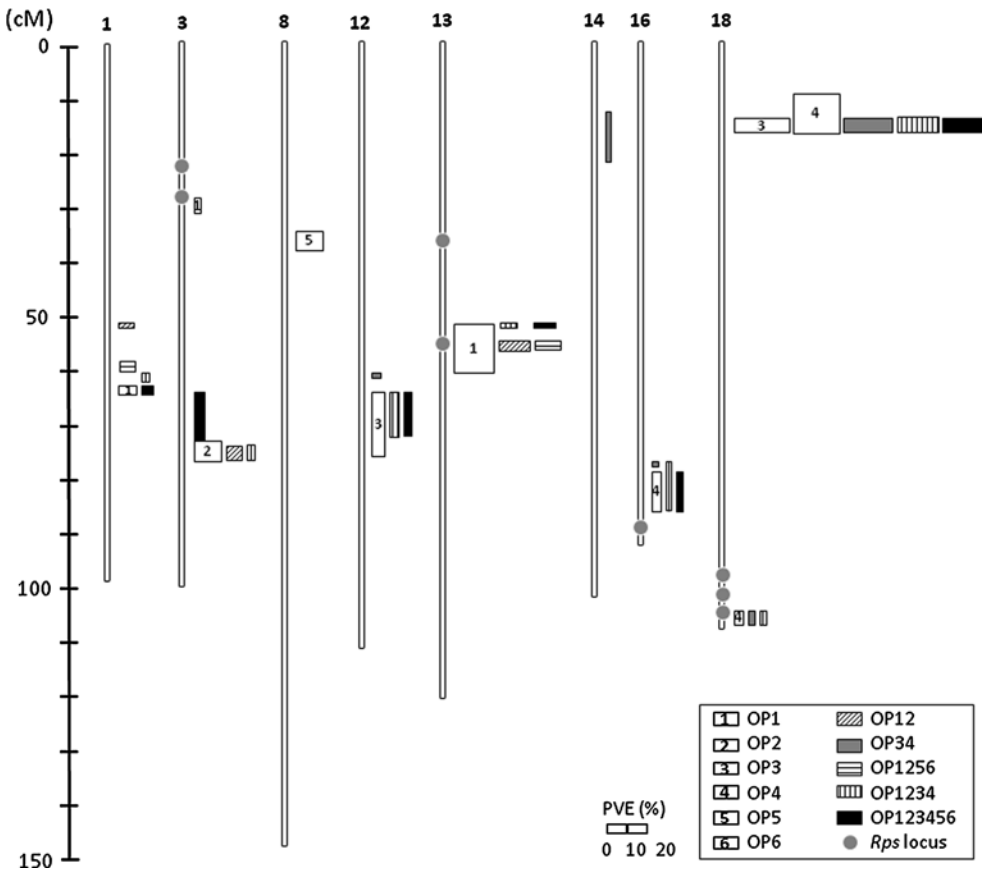

Fig. 4 Identification of QTL for partial resistance to *P. sojae* by single population linkage analysis and joint linkage QTL analyses, ICIM and JICIM, conducted in this study. For OP3 and OP4, QTL identified for RDW with combined isolates are displayed. Chromosomes are represented as *vertical bars* and genetic distance (cM) are shown to the *left* of chromosome 1. *Heights of bars* indicate the interval of LOD peak for each QTL and the *width of bars* represent the phenotypic variance (%) explained by each QTL. Known *Rps*-genes were also presented as *gray circles* on the chromosomes

Table 6). Only a minor QTL on chromosomes 6 was not detected in the combined analysis (supplementary Table 6). PI4 provided resistance alleles for the QTL on chromosomes 13, 14, 16, and 18 (8–16 cM), the resistance allele for other loci was contributed by OX20-8.

Effect of standardization of data on QTL analysis using ICIM

The BLUP values (BR) calculated from lesion length measurements by tray tests and RDW of the layer tests had unequal variances, which were significantly different among populations, with values that ranged from 9.5 to 70.1. Four methods of standardization were applied to the BLUP values or raw measurements (Table 2). Standardized values exhibited unequal variances among populations for BCS, BPS, and CSB (Levene's test; $P < 0.0001$), with only PSB resulting in homogeneity of variances among populations (Table 2). When compared with QTL identified with ICIM of BR, QTL identified with ICIM of data from the four methods of standardization resulted in similar LOD score, PV (%), additive effect (*a*), and genomic location (supplementary Table 7). No false negative QTL were identified with any method of standardization, all QTL identified with BR were also detected in the ICIM in each population (Table 2; supplementary Table 7). In comparison to QTL identified with ICIM of BR, two additional QTL, considered false positives, were detected in OP4 with BCS and BPS (Table 2; supplementary Table 7). PSB, exhibiting equal variance among populations and no false positive or false negative QTL relative to BR, was selected for subsequent analyses of combined populations with JICIM.

Effectiveness of joint linkage QTL analysis using JICIM

Using PSB data, joint linkage QTL analysis was evaluated for its effectiveness in QTL detection under four scenarios (Table 1). With the first scenario of joint linkage QTL analysis, pairs of two populations with the fewest confounding conditions were combined and designated as OP12, OP34, or OP56. The QTL detected through JICIM with the paired populations closely mirrored those identified through ICIM with the individual populations; although, there were several exceptions (Fig. 4; Table 4a). JICIM with OP12 failed to detect one QTL, which had been detected in OP1 (28–31 cM chromosome 3) (Table 4b). The only significant QTL detected through ICIM of OP5 (chromosome 8) was not detected via JICIM in OP56 (Table 4b). In addition to the five QTL associated with RDW detected by ICIM in OP3 and OP4 (Fig. 4; supplementary Tables 5, 6), a sixth QTL was identified on chromosome 14 in JICIM with OP34 (Fig. 4; Table 4c). While this QTL was not significant in either population by ICIM analysis, this locus did exhibit moderate additive effects (−0.16 and −0.17) and LOD scores (2.5 and 2.2) in OP4 and OP4, respectively (Table 4c).

With the second scenario, OP1, 2, 5, and 6 populations were combined (designated as OP1256) to evaluate the effectiveness of joint linkage QTL analysis where RIL populations differed in the inbreeding generations, as well as genotyped with SNP marker sets that overlapped only partially. Two QTL identified on chromosome 1 and 13 (Fig. 4; Table 5a) were previously detected in OP1 by ICIM. Joint linkage QTL analysis failed to detect two QTL which had been identified by ICIM in OP1 (28–31 cM chromosome 3) and OP2 (74–77 cM chromosome 3) as well as the single QTL that had been identified on chromosome 8 in OP5 (Table 5b).

The third scenario was a joint linkage QTL analysis of a combined population of OP1–4 (OP1234), with the confounding conditions of different phenotypic assay methods and measured traits. Seven QTL previously identified by ICIM in OP1–4 were also detected via JICIM with the combined population (Fig. 4; Table 5a). A single QTL on chromosome 3 (28–31 cM) which had been detected with ICIM of OP1 was missed (Table 5b). The QTL on chromosome 14, which was additionally detected in JICIM with OP34, was not significant in OP1234.

The fourth scenario combined all the populations, and thus includes all confounding factors among the six populations. A total of six QTL were identified (Fig. 4; Table 6a), all of which had also been identified in the single population linkage analyses conducted. Three QTL, 28–31 cM on chromosome 3, chromosome 8, and 104–107 cM on chromosome 18, identified in individual populations OP1, OP5, and OP4, respectively, by ICIM were not significant in the joint linkage QTL analysis with OP123456 (Table 6b).

In total, over the six combinations of populations, JICIM of the PSB data yielded eight QTL. A parallel analysis conducted with the CSB data yielded less QTL in OP34 and OP1234, but one additional QTL in OP1256 compared to the results from JICIM with the PSB data (supplementary Tables 9–11).

Discussion

Joint linkage QTL analysis and NAM have been used in QTL analysis for the maize NAM population (Chandler et al. 2013; Cook et al. 2012b; Kump et al. 2011; Poland et al. 2011; Tian et al. 2011). JICIM was recently designed specifically for joint linkage QTL analysis of multiple populations derived with a NAM design (Li et al. 2011). To evaluate effectiveness of joint linkage analysis by JICIM in QTL detection, we initially conducted linkage analysis in individual populations by ICIM. Although CIM (Zeng

Table 4 Joint linkage QTL analysis for partial resistance to *P. sojae* by JICIM with OP12, OP34, and OP56

Chr.	Pos.[a]	LOD[b]	Thr[c]	PVE[d]	LOD in each population		PVE in each population		Additive effect[e] in each population	
a. QTL detected via JICIM and previously detected via ICIM of a single population										
OP12[f]					OP1	OP2	OP1	OP2	OP1	OP2
1	50–51	6.9	4.8	6.9	6.8	0.1	7.1	0.2	−0.14	0.02
3	74–77	5.8		7.4	0.1	5.7	0.1	14.0	−0.03	−0.18
13	52–54	19.4		15.1	15.8	3.6	17.9	8.5	−0.23	−0.13
OP34[f]					OP3	OP4	OP3	OP4	OP3	OP4
12	61–62	5.7	4.6	4.1	5.6	0.1	4.8	0.1	0.22	−0.03
16	77–87	6.1		3.3	1.4	4.7	1.2	4.8	−0.11	−0.22
18	13–16	42.2		24.7	25.3	17.0	27.4	21.6	−0.52	−0.46
18	104–107	5.5		3.1	1.0	4.5	0.9	4.6	0.09	0.22
b. QTL not detected via JICIM which were previously detected via ICIM of a single population										
OP12					OP1	OP2	OP1	OP2	OP1	OP2
3	28–31	4.0	4.8	2.9	3.4	0.6	3.5	1.4	−0.19	−0.12
OP56[f]					OP5	OP6	OP5	OP6	OP5	OP6
8	44–48	2.8	4.6	10.5	2.7	0.1	12.9	0.3	−0.36	0.06
c. QTL detected via JICIM which were not detected via ICIM of a single population										
OP34					OP3	OP4	OP3	OP4	OP3	OP4
14	12–22	4.8	4.6	2.7	2.5	2.2	2.7	2.8	−0.16	−0.17

Chr chromosome number

[a] Genetic positions (cM) of flanking markers for the QTL on the Consensus Map 4.0 (Hyten et al. 2010). The names of the flanking markers were noted in supplementary Table 8

[b] LOD score of QTL from joint linkage QTL analysis

[c] LOD threshold determined by a 1,000-permutation test

[d] Phenotypic variance (%) explained by a single QTL

[e] Negative additive effects indicate that PIs confer resistance alleles for QTL

[f] OP12, OP34, and OP56 indicate the paired populations

Table 5 Joint linkage QTL analysis for partial resistance to *P. sojae* by JICIM with OP1256 and OP1234

Chr.	Pos.[a]	LOD	Thr[b]	PVE[c]	LOD in each population				PVE in each population				Additive effect[d] in each population			
a. QTL detected via JICIM and previously detected via ICIM of a single population																
OP1256[e]					OP1	OP2	OP5	OP6	OP1	OP2	OP5	OP6	OP1	OP2	OP5	OP6
1	58–60	9.4	6.0	7.7	6.0	0.0	3.0	0.4	7.1	0.0	15.6	2.0	−0.27	0.01	−0.39	−0.14
13	52–54	18.6		13.2	15.2	3.0	0.0	0.3	19.0	8.2	0.0	1.2	−0.44	−0.28	0.00	−0.11
OP1234[e]																
1	60–62	6.8	5.9	3.5	6.1	0.1	0.2	0.4	6.9	0.3	0.1	0.4	−0.26	0.06	0.04	−0.07
3	74–77	6.7		4.4	0.2	5.4	0.9	0.1	0.3	14.0	0.8	0.1	−0.05	−0.37	0.09	0.03
12	62–71	6.8		4.1	0.1	0.7	6.0	0.1	0.1	1.8	6.1	0.1	0.03	−0.13	0.25	−0.03
13	51–52	20.2		8.7	14.6	3.5	0.3	1.8	18.0	8.4	0.3	1.7	−0.42	−0.29	−0.05	−0.13
16	77–87	6.9		2.5	0.3	0.2	1.0	5.4	0.4	0.6	0.8	5.3	−0.06	−0.08	−0.09	−0.23
18	13–16	45.3		20.4	0.3	0.3	24.0	20.6	0.4	0.7	26.0	25.5	−0.06	−0.09	−0.51	−0.50
18	104–107	6.6		3.1	0.0	0.0	0.9	5.6	0.0	0.1	0.8	5.8	−0.01	−0.04	0.09	0.24
b. QTL not detected via JICIM and previously detected via ICIM of a single population																
OP1256					OP1	OP2	OP5	OP6	OP1	OP2	OP5	OP6	OP1	OP2	OP5	OP6
3	28–31	3.2	6.0	2.1	2.8	0.4	0.0	0.0	3.2	0.9	0.1	0.0	−0.18	−0.10	−0.02	−0.02
3	74–77	5.1		4.8	0.5	4.1	0.0	0.5	0.6	12.3	0.1	2.2	−0.08	−0.35	−0.02	−0.15
8	44–48	3.5		4.5	0.0	0.5	2.9	0.0	0.0	1.4	12.0	0.1	0.01	0.12	−0.35	0.03
OP1234					OP1	OP2	OP3	OP4	OP1	OP2	OP3	OP4	OP1	OP2	OP3	OP4
3	28–31	3.1	5.9	1.4	2.6	0.5	0.0	0.0	2.7	1.1	0.0	0.0	−0.16	−0.11	0.01	0.00

Chr chromosome number, *LOD* log of odds

[a] Genetic positions (cM) of flanking markers of the QTL on the Consensus Map 4.0 (Hyten et al. 2010). The names of the flanking markers were noted in supplementary Table 8

[b] LOD threshold determined by a 1,000-permutation test

[c] Phenotypic variance (%) explained by a single QTL

[d] Negative additive effects indicate that PIs confer resistance alleles for QTL

[e] OP1256 and OP1234 indicate the combined populations

1994) is the most commonly used method for QTL analysis of a bi-parental population, we applied ICIM for single population QTL analysis to limit discrepancies between the mapping algorithms applied to the single and combined populations. In addition, advantages of ICIM over CIM include avoidance of possible increase of sampling variance and the complicated background marker selection process (Li et al. 2007). Furthermore, a prior study reported that QTL identification by ICIM was more consistent for two different measurements of visual assessment of disease severity and among raters (Poland and Nelson 2011).

Effectiveness of joint linkage QTL analysis of multiple populations with confounding conditions

Joint linkage QTL analysis is expected to increase the power to detect QTL and to estimate more precise effects and positions by combining data from multiple populations (Blanc et al. 2006; Li et al. 2011; Negeri et al. 2011; Walling et al. 2000; Yang et al. 2013). This strategy could lead to the detection of QTL which were not identified in individual populations (Buckler et al. 2009). Ideally, combined populations should be equivalent in the markers for which they were genotyped and traits for which phenotypic data were collected (Chandler et al. 2013). However, in practice, these circumstances may not be met over multiple experiments (Walling et al. 2000). Thus, the present study aimed to investigate possible benefits and drawbacks of the joint linkage QTL analysis of six populations with confounding conditions (Table 1).

With the three pairs of populations with the fewest confounding conditions (scenario 1; OP12, OP34, and OP56), QTL detected in the joint linkage analyses were generally similar to the QTL identified in the single population linkage analyses, with a few notable exceptions. The present study demonstrated that additional QTL could be identified when confounding conditions were relatively fewer among combined populations and the parents of the different populations are closely related (i.e., OP34). One additional QTL on chromosome 14 in OP34 was insignificant for RDW in linkage analysis of both OP3 and OP4 (Table 4c). However, as this QTL was significant for RR and PW in OP4 against both isolates (supplementary Table 6), it is likely a real and not spuriously detected QTL. At this locus, LOD scores

Table 6 Joint linkage QTL analysis for partial resistance to *P. sojae* by JICIM with OP123456

Chr.	Pos.[a]	LOD	Thr[b]	PVE[c]	LOD in each population						PVE in each population						Additive effect[d] in each population					
					OP1	OP2	OP3	OP4	OP5	OP6	OP1	OP2	OP3	OP4	OP5	OP6	OP1	OP2	OP3	OP4	OP5	OP6
a. QTL detected via JICIM and previously detected via ICIM of a single population																						
1	63–65	10.9	7.0	5.6	7.2	0.2	0.1	0.5	2.1	0.9	9.2	0.5	0.1	0.6	8.6	3.4	−0.30	0.07	−0.03	0.07	−0.29	−0.18
3	63–73	7.6		3.9	0.4	4.5	2.2	0.3	0.0	0.2	0.5	12.6	1.9	0.4	0.2	0.8	−0.07	−0.35	−0.14	0.06	0.04	0.09
12	62–71	8.1		4.0	0.1	0.6	6.9	0.2	0.2	0.1	0.1	2.2	7.0	0.3	0.9	0.4	0.03	−0.15	−0.26	0.05	−0.09	0.06
13	51	19.9		10.6	15.4	1.6	0.2	2.2	0.1	0.4	19.9	4.6	0.2	2.4	0.3	1.5	−0.45	−0.21	0.04	0.16	0.06	−0.12
16	79–87	7.2		3.6	0.6	0.2	1.2	4.4	0.8	0.0	0.7	0.6	1.0	4.7	3.4	0.0	−0.08	−0.08	0.10	0.22	−0.18	−0.01
18	13–16	39.3		19.4	0.3	0.4	22.4	16.1	0.2	0.0	0.4	1.1	25.4	22.0	0.7	0.0	−0.06	−0.10	0.50	0.47	0.08	−0.02
b. QTL not detected via JICIM and previously detected via ICIM of a single population																						
3	28–31	4.7	7.0	2.2	3.4	0.5	0.2	0.5	0.0	0.0	3.9	1.6	0.2	0.5	0.1	0.0	−0.20	−0.13	−0.05	0.07	0.04	−0.01
8	44–48	3.3		2.0	0.3	0.3	0.9	0.1	1.7	0.1	0.4	1.2	0.7	0.1	6.9	0.2	0.06	0.11	−0.09	0.04	−0.26	0.05
18	104–107	6.6		3.5	0.2	0.0	0.6	4.1	0.3	1.4	0.2	0.0	0.5	4.7	1.4	5.2	−0.05	−0.01	−0.07	−0.22	0.12	0.23

Chr chromosome number, *LOD* log of odds

[a] Genetic positions (cM) of flanking markers of the QTL on the Consensus Map 4.0 (Hyten et al. 2010). The names of the flanking markers were noted in supplementary Table 8

[b] LOD threshold determined by a 1,000-permutation test

[c] Phenotypic variance (%) explained by a single QTL

[d] Negative additive effects indicate that PIs confer resistance alleles for QTL

in each population separately were insignificant, although greater than half of the LOD threshold. In such cases, the increased population size through combining the two populations (367 and 338 RILs) may have contributed to the detection of this additional QTL. Such QTL which are additionally detected in joint linkage analyses are likely to be minor-effect QTL as described in the maize NAM study (Buckler et al. 2009).

Joint linkage QTL analysis alone without single-population analyses conducted in parallel may fail to detect certain QTL. Two QTL on chromosomes 3 (28–31 cM) and 8, which were detected by ICIM of OP1 and OP5, were not significant in any joint linkage QTL analyses which included either of these populations. These two QTL are similar in that they are rare, segregating in only one population, and their LOD scores in the single population are relatively close to each threshold. These results indicate that joint linkage QTL analysis can be limited for the detection of rare QTL with marginal significance. Several rare QTL for kernel color were not identified in the joint linkage QTL analysis of the maize NAM population, despite the likeness among phenotypic and genotypic methods applied to each population (Chandler et al. 2013). This limitation becomes especially important where the parentage among populations is diverse and QTL would, therefore, likely be uncommon among the populations.

The lack of differences between QTL identified through ICIM conducted with BR and QTL identified through ICIM conducted with PSB or CSB for RDW or lesion length in each population provides evidence that standardization of data would have only minimal effect on the joint linkage QTL analysis in the present study. In further support of the minimal effect of data standardization, only one QTL on chromosome 3 (28–31 cM) was not significant with JICIM of OP1234 (scenario 3, heterogeneous methods of phenotypic assay). This QTL was also insignificant in the QTL analysis of OP12 in which the populations did not differ in the methods of phenotypic assay. Thus, combining data from different phenotypic assays has minimal effects on joint linkage QTL analysis. Consequently, in JICIM analyses, it is possible to combine populations screened by different phenotypic assay methods, if differences in variance and direction of score are accounted. However, the power of combining data from different phenotypic assay methods will be limited by the extent to which the different assays are measuring traits controlled by the same gene(s). Confounding factors such as these always need to be considered in the interpretation of results (Walling et al. 2000). Wang et al. (2012) addressed the question of similarity in resistance mechanism assayed in the tray and layer tests by comparing these two screening methods with a correlation analysis and assessment of co-localization of QTL in a Conrad × Sloan RIL population. Although the correlation between lesion length and RFW was low to moderate, with correlation coefficients ranging from 0.36 to 0.43, the majority of the QTL co-localized between methods (Wang et al. 2012). Thus, this supports combining of data from the tray and layer tests to measure partial resistance to *P. sojae* in a joint linkage QTL analysis.

Overall, joint linkage QTL analyses resulted in identification of a similar number of QTL that were identified in the single population analyses. In comparison, joint linkage QTL analysis using the entire maize NAM population, which consists of 25 nested RIL populations, identified twice as many QTL as were identified in QTL analyses of the single maize RIL populations (Buckler et al. 2009). However, the present study utilized only six populations and this resulted in a smaller relative advantage in increased population size, recombination, and genetic diversity of parents of populations. The results of the present study agree with previous studies conducted for joint linkage QTL analysis using two to ten populations with a NAM design (Chandler et al. 2013; Li et al. 2011; Yang et al. 2013). In these studies, the identified QTL by joint linkage analysis were mostly in accordance with their single-population analyses in terms of numbers of QTL with only a few missed or additional QTL.

Genetic architecture of partial resistance to *P. sojae*

This study identified a total of 16 genetic regions to which QTL for resistance to *P. sojae* localized in five populations and represents the first description of three of the five populations (OP3, OP4, and OP5). No QTL were identified in the sixth population, OP6. Four of the sixteen regions were novel in terms of their genetic location, the remaining QTL co-localized with previously reported QTL for partial resistance to *P. sojae* (Han et al. 2008; Li et al. 2010; Tucker et al. 2010; Wang et al. 2010, 2012; Lee et al. 2013a, b). The QTL on chromosome 12, which was identified for all four traits in OP3 (Fig. 4; supplementary Table 5), was first reported for resistance to *P. sojae* in this study and co-localizes with a previously reported QTL for larval growth of corn earworm (Terry et al. 2000). The QTL on chromosome 4 in OP3 and chromosome 16 in OP3 and OP4 were also novel QTL; although, each was limited in significance to either a single isolate and/or a subset of the four traits (Fig. 4; supplementary Tables 5, 6). While the QTL region on chromosome 4 has not been previously associated with a pest or disease resistance trait, the chromosome 16 locus co-localized with QTL for resistance to brown stem rot (BSR) and soybean cyst nematode (SCN) (Bachman et al. 2001; Guo et al. 2005). The novel QTL on chromosome 9, identified for RR, RFW, and RDW against both isolates in OP4 (Fig. 4; supplementary Table 6), co-localized with a

QTL for *Sclerotinia sclerotiorum* (Lib.) de Bary (Kim and Diers 2000).

OP3 and OP4 were each evaluated with two isolates of *P. sojae* and isolate-specific QTL were detected in these populations. Quantitative or partial resistance is generally considered to be effective against a wide range of strains of a pathogen (Johnson 1984; Kou and Wang 2010, 2012; St Clair 2010). However, it has also been documented that QTL may function differentially across isolates of a pathogen (Calenge et al. 2004; Darvishzadeh et al. 2007; Marcel et al. 2008; Kou and Wang 2010; St Clair 2010; Gonzalez et al. 2012; Wang et al. 2012). Recent studies that compared the specificity of QTL isolates of *Puccinia hordei* reported evidence of isolate-specific QTL in the barley—*P. hordei* system (Marcel et al. 2008; Gonzalez et al. 2012). "Minor gene-for-minor gene" interactions were proposed to explain isolate-specific QTL in a similar manner as the gene-for-gene theory for hypersensitive resistance (Flor 1956; Parlevliet and Zadoks 1977). Two QTL, *Rphq3* and *Rphq11*, were effective towards isolate 1.2.1 of *P. hordei* and explained 37 % of PV, but were insignificant to isolates Co-04 and 28.1 (Gonzalez et al. 2012). In the present study, a significant genotype × isolate interaction effect was observed in OP3 (supplementary Table 3). One QTL on chromosome 4 from OP3 was significant for isolate 1.S.1.1 of *P. sojae*, while it was not detected against isolate OH30 (supplementary Table 5). Isolate-specific QTL were also identified in OP4; QTL on chromosomes 6, 9, 13, and 18 (104–107 cM) were significant for only one of the two isolates of *P. sojae* for two or more traits (supplementary Table 6).

QTL with major effect

In the present study, the QTL on chromosome 18 (13–16 cM) was highly significant for all traits, in both single and joint linkage QTL analyses, and had the largest effect on partial resistance to *P. sojae* in OP3 and OP4. A previously identified QTL for *P. sojae* lesion length co-localized with this QTL on chromosome 18 (Tucker et al. 2010). The QTL identified by Tucker et al. (2010) explained approximately 10 % of PV in an intraspecific cross between a soybean cultivar and an accession of *Glycine soja*. As QTL of large effect have not been commonly identified in soybean—*P. sojae* interactions, this may represent a unique resistance mechanism, such as the root-specific incomplete resistance expressed by the race-specific *R*-gene, *Rps2* (Thomison et al. 1991; Mideros et al. 2007). Incomplete resistance conferred by an *R*-gene may be elucidated by assessing regulatory patterns of key signaling genes, such as *EDS1* and *NDR1* that function downstream of *R*-gene mediated resistance (Aarts et al. 1998). Co-regulation of this pathway with QTL-mediated resistance may indicate that the QTL is conferred by a defeated/weak form of an *R*-gene, a mechanism of partial resistance highlighted in a review by Poland et al. (2009). In support of this hypothesis, the genomic region to which the QTL on chromosome 18 is mapped includes a cluster of resistance gene analogs (McHale et al. 2012). However, other mechanisms of resistance are also likely. A recent study has reported a novel mechanism involving copy number variation by which resistance to SCN is mediated at the *Rhg1* locus (Cook et al. 2012a), located approximately in the same interval of this chromosome 18 QTL. Thus, this QTL may be an interesting locus to investigate for better understanding of molecular mechanisms of partial resistance to *P. sojae*.

In summary, we report that joint linkage QTL analysis can have power to detect QTL additional to those detected by single population QTL analysis. Yet, the effectiveness of joint linkage QTL analysis may be limited when allelic variation for a QTL exists in only one population. Integrating different measurements of partial resistance to *P. sojae* in a combined analysis did not negatively affect the results of joint linkage QTL analysis (e.g., JICIM1234), when BLUP values were standardized on the basis of the population mean and variance (PSB). In addition, we reported genotype × isolate interactions as well as a locus with major quantitative effects on resistance against *P. sojae*. The present research is a case study that provides valuable information for researchers interested in joint linkage QTL analysis of data from multiple populations with heterogeneous assay conditions.

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

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