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ORIGINAL ARTICLE

Genetic mapping and development of co-segregating markers of *RpsQ*, which provides resistance to *Phytophthora sojae* in soybean

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Abstract

Key message **The *RpsQ* Phytophthora resistance locus was finely mapped to a 118-kb region on soybean chromosome 3. A best candidate gene was predicted and three co-segregating gene markers were developed.**

Abstract Phytophthora root rot (PRR), caused by *Phytophthora sojae*, is a major threat to sustainable soybean production. The use of genetically resistant cultivars is considered the most effective way to control this disease. The Chinese soybean cultivar Qichadou 1 exhibited a broad spectrum resistance, with a distinct resistance phenotype, following inoculation with 36 Chinese *P. sojae* isolates. Genetic analyses indicated that the disease resistance in Qichadou 1 is controlled by a single dominant gene. This gene locus was designated as *RpsQ* and mapped to a 118-kb region between BARCSOYSSR_03_0165 and InDel281 on soybean chromosome 3, and co-segregated with Insert11, Insert144 and SNP276. Within this region, there was only one gene Glyma.03g27200 encoding a protein with a typical serine/threonine protein kinase structure, and the expression pattern analysis showed that this gene induced by *P. sojae* infection, which was suggested as a best candidate gene of *RpsQ*. Candidate gene specific marker Insert144 was used to distinguish *RpsQ* from the other known *Rps* genes on chromosome 3. Identical polymerase chain reaction amplification products were produced for cultivars Qichadou 1 (*RpsQ*) and Ludou 4 (*Rps9*). All other cultivars carrying *Rps* genes on chromosome 3 produced different PCR products, which all lacked a 144-bp fragment present in Qichadou 1 and Ludou 4. The phenotypes of the analyzed cultivars combined with the physical position of the PRR resistance locus, candidate gene analyses, and the candidate gene marker test revealed *RpsQ* and *Rps9* are likely the same gene, and confer resistance to *P. sojae*.

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Introduction

Phytophthora root rot (PRR), caused by *Phytophthora sojae* M. J. Kaufmann and J. W. Gerdemann, is one of the most economically destructive diseases of soybean (Kasuga et al. 1997; Schmitthenner 1999; Sugimoto et al. 2011). *Phytophthora sojae* is a soil-borne oomycete that is capable of infecting soybean plants at all developmental stages, resulting in symptoms including damping-off, root and stem rot, leaf yellowing, and wilting (Schmitthenner 1985; Tyler et al. 2007; Gunadi 2012; Lee et al. 2013). Phytophthora root rot was first observed in Indiana, USA in 1948, and has since been detected in all major soybean-producing areas worldwide (Kaufmann and Gerdemann 1958; Schmitthenner 1985; Anderson and Buzzell 1992; Jee et al. 1998; Grau et al. 2004; Dorrance and Grünwald 2009). In China, the disease was first reported in 1991 in Heilongjiang province, which is a major soybean-growing region (Shen and Su 1991). It subsequently spread to several other regions in Heilongjiang and Fujian provinces (Chen et al. 2004; Zhang et al. 2010). Globally, the annual economic

losses resulting from PRR-infected soybean plants exceed US$1–2 billion (Wrather and Koenning 2006).

Generating Phytophthora-resistant soybean plants through the use of dominant *Rps* genes is the most economical and environmentally safe method to prevent this disease (Dorrance et al. 2003). To date, 27 *Rps* genes/alleles associated with 21 loci distributed on eight soybean chromosomes have been reported (i.e., *Rps1a, Rps1b, Rps1c, Rps1d, Rps1k, Rps2, Rps3a, Rps3b, Rps3c, Rps4, Rps5, Rps6, Rps7, Rps8, Rps9, Rps10, Rps11, RpsYB30, RpsYD25, RpsSu, RpsZS18, RpsSN10, RpsYD29, RpsUN1, RpsUN2, RpsJS*, and the *Rps* gene in the Waseshiroge soybean cultivar) (Zhu et al. 2007; Yao et al. 2010; Yu et al. 2010; Ortega 2011; Sugimoto et al. 2011; Sun et al. 2011; Wu et al. 2011; Lin et al. 2013; Zhang et al. 2013a, b; Ping et al. 2015).

The continuous use of Phytophthora-resistant cultivars leads to the breakdown of resistance over time because of the generation of new virulent *P. sojae* strains (Abney et al. 1997; Kaitany et al. 2001; Xue et al. 2015). Mutations and outcrossings between different strains increase the diversity of *P. sojae* populations (Förster et al. 1994). In China, the diversity among *P. sojae* populations is complex (Zhu et al. 2003; Cui et al. 2010; Zhang et al. 2010). Therefore, new *Rps* genes are constantly required to ensure sustainable disease control (Sugimoto et al. 2012).

The *Rps* genes follow a gene-for-gene model, and trigger a defense mechanism that elicits a hypersensitive response (Parlevliet 2002; Tyler 2007; Wang et al. 2011). Among the mapped *Rps* genes in the soybean genome, only three genes have been cloned, namely *Rps1k, Rps2*, and *Rps4*. These genes encode nucleotide-binding site, leucine-rich repeat (NBS-LRR) proteins, which are the most common R gene types in plants (Sandhu et al. 2004; Graham et al. 2002). Based on the sequence preceding the NBS domain, *Rps1k* and *Rps4* are similar to a member of the coiled-coil NBS-LRR class, while *Rps2* is considered to belong to the Toll/Interleukin1 receptor NBS-LRR class. Additionally, *RpsYD29, RpsYD25, RpsUN1*, and *RpsUN2* are present in regions rich in NBS-LRR genes according to the information in the SoyBase database (Zhang et al. 2013b; Fan et al. 2009; Lin et al. 2013). Recently, Zhang et al. (2013a) mapped *Rps10* in the genome of the Chinese soybean cultivar Wandou 15, and identified two candidate genes. The conserved sequences of the *Rps10* candidate genes were determined to encode a serine/threonine kinase and an LRR domain.

There is an abundance of sources of Phytophthora-resistant cultivated soybeans and wild soybeans in China (Zhu et al. 2006; Xia et al. 2011a; Zhong et al. 2015). Soybean cultivar Qichadou 1 was bred at the Shandong Academy of Agricultural Sciences and released in 1995. Qichadou 1 is an elite cultivar with superior yield potential, and produces soybeans rich in fatty acids and proteins. It is also resistant to soybean cyst nematodes (races 1 and 3) and soybean mosaic virus. We previously found that Qichadou 1 plants exhibited a broad-spectrum resistance to *P. sojae* (data not shown).

The objectives of this study were to (1) characterize the inheritance of Phytophthora resistance in Qichadou 1 soybean, (2) finely map the *Rps* gene(s) with molecular markers and identify candidate gene(s), and (3) develop co-segregating candidate gene markers for *P. sojae* resistance in soybean, which would be relevant for molecular marker-assisted selection during breeding.

Materials and methods

Plant resources

A segregating population comprising 207 F_2 individuals was generated by selfing a single F_1 hybrid derived from a cross between Jikedou 2 (susceptible) and Qichadou 1 (resistant) soybean cultivars. The $F_{2:3}$ families were developed through single seed descent of each F_2 individual under field conditions in 2013. The seeds of each $F_{2:3}$ family were harvested separately for genetic analyses and disease evaluations.

The following 19 *P. sojae*-resistant soybean differentials carrying unique *Rps* genes were tested to determine their reactions to the *P. sojae* isolates used in this study: Harlon (*Rps1a*), Harosoy 13XX (*Rps1b*), Williams 79 (*Rps1c*), PI 103091 (*Rps1d*), Williams 82 (*Rps1k*), L76–1988 (*Rps2*), L83–570 (*Rps3a*), PRX 146–36 (*Rps3b*), PRX 145–48 (*Rps3c*), L85–2352 (*Rps4*), L85–3059 (*Rps5*), Harosoy 62XX (*Rps6*), Harosoy (*Rps7*), PI 399073 (*Rps8*), Ludou 4 (*Rps9*), Wandou 15 (*Rps10*), Yudou 25 (*RpsYD25*), Yudou 29 (*RpsYD29*), and Youbian 30 (*RpsYB30*). Williams (*rps*) was used as a susceptible control. All soybean cultivars or lines were obtained from the China National Gene Bank at the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences.

Phytophthora sojae isolates and phenotypic assessments

A total of 36 *P. sojae* isolates that differed in terms of virulence were used for phenotypic assessments of Qichadou 1, Jikedou 2, Williams, and the 19 soybean differentials. The soybean plants were inoculated using a hypocotyl inoculation technique (Haas and Buzzell 1976) (Supplementary Table 1). Briefly, all isolates were cultured on carrot agar medium and incubated at 25 °C in darkness for 7–10 days. A mycelial slurry was made by mixing the colonized agar and pushing it through a 10-ml syringe during the inoculation of soybean plants. The highly virulent Ps41-1 *P. sojae*

isolate was used for phenotypic evaluations of the $F_{2:3}$ population.

For disease evaluations, 15 soybean seeds of each differential were planted in vermiculite-filled paper cups (10-cm diameter) with bottom drainage and incubated in a greenhouse. For genetic analyses and gene mapping, 20 seeds of each $F_{2:3}$ family were sown in paper cups. After 10 days, a mycelial slurry was used to inoculate hypocotyls that had been wounded with a 1-cm slit made by a syringe approximately 1 cm below the cotyledons. Inoculated seedlings were incubated in a misting room (100% relative humidity) at 25 °C for 2 days, and then placed in a greenhouse at 25 °C. Six days after inoculation, disease reactions were scored based on the percentage of dead seedlings.

Families, cultivars, or lines with less than 21% dead seedlings were considered resistant, while those with 21–79% or more than 79% dead seedlings were classified as segregating or susceptible, respectively (Gordon et al. 2006). Phenotypic assays were repeated three times.

DNA extraction and pooling for bulk segregant analysis

Genomic DNA was extracted from healthy soybean leaves sampled from the parents and F_2 plants using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's protocols. For bulked segregant analysis, resistant and susceptible bulks were prepared by mixing equal amounts of DNA from either 10 homozygous-resistant or 10 homozygous-susceptible F_2 individuals. Sample concentrations were adjusted to 20 ng/μl (Michelmore et al. 1991).

Molecular marker development and analysis

The bulked segregant analysis was employed for screening the polymorphisms between two contrasting parents using three types of PCR-based markers (Michelmore et al. 1991; Cregan et al. 1999). For SSR markers, the primer sets evenly distributed throughout the soybean genome were selected from the SoyBase database (http://www.soybase.org/) (Song et al. 2010). For further linkage analysis, the whole genome re-sequencing of the resistant (Qichadou 1) and susceptible (Jikedou 2) parents were performed on an Illumina HiSeqTM 4000 at Biomarker Technologies Corporation in Beijing, China. Thirty-five InDel (Insertion/Deletion polymorphisms) and twenty-eight single nucleotide polymorphism (SNP) markers were developed based on the polymorphisms of targeted genomic sequence between two parents using Primer Premier 5.0 software. Polymorphic markers were analyzed further to genotype the entire $F_{2:3}$ mapping population.

For SSR and InDel markers, polymerase chain reaction (PCR) amplifications were completed in 10 μl reaction mixtures containing 20 ng genomic DNA, 5 μl 2×PCR MasterMix (Tiangen Biotech), and 0.25 μl primers (10 μM each). The PCR program was as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 53–61 °C (depending on primer pairs) for 30 s, and 72 °C for 30 s; 72 °C for 10 min. Samples were then cooled to 4 °C. The amplicons were resolved on 8% non-denaturing polyacrylamide gels and stained with ethidium bromide.

For SNP markers, PCRs were performed in a 10 μl volumes with 20 ng genomic DNA, 5 μl 2×PCR MasterMix (Tiangen Biotech), 1 μl 10×LC Green Plus (Biofire Diagnostics, Salt Lake City, UT, USA), 0.25 μl primers (10 μM each) and 10 μl mineral oil. The PCR reaction were performed as follows: 94 °C for 5 min; 50 cycles of 94 °C for 30 s, 61 °C for 30 s, and 72 °C for 30 s; 72 °C for 10 min. The melting-peak curves were acquired at temperatures ranging from 65 °C to 95 °C and analyzed using LightScanner software (version 2.0) (Idaho Technology, Salt Lake City, UT) according to the manufacturer's instructions.

Data analysis and construction of genetic maps

Chi square (χ^2) analyses were completed to determine the goodness-of-fit of the observed segregations with expected genetic ratios in the mapping population. A genetic linkage map for the target locus was then constructed using MapMaker 3.0 with a logarithm of odds threshold of 3.0 (Lincoln et al. 1993).

Quantitative real-time PCR analysis

The physical positions of the markers tightly linked to *RpsQ* were determined based on the soybean genome reference sequences using the BLAST program (http://soybase.org/SequenceIntro.php; http://www.Phytozome.net). The candidate genes present in the target regions were also analyzed using the BLASTN search program and the soybean database.

To determine the transcript abundance of the candidate gene in response to *P. sojae*, 10-day-old seedlings of Qichadou1 were inoculated with Ps41-1 using a standard hypocotyl inoculation method and a mock control. The stems were harvested at 0, 6, 12, 24, 36 and 48 h post inoculation and stored at −80 °C for further use. The roots, stems and leaves were also collected without inoculation for tissue-specific transcript abundance. Total RNA was isolated using RNAprep Pure Plant Kit (Tiangen Biotech, Beijing, China) and first-strand cDNA was synthesized using a PrimeScript^{TMRT} Reagent Kit (TaKaRa, Japan). Two

candidate gene specific primers (F: CACACTTGCAGG CTTTTGTCT; R: GAACTCTCCTGGATGTCTTCCC) were used to determine its expression level. Real-time PCR was performed using the CFX96 Touch™ Real-Time PCR Detection System (Biobad, USA) with the SYBR® Premix Ex TaqII(TliRNaseH Plus) (TaKaRa, Japan) according to the manufacturer's instructions on the CFX96 Touch™ Real-Time PCR Detection System (Biobad, USA). The constitutive GmACT11 gene amplified with specific primers (F: ATCTTGACTGAGCGTGGTTATTCC; R: GCT GGTCCTGGCTGTCTCC) was used as an internal control. The gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. For each sample, three technological replications were conducted.

Isolation of the candidate gene

To generate genomic DNA fragments of the candidate genes for the parents, the 5′- and 3′-untranslated regions (UTRs) and gDNA were separately amplified using primers designed according to the candidate gene models. The PCR assay was completed using *TransTaq*® HiFi DNA Polymerase (TransGen Biotech, Beijing, China) according to a standard protocol. The amplicons were purified from agarose gels using the Universal DNA Purification Kit (TianGen Biotech). The purified fragments were subcloned into the *pEASY*®-T1 cloning vector (TransGen Biotech) and sequenced. Sequences were assembled and manually edited using DNAMAN 7.0 software. Syntenic sequences were compared between the resistant and susceptible genotypes using the MultAlin online tool (http://bioinfo.genotoul.fr/multalin/multalin.html). The primer sets are listed in Table 1.

Validation of the candidate gene marker

Based on the physical position of Insert144, Insert144 was in the cording region of the candidate gene and considered as candidate gene specific marker. To validate its utility, the marker was then used to check the allelism of *RpsQ* in the vicinity of its locus using the two parental lines and nine *Rps* gene isogenic lines (i.e., *Rps1a, Rps1b, Rps1c, Rps1d, Rps1k, Rps7, Rps9, RpsYD25* and *RpsYD29*).

Results

Qichadou 1 is broadly resistant to *Phytophthora sojae*

Based on phenotypic reactions to *P. sojae* isolates, Qichadou 1 soybean plants were resistant to 30 of 36 isolates tested, indicating this cultivar is broadly resistant to *P. sojae* (Table 2). The reaction of Qichadou 1 to the 36 *P. sojae* isolates was not consistent with the reactions of the 19 differentials, although it was closest to that of Ludou 4 (*Rps9*), which was completely resistant to 26 isolates (Table 2). Qichadou 1 and Ludou 4 plants were affected differently by only four *P. sojae* isolates (i.e., PsAH5, PsJS10, PsJS6, and PsJMS2). Additionally, Qichadou 1 plants were more broadly resistant to *P. sojae* than the 19 differentials, except for Yudou 25 (*RpsYD25*) and Williams 82 (*Rps1k*), which were resistant to 33 and 30 isolates, respectively (Table 2).

Phenotypic analysis of the mapping population

Six days after inoculation, the reactions of the parents and the derived mapping population to *P. sojae* isolate Ps41-1 were as expected. All Jikedou 2 seedlings were dead, indicating susceptibility to isolate Ps41-1, whereas all Qichadou 1 seedlings were completely resistant, with only hypersensitive necrosis observed at infection sites.

The PRR phenotypes of the $F_{2:3}$ mapping population comprising 207 families revealed there were 52 homozygous resistant, 101 heterozygous, and 54 susceptible families. A χ^2 test indicated that this distribution matched a theoretical 1 RR: 2 Rr: 1 rr segregation ratio ($\chi^2 = 0.16$, $p = 0.92$) (Table 3). The results suggested the PRR resistance in Qichadou 1 plants was controlled by a single dominant gene, which was designated as *RpsQ*.

Genetic mapping of *RpsQ*

To map *RpsQ* in the Qichadou 1 genome, random SSR markers selected from the SoyBase database (http://www.soybase.org/) were first used to screen the parents and the bulks prepared for bulk segregant analysis. We focused on the chromosome harboring the *Rps* gene. Four markers (i.e., BARCSOYSSR_03_0012, Satt631, BARCSOYSSR_03_0204, and BARCSOYSSR_03_0250) on

Table 1 Primer pairs used for sequencing

Marker name	Forward primer (5′–3′)	Reverse primer (5′–3′)	Tm (°C)	Expect size (bp)
5′-UTR	CAGAGTCAAGTCAACCGTGC	TCTGCTGGTATGGCACCTTC	58	1000
gDNA	TGTTGGGCACTCGGTTGTTA	GAGTGTCACATCATCCCAAATGT	60	3330
3′-UTR	GCACTAGCATTGGCTTGCTT	TGCAACCCGTGCATTCAATTT	58	950

Table 2 Responses of 21 soybean cultivars/lines to 36 *Phytophthora sojae* isolates

Cultivar/line	Rps gene	Reaction type[a]	No. of resistance to isolates
Williams	rps	SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS	0
Harlon	Rps1a	RSRSRRRSSRSSRRRRSRSSSRRRRSRSRRSRRSSS	20
Harosoy 13XX	Rps1b	RRRRRSRRRRRSSRRRSSRRRSSSRRSRRRSRRSSR	24
Williams 79	Rps1c	RRRRRRSRSRRSRRRRSRSSSRRRRRRRRRRRRSS	27
PI 103091	Rps1d	RSRSRRSSSRSRSRRRSRSSSRSRRSSRRSSSSRSS	16
Williams 82	Rps1k	RRRRRRRRRRRRRRRRSRSSRRRRRRRRRSRRRRSS	30
L76–1988	Rps2	SSRRRSSRRSSRRRSSSRSSRRRSRRSSSSSSSSRS	15
L83–570	Rps3a	RRRRRRRRRRSRSSRSRRSRRRRSSRRSRSSRSSSSS	21
PRX 146–36	Rps3b	RRRRRRRSRRSSSRRRSSRRSRSRRSSRSSSRSSSS	19
PRX 145–48	Rps3c	RRRRSSSRSRSSSRSSSSSSSSRSSSSSSSSSSSSS	8
L85–2352	Rps4	RRRRSRRRRRRSRRSSRSRRRRSRSRSRSSSSRSSS	21
L85–3059	Rps5	RRSRRSRRRSRRSRSSRSRRRRSRSRSRSRSSSSSS	19
Harosoy 62XX	Rps6	RRRRRRRRRRSRSSSSSRSRRRSSRSSSSSSSSSSS	15
Harosoy	Rps7	RRSRRRSRSRRRSSSSSSRSRSRSRSSSSSRSRRSS	16
PI 399073	Rps8	RRSSRRRRRRRSRRRRRRSRRSSRRSSSSSRSRSRS	22
Ludou 4	Rps9	RRRRRRRRRRRRRRRSRRSRRRRRRRRRSSSSSSSS	26
Wandou 15	Rps10	RSRSRSRSRRRRRRRRSRRRSRRRSSRSRRSRSR	25
Yudou 25	RpsYD25	RRRRRRRRRRRRRRRRRRSRRRRRRRRRRRRRSRRS	33
Yudou 29	RpsYD29	RSSRSSRRSRSRRRRRRSRSSRRSSRRSRRRRSSSR	21
Youbian 30	RpsYB30	RRSRRRSRRRRRSRSSRSRRRRSRSRSRSSSSSSSS	19
Qichadou 1	RpsQ	RRRRRRRRRRRRRRRRRRRRRRRRRRRRSSSSRRSS	30

Ps13-2, PsUSAR2, Ps13-1, PsAH, Ps13-4, PsAH4, PsHLJ5, Ps41-1, PsGS8, PsJS12, PsJMS2, PsAH1, PsSX1, PsAH5, Ps13-14, Ps13-5, PsJA08-1, Ps13-3, PsJA08-3, PsMC1, PsAH3, PsAH6, PsJL5, Ps13-12, Ps13-6, PsNKI, Ps13-13, Ps13-7, PsJS6, PsJS10, PsJS2, and Ps13-9. R: resistant; S: susceptible

[a]Reaction type is based on a combination of reactions to 36 isolates, in the following order: PsJL1-2, PsGZ-2, PsFJ, PsJS7

Table 3 Segregation of resistance to *P. sojae* isolate Ps41-1 in the $F_{2:3}$ families derived from the Qichadou 1 × Jikedou 2 cross

Cultivar and the cross	Generation	Amount	Observed number			Except ratio and goodness of fit		
			R	Rs	S	(R:Rs:S)[a]	$\chi 2$	P
Jikedou 2	P_1	20	–	–	20			
Qichadou 1	P_2	20	20	–	–			
Jikedou 2 × Qichadou 1	$F_{2:3}$	207	52	101	54	1:2:1	0.16	0.92

[a]*R* resistant; *Rs* segregating; *S* susceptible

chromosome 3 were polymorphic between the parents and DNA bulks (Table 4; Fig. 1a). Linkage analyses revealed *RpsQ* was linked to these four polymorphic markers, and was flanked by Satt631 and BARCSOYSSR_03_0204. Thirty-four known SSR markers in the target region between Satt631 and BARCSOYSSR_03_0204 were screened. We found that three polymorphic SSR markers, BARCSOYSSR_03_0165, BARCSOYSSR_03_0176 and BARCSOYSSR_03_0184, were linked to *RpsQ* (Fig. 1a).

To further delimit the target region, 35 InDel markers were secondly to screen the parents as well as two contrasting bulked and four polymorphic InDel markers (i.e., Insert11, Insert144, InDel281 and InDel286) were used to genotyping the mapping population. Thus, *RpsQ* was delimited to the BARCSOYSSR_03_0165-InDel281 interval of the short arm of the Chromosome 3, and co-segregated with Insert11and Insert144 (Fig. 1a). Lastly, 28 SNP markers were developed and polymorphic SNP marker SNP276 was also co-segregated with the phenotype of the mapping population (Fig. 1a). The final region containing *RpsQ* span approximately 118 kb (i.e., physical distance) according to the reference sequence for soybean cultivar Williams 82 (Fig. 1b, c).

Table 4 Sequence details for the polymerase chain reaction-based markers used for mapping *RpsQ*

Marker name	Forward primer (5′–3′)	Reverse primer (5′–3′)	Start site	Stop site	Product (bp)
BARCSOYSSR_03_0112	GGTATCAGGTGGAAGGACGA	TCACCGTTCTTTGTTTTTGCT	1983846	1984117	272
Satt631	GGTAGATCCAGGAGCTTGAGT CAG	GCGCATCTCACTGCACTTGATTTT	2943883	2944031	152
BARCSOYSSR_03_0165	GATTTGAATTGGCGCCTTTA	CAACCTAAATTTGGTGTGACTTTT	2968566	2968757	192
Insert11	AGCACACTTACAAAGCTTACCG	AGCACTAGTTGTGTCAAACTCC	2997143	2997374	221
Insert144	TGGAATGAGTCTTAGGGGAAGC	ACCGGTGATTGAATTGTTGGAG	2997106	2997549	437
SNP276	AAAAACAACTAAAGCCTTATC CCAC	TTTCCATGACTTCTTTGGATTACT G	3031924	3032155	232
InDel281	TCTCAAAAGTGGTTTCATTCGG	TTCAAAAATAAAAGGGAATCA TAAT	3087418	3087579	171
InDel286	TTTGGGTGGGTTTATGATTTTATT A	CTACTATCGCAGGTTATTGCC ATTG	3120385	3120585	201
BARCSOYSSR_03_0176	GCACACAATAACTCAAAAATC CTTT	TGTGGAGAATACAAATACAGA TTGA	3153294	3153592	299
BARCSOYSSR_03_0184	GAGATTCATGAGAAGGGCCA	CTCCCCGTGTTAGGTGTTGT	3254518	3254658	141
BARCSOYSSR_03_0204	GCGACGCGCTAGTCTTATTT	GCGGATGGCTTTTACTTT	3488616	3488905	289
BARCSOYSSR_03_0250	AAAACCTCGTTCCCACTGTT	TCTTCCTTGGACTCCTCGAA	4285031	4285276	246

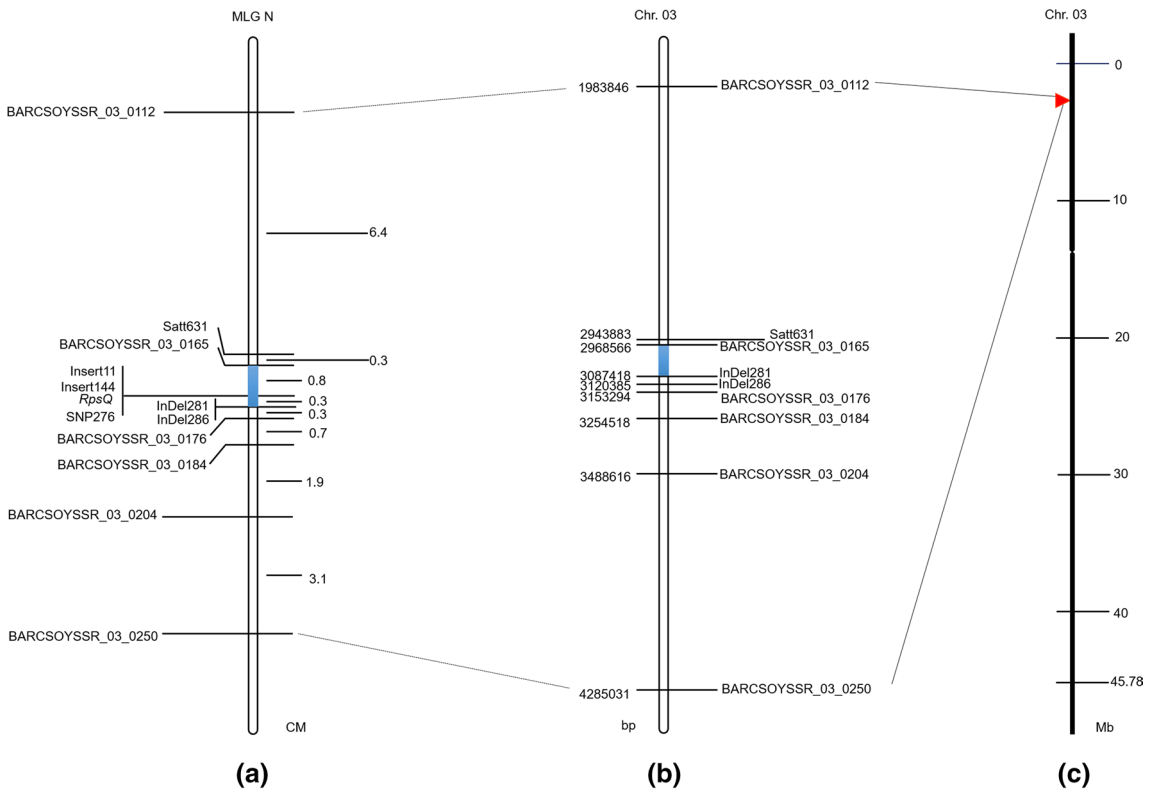

Fig. 1 Genetic and physical map of *RpsQ* on chromosome 3. **a** Genetic linkage map of *RpsQ*. Molecular markers are on the left side of the map, while genetic distances (cM) are on the right side. *Blue shading* indicates the *RpsQ* target region. **b** Physical positions of molecular markers on chromosome 3. Loci names are on the right side of the map, while physical positions of molecular markers are provided on the left side. *Blue shading* indicates the *RpsQ* interval. **c***RpsQ* region on the short arm of chromosome 3, with a red triangle indicating the *RpsQ* locus. (Color figure online)

Candidate gene analysis

Based on the gene annotations available in the Phytozome genomics resource (https://phytozome.jgi.doe.gov/pz/portal.html), 11 predicted genes were detected within the 118-kb target region (Supplementary Table 2) and two (i.e., Glyma.03g27000 and Glyma.03g27100) of them encode proteins with unknown function. By genomic-sequence comparison of the two parental lines, five predicted genes (i.e., Glyma.03g27200, Glyma.03g27400, Glyma.03g27500, Glyma.03g27600 and Glyma.03g27900) with non-synonymous variants in coding region were found, while the other four genes (i.e. Glyma.03g27300, Glyma.03g27700, Glyma.03g27800 and Glyma.03g28000) show synonymous variants in coding region. Among these five predicted genes, Glyma.03g027200 encoded a serine/threonine protein kinase that functioned as a receptor-like kinase (RLK) involved in signaling and plant defense activities (Afzal et al. 2008). Therefore, this gene was most likely the candidate gene of *RpsQ*.

To characterize the expression of the candidate gene to *P. sojae* in Qichadou1, a quantitative real-time PCR was performed. The examination of tissue-specific transcript abundance in Qichadou 1 showed that the candidate gene was constitutively and highly expressed in stems, followed by roots and leaves (Fig. 2a). The transcripts of the candidate gene rapidly increased in stem after *P. sojae* infection, reaching a maximum level at 24 h, followed by a rapid decline (Fig. 2b). According to the results, Glyma.03g027200 was suggested as a best candidate gene of *RpsQ*.

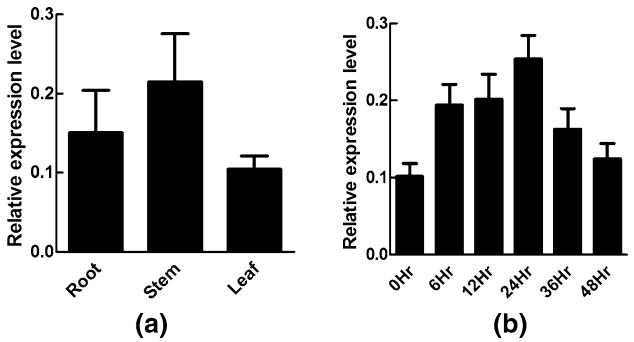

Fig. 2 Expression patterns of the candidate gene in Qichadou 1. The infected samples were collected at 0, 6, 12, 24, 36, and 48 h post inoculated with isolate Ps41-1. **a** Expression patterns of the candidate gene mRNA level in various tissues of Qichadou 1. Roots, stems, and leaves were harvested from 10-day-old seedlings. **b** The candidate gene expression in stems of Qichadou 1 upon *P. sojae* infection. The infected samples were collected at 0, 6, 12, 24, 36 and 48 h post inoculated with isolate Ps41-1. The soybean GmACT11 gene was used as an internal reference. Values are mean ± SD of three biological replicates

Genomic sequence analysis

The genomic DNA sequences of the *RpsQ* candidate genes in the parents were determined based on the Glyma.03g027200 sequence, which was compared among the Qichadou 1, Jikedou 2, and Williams 82 cultivars (Supplementary Fig. 1). The *RpsQ* candidate gene in the Qichadou 1 genome was named *STKQ*, and its allele in the Jikedou 2 genome was referred to as *stkq*. The *stkq* sequence was identical to the corresponding sequence in the Williams 82 genome. The genomic *STKQ* sequence was 98% identical to *stkq* and Glyma.03g027200, which comprised a 173-bp 5′-UTR, a 3142-bp coding region, and a 260-bp 3′-UTR. There were 258 changes to the genomic *STKQ* sequence [i.e., single nucleotide polymorphisms (SNPs) and insertion/deletions (InDels)] (Supplementary Fig. 1). Moreover, the mean nucleotide diversity was higher at non-coding sites than at coding sites. Significant sequence differences included an 11-bp insertion in the 5′-UTR and a 144-bp insertion in the coding region of *STKQ*. The sequence of the 144-bp insertion was highly similar to a Glyma.03g027200 fragment (from position 734 bp to 878 bp), with the differences represented by a few SNPs (Supplementary Fig. 1). This suggested that the 144-bp insertion may have resulted from sequence duplications.

Analysis of candidate gene markers

Insert11 and Insert144 were codominant markers that co-segregates with the *RpsQ* (Figs. 1a, 3a, b), and were developed corresponding to the 11-bp (i.e., 5′-UTR) and 144-bp (i.e., *STKQ* coding region) insertions in Glyma.03g27200, respectively. The Insert11 InDel marker resulted in the PCR amplification of 221-bp and 232-bp fragments in Jikedou 2 and Qichadou 1 cultivars, respectively (Fig. 3a, b). Insert144 produced 437-bp and 581-bp fragments in Jikedou 2 and Qichadou 1, respectively (Fig. 3a, b). The two candidate gene markers were tested in the mapping population, and the results suggested that Insert11 and Insert144 can be used in marker-assisted selection (MAS) breeding. The *RpsQ* locus was on the short arm of chromosome 3, which contains 11 PRR resistance genes (i.e., *Rps1a, Rps1b, Rps1c, Rps1d, Rps1k, Rps7, Rps9, RpsYD25, RpsYD29, RpsUN1*, and the Waseshiroge *Rps* gene). To differentiate among these resistance genes, DNA was extracted from the differentials carrying all genes except *RpsUN1* and the Waseshiroge *Rps* gene and analyzed using Insert144 (Fig. 4). The Qichadou 1 (*RpsQ*) and Ludou 4 (*Rps9*) 581-bp amplification products were identical. The amplicons for the other cultivars were similar to that for Jikedou 2, with all lacking the 144-bp fragment. These results suggested that *RpsQ* and *Rps9* might represent the same gene.

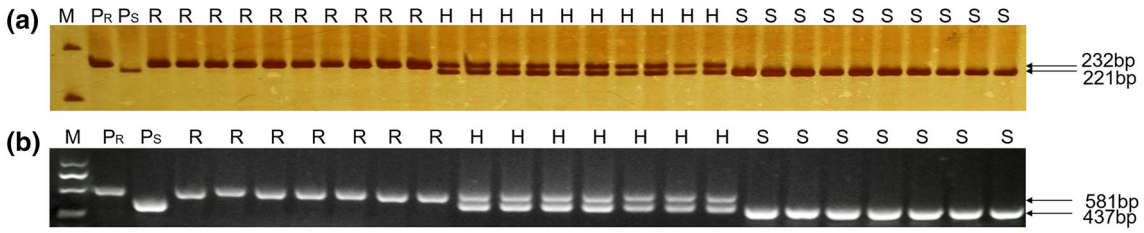

Fig. 3 Polymerase chain reaction analysis of progenies derived from the Jikedou 2 (susceptible) × Qichadou 1 (resistant) cross using the Insert11 and Insert144 candidate gene markers. **a** Co-segregation of Insert11 with the *RpsQ* locus in the parents and their 30 $F_{2:3}$ progenies. **b** Co-segregation of Insert144 with the *RpsQ* locus in the parents and their 21 $F_{2:3}$ progenies. *M* marker; P_R Qichadou 1; *Ps* Jikedou 2; *R* resistant homozygote; *H* heterozygote; *S* susceptible homozygote

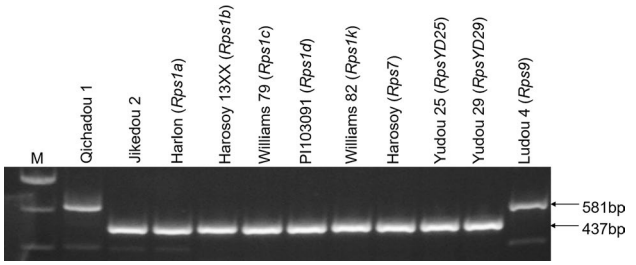

Fig. 4 Polymerase chain reaction amplification products using Insert144 primers and genomic DNA prepared from 11 soybean cultivars respectively carrying a unique *Rps* on chromosome 3. M: D2000 DNA marker

Discussion

In this study, we determined the Chinese soybean cultivar Qichadou 1 carried the *RpsQ* locus, which conferred broad spectrum Phytophthora resistance. This locus was mapped to a 118-kb region flanked by BARCSOYSSR_03_0165 and InDel281 on the short arm of chromosome 3 and co-segregated with Insert11 and Insert144 and SNP276. *RpsQ* was located in a region nearby 11 other known *Rps* genes, including five *Rps1* alleles (Bernard et al. 1957; Mueller et al. 1978; Lin et al. 2013; Sun et al. 2014), *Rps7* (Buzzell and Anderson 1992; Weng et al. 2001), *Rps9* (Wu et al. 2011), *RpsYD25* (Fan et al. 2009; Sun et al. 2011), *RpsYD29* (Zhang et al. 2013b), *RpsUN1* (Lin et al. 2013), and a Waseshiroge *Rps* gene (Sugimoto et al. 2011). To differentiate among these genes, the resistance spectrum of Qichadou 1 and 19 differentials known to carry *Rps* genes was investigated. Qichadou 1 exhibited a broad-spectrum resistance, which was most similar to the reaction of Ludou 4. Qichadou 1 was derived from a cross between the Peking and Ludou 4 cultivars. Peking is an important source of soybean cyst nematode resistance (races 1, 3, and 5) (Qiu et al. 1997), but it is susceptible to PRR (Xia et al. 2011b). In contrast, Ludou 4 is a *P. sojae*-resistant cultivar that carries *Rps9* on chromosome 3 (Wu et al. 2011). Therefore, we hypothesized that *RpsQ* in the Qichadou 1 genome may have been inherited from the resistant parent, Ludou4. This suggests that *RpsQ* and *Rps9* might be the same gene. However, the level of disease resistance in Qichadou 1 and Ludou 4 plants is not the same, likely because of differences in the genetic backgrounds between these two cultivars. A similar phenomenon was previously reported for other resistance genes, including *Xa26* and *Xa3* (Xiang et al. 2006). The level of resistance provided by *Xa26* differs among lines and growth stages, and *Xa26* symbolized *xa9*, causing dominance reversal at booting stages (Sidhu and Khush 1978).

A comparison between *Rps* genes and *RpsQ* regarding physical positions revealed that *RpsQ* was located at the *Rps9* locus, which covers a 548-kb fragment, and was not close to the other *Rps* genes on chromosome 3. Furthermore, the candidate gene for *RpsQ* and *Rps9* appears to be the same, because only Glyma.03g027200 encodes an LRR receptor kinase-like protein involved in disease resistance. The genomic sequence of the *Rps9* candidate gene was determined based on the Ludou 4 genome, and a few SNPs were detected in the 3′- and 5′-UTRs during comparisons with *STKQ* in Qichadou 1 (Supplementary Fig. 1). Additionally, the Insert144 candidate gene marker generated the same amplicons for Ludou 4 and Qichadou 1, indicating *RpsQ* and *Rps9* were likely the same gene.

According to deduced amino acid sequences, *STKQ* encoded a serine/threonine protein kinase, and belonged to the RLK class of resistance genes (Hulbert et al. 2001; Liu et al. 2007; Afzal et al. 2008). Plant RLK genes associated with diverse developmental pathways and pathogen recognition processes have been well studied (Li et al. 2006; Liu et al. 2007). Regarding plant–pathogen interactions, previous studies have concluded that *Xa21* confers a high level of resistance to *Xanthomonas oryzae* in rice (Song et al. 1995), *FLS2* regulates flagellin binding (Gómez-Gómez and Boller 2000), and *SERK3/BAK1* modulates pathogen-associated molecular pattern-triggered immunity (Heese et al. 2007). Most RLKs contain extracellular repeats and an intracellular kinase, which enable the perception of elicitors and transmission of

signals through phosphorylations, respectively (Morillo and Tax 2006). The *RpsQ* candidate gene consisted of an LRR extracellular domain and a serine/threonine kinase intracellular domain. Leucine-rich repeat domains vary considerably and evolve faster under diversifying selection pressures. This results in a versatile structural framework for the perception of extracellular signals, which influences resistance specificity (Braun and Walker 1996; Bai et al. 2002; Hulbert et al. 2001). In this study, segmental duplications occurred in the *RpsQ* candidate gene, thereby increasing the number of LRR-repeat units, which may have led to enhanced resistance to *P. sojae* (Dogimont et al. 2014; Ellis et al. 1999). The kinase domain affects protein phosphorylation-mediated signal transduction cascades, resulting in specific plant responses (Gachomo et al. 2003; Li et al. 2006). Three SNPs in the coding sequences resulted in three amino acid differences in the serine/threonine kinase motif between the *RpsQ* candidate gene in Qichadou 1 and its allele in Jikedou 2, which may be responsible for the differences in defense responses to *P. sojae*. A recent study regarding *Rps* genes in soybean concluded the *Rps10* candidate gene encoded an LRR-RLK containing a serine/threonine kinase domain (Zhang et al. 2013a), with a similar structure to that of the protein encoded by the *RpsQ* candidate gene. Because of the complexity of disease resistance mechanisms, the activities of the *RpsQ* candidate gene that affect disease resistance require further characterization.

The use of *Rps*-containing soybean cultivars is the most effective and environmentally friendly strategy for managing PRR (Walters 2009). During the development of disease-resistant cultivars using marker-assisted selection, several genes with complementary disease resistance activities should be incorporated into an elite cultivar to delay the breakdown of resistance (Dorrance and Schmitthenner 2000; Pathan and Sleper 2008; Saghai Maroof et al. 2008). In this study, the Chinese soybean cultivar Qichadou 1 was resistant to 30 *P. sojae* isolates, and the presence of *RpsQ* made it a good resource for breeding new soybean cultivars. Moreover, three co-segregated markers specific for *RpsQ* were developed and validated in the mapping population. These results are relevant for the introgression of *RpsQ* into new commercial cultivars through marker-assisted selection or *Rps* gene pyramiding. Future studies will focus on the characterization of *RpsQ* activities during the interaction between soybean plants and *P. sojae*.

Author contribution statement YL and ZZ conceived the study and designed the experiments. ZZ developed the genetic populations. YL, CZ and XW performed research. YL, SS, CZ, XW and ZZ analyzed data. YL, SS and ZZ organized the figures and prepared the manuscript.

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Compliance with ethical standards

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

Ethical standards The experiments were performed in accordance with all relevant Chinese laws.

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