Theor Appl Genet (2010) 121:651–658
DOI 10.1007/s00122-010-1337-2

ORIGINAL PAPER

Pyramided QTL underlying tolerance to *Phytophthora* root rot in mega-environments from soybean cultivars 'Conrad' and 'Hefeng 25'

Xiuping Li · Yingpeng Han · Weili Teng · Shuzheng Zhang · Kangfu Yu · Vaino Poysa · Terry Anderson · Junjie Ding · Wenbin Li

Received: 28 December 2009 / Accepted: 1 April 2010 / Published online: 14 April 2010

Abstract *Phytophthora* root rot (PRR) of soybean (*Glycine max* (L.) Merr.) is the second most important cause of yield loss by disease in North America, surpassed only by soybean cyst nematode (Wrather et al. in Can J Plant Pathol 23:115–121, 2001). Tolerance can provide economically useful disease control, conditioning partial resistance of soybean to PRR. The aims of this study were to identify new quantitative trait loci (QTL) underlying tolerance to PRR, and to evaluate the effects of pyramided or stacked loci on the level of tolerance. A North American cultivar 'Conrad' (tolerant to PRR) was crossed with a northeastern China cultivar 'Hefeng 25' (tolerant to PRR). Through single-seed descent, 140 $F_{2:5}$ and $F_{2:6}$ recombinant inbred lines were advanced. A total of 164 simple sequence repeat (SSR) markers were used to construct a genetic linkage map. The percentage of seedling death was measured over 2 years (2007 and 2008) in the field at four naturally infested locations in Canada and China following additional soil infestation and in the greenhouse following inoculation with *Phytophthora sojae* isolate. A total of eight QTL underlying tolerance to PRR were identified, located in five linkage groups (F, D1b+w, A2, B1, and C2). The phenotypic variation contributed by the loci ranged from 4.24 to 27.98%. QPRR-1 (anchored in the interval of SSR markers Satt325 and Satt343 of LG F), QPRR-2 (anchored in the interval of Satt005 and Satt600 of LG D1b+w), and QPRR-3 (anchored in the interval of Satt579 and Sat_089 of LG D1b+w) derived their beneficial allele from 'Conrad'. They were located at chromosomal locations known to underlie PRR tolerance in diverse germplasm. Five QTL that derived beneficial alleles from 'Hefeng 25' were identified. The QTL (QPRR-1 to QPRR-7) that were detected across at least three environments were selected for loci stacking and to analyze the relationship between number of tolerance loci and disease loss percentage. The accumulation of tolerance loci was positively correlated with decreases in disease loss percentage. The pyramid of loci underlying tolerance to PRR provided germplasm useful for crop improvement by marker-assisted selection and may provide durable cultivar tolerance against the PRR disease.

Communicated by D. Lightfoot.

X. Li and Y. Han have equal contributions to the paper.

X. Li · Y. Han · W. Teng · S. Zhang · W. Li (✉)
Soybean Research Institute, Key Laboratory of Soybean Biology in Chinese Ministry of Education,
Northeast Agricultural University, 150030 Harbin, China
e-mail: wenbinli@neau.edu.cn

K. Yu · V. Poysa · T. Anderson
Greenhouse and Processing Crops Research Centre,
Agriculture and Agri-Food Canada,
Harrow, ON N0R 1G0, Canada

X. Li
College of Life Sciences, Jiamusi University,
154007 Jiamusi, China

J. Ding
Agricultural Academy of Heilongjiang Province,
154008 Jiamusi, China

Introduction

Phytophthora root rot (PRR) of soybean (*Glycine max* (L.) Merr.), caused by *Phytophthora sojae* was first known as a soil-borne disease of unknown etiology in northeast Indiana, USA, in 1948 (Schmitthenner 1989). PRR is the second most important cause of seed yield loss by disease

in North America, surpassed only by soybean cyst nematode (Wrather et al. 2001). PRR has been especially severe in low areas, poorly drained, and clay soils. PRR has been found in most soybean-growing regions (Bernard et al. 1957; Kaufmann and Gerdemann 1958; Hildebrand 1959; Ryley et al. 1998; Jee et al. 1998; Su and Yao 1993). Furthermore, PRR was identified in 22–25% of soybean-growing regions in Heilongjiang Province of China, causing 50–80% of yield loss in certain years with low temperatures and high rainfalls (Han et al. 2008).

Over 50 races of *P. sojae* have been reported based on resistant cultivar differentials (Drenth et al. 1996; Abney et al. 1997; Leitz et al. 2000; Malvick and Grunden 2004). The number of races has continually increased by mutation or outcrossing driven by interaction with the PRR resistance loci bred into the soybean host cultivars (Bhat and Schmitthenner 1993; Irwin et al. 1995). Current control strategies, such as fungicides, calcium application, soil drainage, and tillage practices, were not effective to control this disease (Sugimoto et al. 2005). Hence, selecting resistant cultivars is an important method to control PRR (Burnham et al. 2003a).

Fourteen dominant host-resistant genes of PRR (*Rps1a*, *Rps1b*, *Rps1c*, *Rps1d*, *Rps1k*, *Rps2*, *Rps3a*, *Rps3b*, *Rps3c*, *Rps4*, *Rps5*, *Rps6*, *Rps7*, and *Rps8*) have been identified at eight different loci, nine of them (*Rps1a*, *Rps1b*, *Rps1c*, *Rps1k*, *Rps2*, *Rps3a*, *Rps4*, *Rps6*, and *Rps7*) have been integrated into public and private cultivars (Burnham et al. 2003b). However, not a single gene could control all races of the pathogen. Moreover, single gene resistance has been suggested to follow "gene for gene" mechanism, and intensive use of race-specific resistance for control has promoted selection for new *P. sojae* races that are virulent to cultivars carrying these resistance genes (Hartman et al. 1999; Drenth et al. 1996). Tolerance or partial resistance may be more durable than single gene resistance (Tooley and Grau 1982, 1984a; Mundt 2000; Zhu et al. 2000; Njiti et al. 2002). Since partial resistance exerts a lower selection pressure on the pathogen population it should be more durable than general race-specific resistance. Furthermore, partial resistance often do not have negative effect on yield in the absence of PRR infection (Tooley and Grau 1984b; Yuan et al. 2002; Dorrance et al. 2003).

Selecting cultivars with tolerance to PRR has been difficult requiring evaluation of the tolerance in multiple environments. Field tests are unpredictable, unstable, time consuming, and labor intensive. Molecular markers offer a faster and more accurate approach to guide the selection, since selection can be based on genotype rather than solely on phenotype (Prabhu et al. 1999). The use of molecular markers for indirect selection of important agronomic traits, or marker-assisted selection (MAS) can improve the efficiency of traditional plant breeding.

Cregan et al. (1999) and Song et al. (2004) developed an integrated genetic linkage map of soybean containing 1,849 markers in one or more of five different populations and aligned the linkage groups (LG) into a consensus map of 20 LGs that correspond to the 20 pairs of soybean chromosomes (Zou et al. 2003). This information had greatly facilitated MAS in soybean breeding.

To date most quantitative trait loci (QTL) associated with tolerance to PRR were identified in the cultivar Conrad. Burnham et al. (2003b) used three recombinant inbred line (RIL) populations with the cultivar Conrad as the tolerant parent and identified two putative QTLs on LG F and D1b+w. The QTL on LG F explained 34.4, 35.0, and 21.4% of the phenotypic variation for the three populations, respectively. The QTL on MLG D1b+w explained 10.6, 15.7, and 20.7% of the variation for the three populations, respectively. Weng et al. (2007) identified one putative QTL, QSatt 414–596 on LG J, associated with the PRR tolerance in Conrad using a RIL population and the field data. This QTL explained about 20% of the phenotypic variation. Han et al. (2008) identified three additional putative QTL (linked to Satt509, Satt334, and OPL18800/SCL18659), associated with the PRR tolerance in Conrad using the same RIL population as Weng et al. (2007). All these studies focused on North American soybean germplasm. The QTL associated with PRR tolerance in Chinese germplasm and the effects of pyramids or stacks of PRR tolerance loci, derived from both North American and northeastern China germplasm, have not been reported to date.

The objectives of the present study were to identify QTL underlying tolerance to PRR using RILs derived from the cross of 'Conrad' × 'Hefeng 25' in multiple environments with SSR markers, and to evaluate the pyramids of loci for potential use in developing tolerant lines with durable control of PRR.

Materials and methods

Plant materials

As much as 140 RILs were advanced as bulks following single-seed descent (SSD) at the $F_{2:5}$ and $F_{2:6}$ from a cross between 'Conrad' (Fehr et al. 1989, tolerant to PRR) and 'Hefeng 25' (a northeastern Chinese cultivar, tolerant to PRR). Lines were advanced in field with no history of PRR to avoid unintentional selections.

Inoculation and disease susceptibility evaluation

Tolerance to PRR in field was tested at four locations across northeastern China and central Canada (Jiamusi,

Huachuan, Huanan, and Woodslee) in 2007 and (Jiamusi, Huachuan, Jixian, and Woodslee) in 2008. The Woodslee site in Ontario of Canada has been used to evaluate tolerance to PRR under field condition since 1975. Other testing sites in northeastern China have been shown to have a high incidence of PRR in past surveys. Seeds were planted in early May in 2007 and 2008 with rows 3 m long, 0.65 m apart, and with a space of about 6 cm between each plant. Two row plots were used in a complete randomized design with three replicates. The surface soil was mixed with two strains of laboratory grown *Phytophthora sojae*. Furthermore, tolerance to PRR in the greenhouse was also evaluated with the two PRR isolates collected from both Jiamusi and Woodslee in 2007 according to the procedure described by Han et al. (2008). Each RI line was inoculated three times with the isolates. The total of germinated plants and the numbers of dead plants were recorded at germination and the R3 stage, respectively. Disease loss percentage (%) per RI line was calculated as follows [(total number of germinated plants − living plants after inoculation with *Phytophthora sojae*)/(total number of germinated plants)] × 100.

SSR analysis

Total genomic DNAs of plants were isolated from freeze-dried leaf tissue by the CTAB method (Doyle and Doyle 1990). SSR analysis was performed with the primers developed by Cregan et al. (1999). PCR was performed in 20 μl reactions containing 2 μl genomic DNA (25 ng/μl), 1.5 μl $MgCl_2$ (25 mM), 0.3 μl dNTP mixtures (10 mM), 2 μl 10 × PCR buffer, 2 μl SSR primer (2 μM), 0.2 μl *Taq* polymerase enzyme (10 units/μl), and 12 μl double-distilled water. The amplification temperature profiles were: 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 47°C, 30 s at 72°C, then 5 min at 72°C. After amplification, the PCR product was mixed with loading buffer (2.5 mg/ml bromophenol blue, 2.5 mg/ml diphenylamine blue, 10 mM EDTA, 95% (v/v) formamide), and denatured for 5 min at 94°C, and then put on ice for 5 min. The denatured PCR products were separated on 6% (w/v) denaturing polyacrylamide gel and visualized by silver staining (Trigiano and Caetano-Anolles 1998).

Data analysis

Broad-sense heritability of tolerance to PRR was computed as $h^2 = h_g^2/(h_g^2 + h_e^2/n)$, where h_g^2 and h_e^2 were the estimates of genetic and residual variance derived from the expected mean squares of the variance and n was the number of replications (Blum et al. 2001). The frequency distribution of PRR tolerance of RIL was analyzed using the SAS procedure (PROC. Shapiro-wilk.SAS).

Mapmaker/EXP version 3.0 b (Lander et al. 1987) was used for genetic linkage analysis. The genetic linkage map was constructed using Mapchart 2.1 (Voorrips 2002). QTL was performed using QTL Cartographer 2.0 (Zeng, 1993) with composite interval mapping (CIM) module (Basten et al. 1996). Window size was 5 cM (Haldane units) and the walk speed was 1 cM. The threshold of LOD score for evaluating the statistical significance of QTL effects was determined by 1,000 permutations using the Zmapqtl program in QTL Cartographer (Churchill and Doerge 1994). A LOD value corresponding to an experiment-wise threshold of $a = 0.05$ was used to declare QTL as significant. The estimate of the QTL position was the point of maximum LOD score in the region under consideration.

Results

Phenotypic analysis of PRR tolerance

Phytophthora root rot tolerance of 'Conrad' is higher than that of 'Hefeng 25' in most tested environments or greenhouse conditions (Table 1). Among the RILs, disease loss percentage was significantly different among field or greenhouse tests with isolates from Jiamusi and Woodslee. The variation of disease loss percentage in the greenhouse tests was much wider than that in the fields. Both skewness and kurtosis values of PRR tolerance were less than 1.0, suggesting that the segregation of this trait fit a normal distribution. Broad-sense heritability of PRR tolerance was relatively low at 0.17–0.46 (Table 1).

Linkage analysis

A total of 684 SSR markers were used to detect polymorphisms between the two parents. Of that, 164 SSR markers were polymorphic among RILs and mapped onto 12 linkage groups according to Cregan et al. (1999) and Song et al. (2004). The map developed encompassed 3,160.28 cM with an average distance of 19.27 cM between markers (data not shown).

QTL analyses of PRR tolerance

A total of eight QTL underlay PRR tolerance (Table 2). QPRR-1, anchored in the interval of Satt325 and Satt343 of LG F could explain 9.21, 9.23, and 10.24% of the phenotypic variation of PRR tolerance to the Woodslee site isolate both in greenhouse and field of 2007 and in field of 2008, respectively. QPRR-2, located in the interval of Satt005–Satt600 of LG D1b+w, was associated with PRR tolerance in four field environments including Woodslee (in 2007), Huachuan (in 2007), Jiamusi (in 2008), and

Table 1 The loss percentage caused by PRR for parents and RILs in greenhouse and field

Year	Site	Identification type	Parents		Average	Range	CV[a]	Kurtosis	Skewness	BSH[a]
			'Conrad'	'Hefeng 2'						
2007	Woodslee	Field	3.27	3.38	32.69	3.28–84.16	55.29	−0.2191	−0.3992	0.38
	Huachuan	Field	0	3.25	27.71	0–82.22	50.28	−0.3403	−0.1952	0.46
	Huanan	Field	0	0	23.91	0–56.52	40.31	0.9695	0.3142	0.30
	Jiamusi	Field	0	0	34.84	0–52.00	37.03	0.5451	0.3631	0.28
	Jiamusi	Greenhouse	6.67	16.67	52.97	0–100.00	43.85	−0.3895	−0.2356	0.30
	Woodslee	Greenhouse	20	20	46.69	0–100.00	51.09	0.4873	0.2896	0.44
2008	Woodslee	Field	1.01	2.59	13.52	0–31.29	30.03	−0.8765	0.4973	0.17
	Jiamusi	Field	1.14	1.41	13.97	0–29.13	20.19	−0.3873	−0.2013	0.22
	Huachuan	Field	0.727	3.73	22.28	0–51.93	34.06	0.8733	0.7363	0.38
	Jixian	Field	0.27	1.6	13.14	0–37.34	19.27	0.3844	0.8732	0.20

[a] Coefficient of variation

[b] Broad-sense heritability

Jixian (in 2008). The QTL explained 11.28, 21.98, 21.52, and 14.56% of the phenotypic variation, respectively. QPRR-3, identified in the interval of Satt579–Sat_089 of LG D1b+w, was associated with PRR tolerance in five environments (Woodslee, 2007 in greenhouse; Woodslee, 2007 in field; Huachuan, 2007 in field; Jiamusi, 2008 in field and Huachuan, 2008 in field). The QTL explained 19.28, 5.47, 27.98, 8.32, and 18.89% of the phenotypic variation of PRR tolerance, respectively. The beneficial alleles of these three QTL were contributed by 'Conrad'.

QPRR-4 (Satt233–Satt437 of LG A2) was associated with PRR tolerance in six field environments (Woodsleein, 2007; Huachuan, in 2007; Woodslee, 2008; Jiamusi, 2008; Huachuan, 2008 and Jixian, 2008), explaining 4.98, 14.1, 6.79, 17.02, 16.43, and 8.56% of the phenotypic variation of PRR tolerance, respectively. QPRR-5, detected in the interval of Satt484–Satt453 of LG B1, was associated with four field environments (Huachuan in 2007, Woodslee in 2008, Huachuan in 2008 and Jixian in 2008). The QTL explained 14.8, 11.95, 5.24, and 7.64% of the phenotypic variation of PRR tolerance, respectively. QPRR-6 (Satt489–Satt100 of LG C2) was associated with PRR tolerance in three environments (Jiamusi, 2007 in the greenhouse; Woodslee, 2008 in the field and Jiamusi, 2008 in the field), explaining 7.56, 5.35, and 21.78% of the phenotypic variation for PRR tolerance, respectively. QPRR-7, anchored on the interval of Satt277–Satt365 of LG C2, could explain 21.76, 13.72, 9.34, and 11.76% of the phenotypic variation of PRR tolerance in Woodslee (2007, greenhouse), Huanan (2007, field), Jiamusi (2007, field), and Jixian (2008, field), respectively. QPRR-8, detected in the interval of Satt460–Satt307 of LG C2, could explain 4.24 and 7.76% of the phenotypic variation of PRR tolerance in Huanan (2007, field) and Jiamusi (2007, field), respectively. These five QTL were the newly found loci that were associated with PRR tolerance in soybean and the beneficial alleles were contributed by the Chinese cultivar 'Hefeng 25'.

The relationship analyses between number of tolerant loci and disease loss percentage

Quantitative trait loci that were detected in at least three environments were selected to analyze the pyramid effect of tolerance loci. The disease loss percentage of RI lines with the maximum accumulation of PRR tolerant loci from both parents was very low (ranged from 7.78 to 23.33%). In contrast, the disease loss percentage of RI lines without any tolerance loci was very high (ranged from 66.67 to 91.67%). The RILs with all the tolerance loci either from 'Conrad' or 'Hefeng 25' showed a moderate loss rate (Table 3), indicating that plant tolerance to PRR was correlated with the number of loci.

Discussion

Phytophthora root rot causes huge yield losses under appropriate environmental conditions (Wrather et al. 2001). Tolerance or partial resistance to PRR has provided an economically useful disease control and is one of the breeding objectives in current soybean improvement programs (Dorrance et al. 2003). Though the PRR tolerance of soybean cultivar 'Conrad' has been known for more than 30 years (Fehr et al. 1989), the late maturity and lower yield prevent its use in northeastern China. 'Hefeng 25' has elite agronomic traits including strong tolerance to PRR (data not shown), and is widely planted in northeastern China. Therefore, pyramids of PRR tolerance loci from 'Conrad' and 'Hefeng 25' may assist breeding of durable

Table 2 QTL associated with PRR tolerance based on the disease loss in the greenhouse and field across 2007 and 2008

LG	Interval	QTL	Locations[a]	cM	R^2(%)[b]	LOD[c]
F	Satt325–Satt343	QPRR-1	Woodslee 07	6.14	9.23	5.28
			Woodslee 07	6.92	9.21	4.02
			Woodslee 08	10.07	10.24	8.85
D1b+w	Satt005–Satt600	QPRR-2	Huachuan 07	1.25	21.98	4.57
			Woodslee 07	2.55	11.28	2.57
			Jiamusi 08	0.81	21.52	7.24
			Jixian 08	3.25	14.56	5.95
	Satt579–Sat_089	QPRR-3	Huachuan 07	2.97	27.98	3.21
			Woodslee 07	2.16	5.47	4.58
			Woodslee 07	2.18	19.28	3.46
			Jiamusi 08	3.73	8.32	3.25
			Huachuan 08	3.12	18.89	4.36
A2	Satt233–Satt437	QPRR-4	Huachuan 07	6.33	14.1	9.86
			Woodslee 07	9.12	4.98	5.39
			Woodslee 08	4.55	6.79	7.32
			Jiamusi 08	1.22	17.02	9.2
			Huachuan 08	3.12	16.43	10.64
			Jixian 08	4.22	8.56	5.14
B1	Satt484–Satt453	QPRR-5	Huachuan 07	4.27	14.80	2.82
			Woodslee 08	0.22	11.95	5.98
			Huachuan 08	0.57	5.24	4.99
			Jixian 08	7.84	7.64	6.02
C2	Satt489–Satt100	QPRR-6	Jiamusi 07	1.47	7.56	7.17
			Woodslee 08	5.17	5.35	6.94
			Jiamusi 08	4.24	21.78	5.57
	Satt277–Satt365	QPRR-7	Hunan 07	9.39	13.72	33.94
			Woodslee 07	10.35	21.76	6.24
			Jiamusi 07	17.05	9.34	7.83
			Jixian 08	20.35	11.76	9.04
	Satt460–Satt307	QPRR-8	Huanan 07	6.29	4.24	2.76
			Jiamusi 07	9.58	7.76	6.36

[a] Woodslee 07: 2007 in Woodslee; Woodslee 08: 2008 in Woodslee; Huachuan 07: 2007 in Huachuan; Huachuan 08: 2008 in Huachuan; Jiamusi 07: 2007 in Jiamusi; Jiamusi 08: 2008 in Jiamusi; Huachuan 07: 2007 in Huanan; Jixian 08: 2008 in Jixian

[b] R^2 *R*-square or the proportion of the phenotypic data explained by the marker locus

[c] *LOD* log of odd score

and tolerant soybean lines. Since PRR tolerance of soybean was difficult to be evaluated directly by phenotype increased selection intensity by marker-assisted selection will improve the selection gain and lead to the success in the development of PRR tolerant cultivar. By early 2010, the QTL analysis of PRR tolerance for northeastern China germplasm was not reported and the evaluation of pyramids of PRR tolerance loci derived from more than one cultivar was not conducted.

Stable phenotypic data are important for QTL mapping (Beavis 1998). PRR outbreaks were significantly influenced by temperature and moisture so that it has been difficult to get stable data of disease loss from even single sites (Tooley and Grau 1982). Hence, experiments in multiple environments, especially mega-environments across China and North America, were critical to evaluate the decrease in disease loss protected by tolerance loci. In the present study, disease loss data were collected from five locations (including Jiamusi, Huachuan, Huanan, Jixian of northeastern China and Woodslee of Canada) and 2 years (2007, 2008). Of the five locations, Woodslee has been used to evaluate PRR tolerance of registered soybean cultivars in Canada since 1975. Other three tested sites have been proven to be typical PRR infested sites in northeastern

Table 3 Effect of pyramids of PRR tolerant loci with beneficial alleles derived both from 'Conrad' and 'Hefeng 25'

Lines	Tolerant loci from 'Conrad'			Tolerant loci from 'Hefeng 25'				No. of tolerance loci	PRR loss percentage
	Satt600 600	Satt579 579	Satt325 325	Satt233 233	Satt484 484	Satt489 489	Satt277 277		
P46	a	a		b	b	b	b	6	7.78
P50	a	a	a	b	b	b	b	6	13.33
P80	a	a	a	b	b	b		6	13.33
P52		a	a	b	b	b		5	18.33
P55		a		b	b	b	b	5	20.56
P58	a	a	a		b	b		5	23.33
P69	a	a			b	b	b	5	23.33
P17								0	75.24
P44								0	66.67
P60								0	75.00
P65								0	75.55
P90								0	91.67
P113								0	85.15
P106				b	b	b	b	4	44.36
P9				b		b	b	3	54.35
P22				b	b		b	3	44.44
P26				b	b	b		3	40.15
P93				b	b	b		3	43.33
P83	a	a	a					3	43.33
P101	a	a	a					3	55.00
P128	a	a	a					3	47.14
P8		a	a					2	42.55
P31	a	a						2	58.06

[a] Existing tolerant locus from 'Conrad'

[b] Existing tolerant locus from 'Hefeng 25'

China (Zhu et al. 2004). Furthermore, tolerance to PRR in the greenhouse was evaluated with two *P. sojae* isolates from Jiamusi and Woodslee in 2007 according to the procedures described by Han et al. (2008). Disease loss percentage of Woodslee and Jiamusi in 2007 through both inoculation methods was consistent (Table 1), suggesting the phenotypic data were highly reproducible.

A total of eight QTL that underlay tolerance to PRR were identified and their phenotypic variation ranged from 4.24 to 27.98%. Three QTL (QPRR-1, and QPRR-2 or QPRR-3) were in genomic regions comparable to the loci identified by Burnham et al. (2003b) and Han et al. (2008), Fig. 1). Both used the same Conrad cultivar as the PRR tolerant parent. The results of Burnham et al. (2003b) and Han et al. (2008) were based on greenhouse tests only and so quite different from both greenhouse and field tests in this study. Here, both LG F and LG D1b+w were associated with PRR tolerance in Conrad, and will be useful for the improvement of PRR tolerance in the field (Tooley and Grau 1984b). The discovery of the five new QTL associated with PRR tolerance was significant (QPRR-4 to QPRR-8). The beneficial alleles of the novel loci underlying PRR tolerance were derived from 'Hefeng 25', a northeastern Chinese cultivar. The basis of the resistance they confer is yet to be determined. Significantly, the accumulation of tolerance loci from 'Conrad' and 'Hefeng 25' in RILs directly increased the PRR tolerance of those soybean lines. The effect of gene stacks was similar to those found in other partial resistance traits (Njiti et al. 2001, 2002).

So far, progress in breeding PRR tolerant cultivars has been slow due to high variability among phenotypic data. The availability of QTL associated with PRR tolerance could facilitate MAS in breeding programs aiming to pyramid tolerance from different soybean genotypes (Prabhu et al. 1999). Knowledge of the locations of QTL controlling tolerance to PRR will allow the design and implementation of more efficient selection schemes to develop soybean variety with durable PRR tolerance (Prabhu et al. 1999; Njiti et al. 1997, 2002; Mundt 2000).

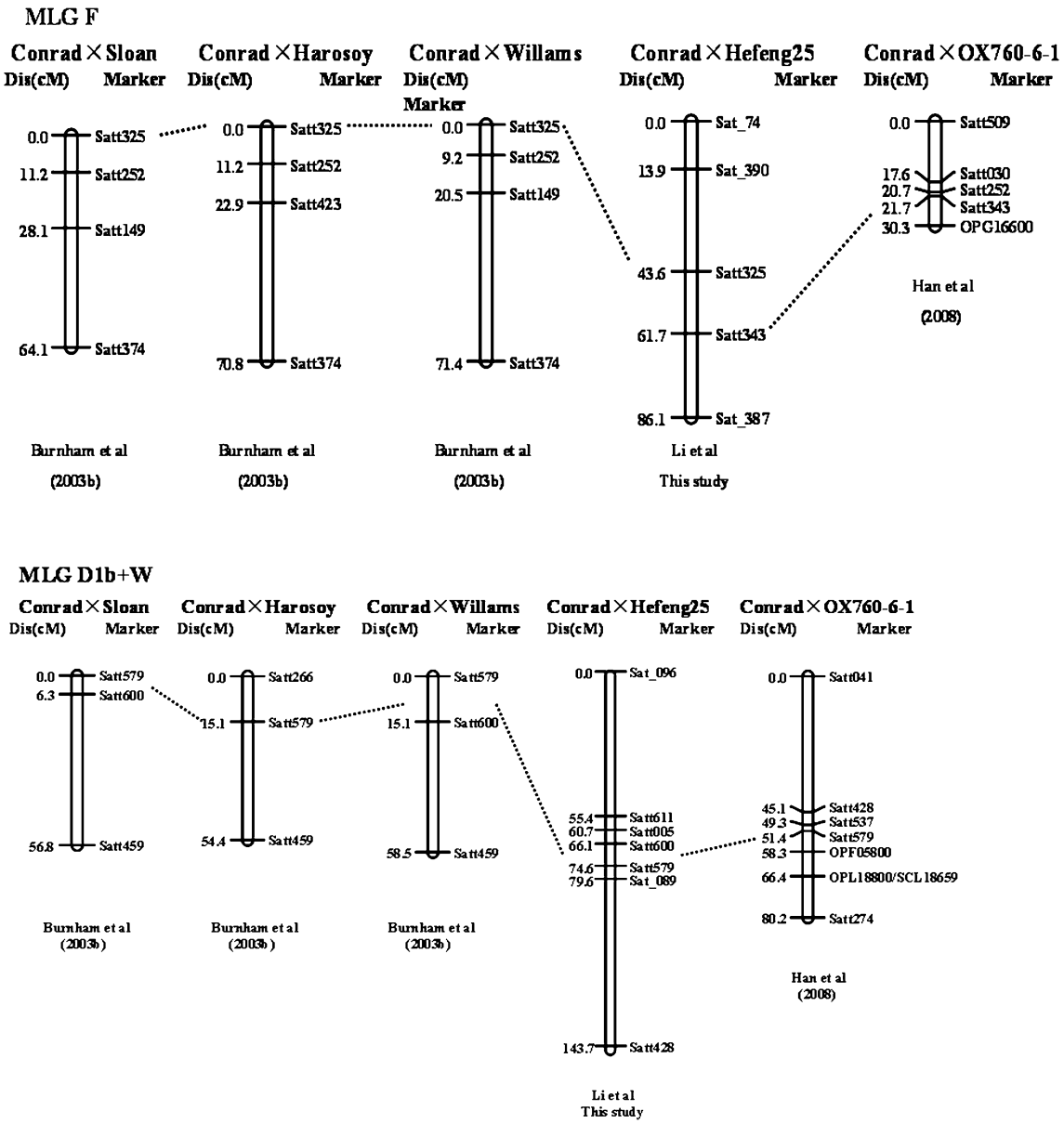

Fig. 1 QTL location comparisons among this study, Burnham et al. (2003b), and Han et al. (2008). The map distances in cMs is shown on the *left*. The QTL locations are indicated on the *right*

Acknowledgments This study was conducted in the Key Laboratory of Soybean Biology of Chinese Education Ministry and Soybean Development Centre of Agricultural Ministry, financially supported by National High Technology Project (2006AA10Z1F1 and 2006AA100104-4), 948 project of Agricultural Ministry of China (2006-G5), National Nature Science Foundation projects (30971810, 60932008), National 973 Project (2009CB118400), Technology project of Education Ministry of Heilongjiang Province (11541025).

References

Abney T, Melgar J, Richards T, Scott D, Grogan J, Young J (1997) New races of *Phytophthora sojae* with *Rps1-d* virulence. Plant Dis 81:653–655

Basten CJ, Weir BS, Zeng ZB (1996) QTL cartographer. North Carolina State University, NC

Beavis WD (1998) QTL analyses: power, precision, and accuracy. In: Paterson AH (ed) Molecular dissection of complex traits. CRC Press, Boca Raton, pp 145–162

Bernard RL, Smith PE, Kaufmann MJ, Schmitthenner AF (1957) Inheritance of resistance to phytophthora root rot and stem rot in the soybean. Agron J 49:391

Bhat R, Schmitthenner A (1993) Genetic crosses between physiologic races of *Phytophthora sojae*. Exp Mycol 17:122–129

Blum A, Klueva N, Nguven HT (2001) Wheat cellular thermo tolerance is related to yield under heat stress. Euphytica 117:117–123

Burnham K, Dorrance A, Francis D, Fioritto R, St. Martin S (2003a) Rps8, a new locus in soybean for resistance to *Phytophthora sojae*. Crop Sci 43:101–105

Burnham K, Dorrance A, VanToai T, St. Martin S (2003b) Quantitative trait loci for partial resistance to *Phytophthora sojae* in soybean. Crop Sci 43:1609–1617

Churchill RW, Doerge GA (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Cregan P, Jarvik T, Bush A, Shoemaker R, Lara K, Kahler A, Kaya N, VanToai T, Lohnes D, Chung J, Specht J (1999) An integrated genetic linkage map of the soybean genome. Crop Sci 39:1464–1490

Dorrance AE, McClure SA, St. Martin SK (2003) Effect of partial resistance on Phytophthora stem rot incidence and yield of soybean in Ohio. Plant Dis 87:308–312

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Drenth A, Whisson S, MacLean D, Irwin J, Obst N, Riley M (1996) The evolution of *Phytophthora sojae* in Australia. Phytopathology 86(2):163–169

Fehr WR, Cianzio SR, Voss BK, Schultz SP (1989) Registration of 'Conrad' soybean. Crop Sci 29:830

Han YP, Teng WL, Yu KF, Poysa V, Anderson T, Qiu LJ, Lightfoot DA, Li WB (2008) Mapping QTL tolerance to *Phytophthora* root rot in soybean using microsatellite and RAPD/SCAR derived markers. Euphytica 162:231–239

Hartman G, Sinclair J, Rupe J (1999) A compendium of soybean diseases, 4th edn. The American Phytopathological Society, St Paul, p 100

Hildebrand A (1959) A root and stalk rot of soybean caused by *Phytophthora megaspema* var. *sojae* var. Can J Bot 37:927–957

Irwin J, Cahill D, Drenth A (1995) *Phytophthora* in Australia. Aust J Agric Res 46:1311–1337

Jee H, Kim W, Cho W (1998) Occurrence of *Phytophthora* root rot on soybean (*Glycine max*) and identification of the causal fungus. Crop Prot 40:16–22

Kaufmann MJ, Gerdemann W (1958) Root and stem rot of soybean caused by *Phytophthora sojae n. sp*. Phytopathology 48:201–208

Lander ES, Green P, Abrahamson J, Baarlow A, Daly MJ, Lincoln SE, Newburg L (1987) MapMaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Leitz R, Hartman G, Pederson W, Nickell C (2000) Races of *Phytophthora sojae* on soybean in Illinois. Plant Dis 84:487

Malvick D, Grunden E (2004) Traits of soybean-infecting Phytophthora populations from Illinois agricultural Welds. Plant Dis 88:1139–1145

Mundt CC (2000) Use of multiline cultivars and cultivar mixtures for disease management. Annu Rev Phytopathol 40:381–410

Njiti V, Gray L, Lightfoot DA (1997) Rate-reducing resistance to *Fusarium solani* f.sp. *phaseoli [nee: glycines]* underlies field resistance to soybean sudden-death syndrome (SDS). Crop Sci 37:1–12

Njiti V, Johnson JE, Torto TA, Gray LE, Lightfoot DA (2001) Inoculum rate influences selection for field resistance to soybean sudden death syndrome in the greenhouse. Crop Sci 41:1726–1731

Njiti V, Meksem K, Iqbal MJ, Johnson JE, Kassem MA, Zobrist KF, Kilo VY, Lightfoot DA (2002) Common loci underlie field resistance to soybean sudden death syndrome in Forrest, Pyramid, Essex, and Douglas. Theor Appl Genet 104:294–300

Prabhu RR, Njiti VN, Johnson JE, Schmidt ME, Klein RJ, Lightfoot DA (1999) Selecting soybean cultivars for dual resistance to cyst nematode sudden death syndrome with two DNA markers. Crop Sci 39:982–987

Ryley M, Obst N, Irwin J, Drenth A (1998) Changes in the racial composition of *Phytophthora sojae* in Australia between 1979 and 1996. Plant Dis 82:1048–1054

Schmitthenner AF (1989) Phytophthora root rot. In: Sinclair JB, Backman PA (eds) Compendium of soybean diseases. APS Press, St Paul, pp 35–38

Song Q, Marek L, Shoemaker R, Lark K, Concibido V, Delannay X, Specht J, Cregan P (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

Su Y, Yao S (1993) The discovery and biological characteristics studies of *Phytophthora megasperma* f.sp.*glycinea* on soybean in China. Acta Phytopathol Sin 23:341–347

Sugimoto T, Aino M, Sugimoto M, Watanabe K (2005) Reduction of Phytophthora stem rot disease on soybeans by the application of $CaCl_2$ and $Ca(NO_3)_2$. J Phytopathol 153:536–543

Tooley PW, Grau CR (1982) Identification and quantitative characterization of rate-reducing resistance to *Phytophthora megasperma* f. sp. glycinea in soybean seedlings. Phytopathology 72:727–733

Tooley PW, Grau CR (1984a) Field characterization of rate-reducing resistance to *Phytophthora megasperma* f. sp. glycinea in soybean. Phytopathol 74:1201–1208

Tooley PW, Grau CR (1984b) The relationship between rate-reducing resistance to *Phytophthora megasperma* f.sp. *glycinea* and yield of soybean. Phytopathol 74:1209–1216

Trigiano RN, Caetano-Anolles G (1998) Laboratory exercises on DNA amplification fingerprinting for evaluating the molecular diversity of horticultural species. HortTechnology 8:413–423

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTL. Heredity 93:77–78

Weng CR, Yu KF, Anderson T, Poysa V (2007) A quantitative trait locus influencing tolerance to *Phytophthora* root rot in the soybean cultivar 'Conrad'. Euphytica 158:81–86

Wrather JA, Anderson TR, Arsyad DM, Tan Y, Ploper LD, Porta-Puglia A, Ram HH, Yorinori JT (2001) Soybean disease loss estimates for the top ten soybean producing countries in 1998. Can J Plant Pathol 23:115–121

Yuan J, Njiti VN, Meksem K, Iqbal MJ, Triwitayakorn K, Kassem MA, Davis GT, Schmidt ME, Lightfoot DA (2002) Quantitative trait loci in two soybean recombinant inbred line populations segregating for yield and disease resistance. Crop Sci 42:271–277

Zeng Z (1993) Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976

Zhu Y, Chen H, Fan J, Wang Y, Li Y, Chen J, Fan J, Yang S, Hu L, Leung H, Mew TW, Teng PS, Wang Z, Mundt CC (2000) Genetic diversity and disease control in rice. Nature 406:718–722

Zhu Z, Huo Y, Wang X, Huang J, Wu X (2004) Analysis of simple sequence repeats markers derived from *Phytophthora sojae* expressed sequence tags. Chin Sci Bull 49(19):2041–2046

Zou J, Lee J, Singh R, Xu SS, Cregan PB, Hymowitz T (2003) Assignment of molecular linkage groups to the soybean chromosomes by primary trisomics. Theor Appl Genet 107:745–750