Theor Appl Genet (2010) 120:1443–1450
DOI 10.1007/s00122-010-1266-0

ORIGINAL PAPER

Fine mapping of a resistance gene to bacterial leaf pustule in soybean

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Received: 29 September 2009 / Accepted: 27 December 2009 / Published online: 20 January 2010

Abstract Soybean bacterial leaf pustule (BLP) is a prevalent disease caused by *Xanthomonas axonopodis* pv. *glycines*. Fine mapping of the BLP resistant gene, *rxp*, is needed to select BLP resistant soybean cultivars by marker-assisted selection (MAS). We used a total of 227 recombinant inbred lines (RILs) derived from a cross between 'Taekwangkong' (BLP susceptible) and 'Danbaekkong' (BLP resistant) for *rxp* fine mapping and two different sets of near isogenic lines (NILs) from Hwangkeumkong × SS2-2 and Taekwangkong × SS2-2 were used for confirmation. Using sequences between Satt372 and Satt486 flanking *rxp* from soybean genome sequences, eight simple sequence repeats (SSR) and two single nucleotide polymorphism (SNP) markers were newly developed in a 6.2-cM interval. Linkage mapping with the RILs and NILs allowed us to map the *rxp* region with high resolution. The genetic order of all markers was completely consistent with their physical order. QTL analysis by comparison of the BLP phenotyping data with all markers showed *rxp* was located between SNUSSR17_9 and SNUSNP17_12. Gene annotation analysis of the 33 kb region between SNUSSR17_9 and SNUSNP17_12 suggested three predicted genes, two of which could be candidate genes of BLP resistance: membrane protein and zinc finger protein. Candidate genes showed high similarity with their paralogous genes, which were located on the duplicated regions obtaining BLP resistance QTLs. High-resolution map in *rxp* region with eight SSR and two SNP markers will be useful for not only MAS of BLP resistance but also characterization of *rxp*.

Communicated by H. T. Nguyen.

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Introduction

Bacterial leaf pustule (BLP), caused by *Xanthomonas axonopodis* pv. *glycines* (*Xag*), is a serious bacterial disease of soybean [*Glycine max* (L.) Merr.]. BLP is widespread in many soybean-growing regions of the world where high temperature and humidity prevail. The area of *Xag*-infected soybean fields in Korea has steadily increased, reaching 88% in 1998 (Lee 1999). The symptoms of BLP are characterized by small, yellow to brown lesions with raised pustules in the center, which cause premature defoliation resulting in 15% loss of yield (Hartwig and Lehman 1951; Laviolette et al. 1970).

BLP was first reported in the USA and resistance was controlled by a single recessive gene in F_3 generation from crosses of BLP resistant variety, CNS (PI 548445), with several susceptible varieties (Hartwig and Lehman 1951). The *rxp*, a resistance gene to BLP, was mapped on chromosome 17 (previously linkage group D2) 3.9 cM away from Satt372 and 12.4 cM from Satt014 (Narvel et al. 2001; Palmer et al. 1992). Quantitative trait loci (QTLs) identification for BLP resistance revealed that Satt372 on chromosome 17 was strongly associated with

resistance to six different isolates of *Xag* under greenhouse and field conditions (Van et al. 2004). Several minor QTLs were also identified depending on the *Xag* isolates, indicating that BLP resistance was mainly controlled by a single major gene tightly linked to Satt372 with several minor genes. Satt486 was also known as the marker significantly associated to BLP resistance (Kim et al. 2004). Three single nucleotide polymorphism (SNP) markers between Satt372 and Satt486 were present in soybean transcript map: BARC-021191-04000, BARC-022037-04263, BARC-040963-07870 (Choi et al. 2007), but the physical location of BARC-040963-07870 was changed to about 400 kb away from the original position in soybean transcript map according to soybean genome sequences (http://www.phytozome.net/soybean.php). Related to that, more markers need to be developed for constructing a high-resolution map near *rxp*.

The strategy of map-based cloning is to find molecular markers linked tightly to the gene of interest. In previous studies, sequencing BACs or chromosome walking was commonly used for identifying the candidate genes (Blair et al. 2003; Chu et al. 2006). Since genomes from various organisms have been fully sequenced, we can now use these sequences as references for identifying candidate genes or developing new markers like *xa24* (Wu et al. 2008). Gene-for-gene theory suggests that a plant has resistance, if it has a specific resistance (*R*) gene against a specific pathogen avirulence gene (Staskawicz et al. 1995). Many *R* genes identified in plants are dominant and most of the dominant *R* genes belong to five classes, e.g. Ser/Thr kinase, transmembrane and leucine-rich repeat (Martin et al. 2003). However, recessive resistance genes did not have prototypes and only few recessive resistance genes including *mlo*, *RRS1-R* and *xa5* were identified (Deslandes et al. 2002; Jiang et al. 2006; Kim et al. 2002). These have different structures and functions and their signal pathway or any part of recessive defense system was uncharacterized. Thus, developing linked markers and fine mapping could be the first step to clone recessive resistance genes.

Marker-assisted selection (MAS) could help breeders to select resistant lines without pathogen inoculation on plants and regardless of environmental conditions. Breeding of resistant cultivars with MAS could be the most economically and environmentally efficient approach which can reduce time-consuming labor and expenses for many selection cycles. However, tightly linked genetic markers for resistance genes are needed for MAS in crop breeding (Mohan et al. 1997). Thus, the objective of this study is to finely map *rxp* by developing new simple sequence repeats (SSR) and SNP markers tightly linked to *rxp*.

Materials and methods

Plant materials and DNA extraction

Danbaekkong is resistant to soybean BLP while Taekwangkong is BLP susceptible cultivar (Han et al. 2007; Park et al. 2008). A population of 227 F_7 recombinant inbred lines (RILs) was developed from a cross of Taekwangkong and Danbaekkong and these RILs were used for linkage mapping. Fine mapping of the BLP resistance gene was confirmed using two different sets of near isogenic lines (NILs). Three Hwangkeumkong-resistant (HR) lines and two Taekwangkong-resistant (TR) lines were generated by backcrossing a *rxp* donor parent SS2-2 with susceptible Hwangkeumkong and Taekwangkong as recurrent parents, respectively. Hwangkeumkong is BLP susceptible and SS2-2 is BLP resistant (Kim et al. 2008). DNA was extracted from young leaves by CTAB method with minor modifications (Allen et al. 2006). Concentration of DNA was determined with a ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE, USA).

Xag inoculation and BLP evaluation

Experiments were carried out in a greenhouse at Suwon, Korea during July and August 2008, where relative humidity averaged 85.6% and 25.7°C was average temperature. The pathogen strain 8ra of *Xag* was cultured in peptone sucrose agar medium 28°C for 48 h. Four-week-old parents and their 227 RILs were inoculated by spraying the bacterial suspension on both sides of leaves using an atomizer (Park et al. 2008). All plant inoculation experiments were repeated twice and each replicate consisted of three leaves. After inoculation with *Xag* in the RILs, the disease scale was determined by the numbers of their lesions (Fig. 1). The lesion numbers were counted 1 week after inoculation. BLP phenotype was visually rated by counting disease spots of each plant after inoculation of *Xag* using a 0–5 scale: 0 is for below 5 disease spots, 1 is for 5–20 spots, 2 is for 20–50 spots, 3 is for 50–80 spots, 4 is for covering half of leaf with spots and 5 is for almost dead leaf. This disease assessment was modified from Kim (2003).

SSR/SNP marker development and PCR conditions

Marker development and fine mapping near the *rxp* region was done in two steps. The first step was performed with SSR markers developed in this study (Table 1). Amplifications were carried out by PTC-225 Thermal cycler (MJ research, Waltham, MA, USA) with Satt372 and Satt486 as described in Soybase (http://soybase.org). For developing new SSR markers between Satt486 and Satt372, the

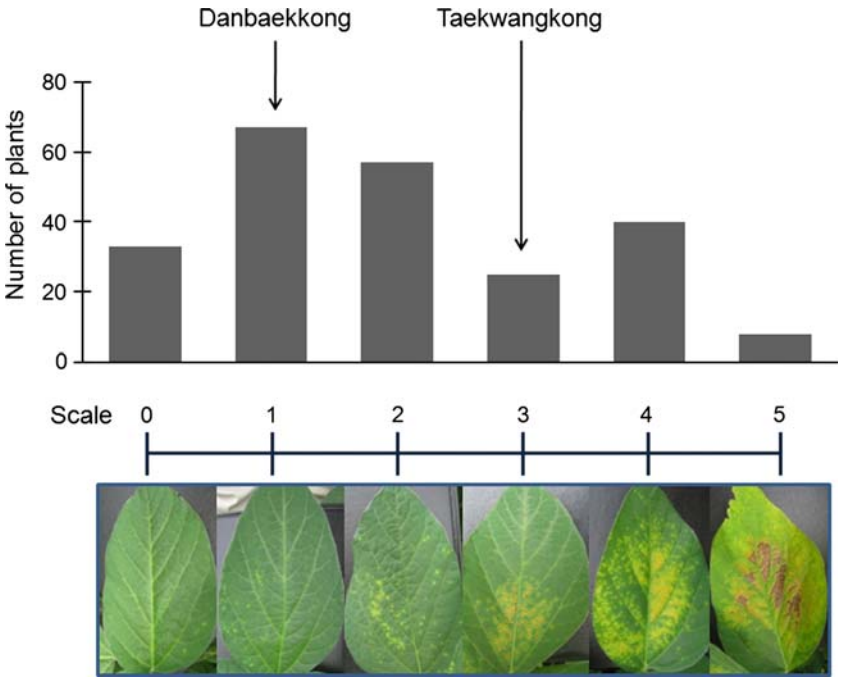

Fig. 1 Disease scale and distribution by lesion numbers. Disease scale was made by counting lesion numbers in RILs. Scale 0 <5 lesions, scale 1 5–20 spots, scale 2 20–50 spots, scale 3 50–80 spots, scale 4 covering half of leaf, scale 5 almost death of leaf. The numbers of plants were counted by the disease scale. Danbaekkong belonged to scale 1 while Taekwangkong was scale 3

sequences were downloaded from Phytozome (http://www.phytozome.net/soybean) and repeat sequences were searched by Sputnik (http://cbi.labri.fr/outils/Pise/sputnik.html) with default parameters except max unit length as 4. The primers for amplification of the searched SSR were designed by primer3 (http://frodo.wi.mit.edu) with default parameters. The SSR amplification consisted of an initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at temperatures mentioned in Table 1 by each primer for 30 s and extension at 72°C for 30 s and final extension at 72°C for 10 min. PCR products for SSR were separated by 8% polyacrylamide gel (Wang et al. 2003).

The second step was carried out with the SNP markers. We selected intron, UTR and intergenic regions for developing SNP markers because those regions showed higher frequency of SNPs than exon (Zhu et al. 2003). Among them, the duplicated regions showing less than 70% in homology were only used for designing primers because their flanking regions were duplicated with high similarity. Primers were tested using genomic DNA of Taekwangkong and Danbaekkong. For identifying SNP, sequencing was performed with the BigDye Terminator (v. 3.1) cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Since PCR products less than 250 bp could lead to the best results, the SNP genotyping primers were redesigned for high-resolution melting (HRM) after SNP was detected. However, PCR products for HRM were longer than 300 bp because SNPs were located in AT-rich regions. PCR for HRM analysis was performed with the presence of the dye 5 μM Syto® 9 (Invitrogen, Sydney, Australia) on a LightCycler® 480 (Roche, Mannheim, Germany). The PCR reactions were performed in 20 μl reaction volume contained 50 ng of genomic DNA, 1 μM of forward and reverse primer, 1× reaction buffer, 0.1 mM dNTP and 0.5 U of *Taq* DNA polymerase (Vivagen, Sungnam, Korea). The HRM amplification consisted of pre-incubation at 95°C for 10 min, followed by 45 cycles of denaturation at 94°C for 10 s, annealing at 62°C for 10 s, and extension at 72°C for 10 s and final extension at 72°C for 10 min. Melting analysis was performed with the additional denaturation at 95°C for 1 min, cooling down with a programmed rate of 2.2°C/s to 40°C for 1 min and a continuous melting of the amplicon with high-resolution data acquisition. The HRM results were obtained with LightCycler® 480 Gene Scanning program as a manufacturer's protocol.

Data analysis

Linkage map was constructed with the Kosambi mapping function using Mapmaker 3.0 (Lander et al. 1987) with the threshold LOD score of 3.0. Composite interval mapping (CIM) was performed by Qgene V. 4.2.2 (Joehanes and Nelson 2008) with scan interval 1. Gene annotation was conducted with gene prediction program FGENESH (http://www.softberry.ru) against *Arabidopsis* database. Each predicted gene was subjected to a BLASTP query of the UniRef database (http://www.ebi.ac.uk/uniref) and a BLASTN query of the EST database (http://www.ncbi.nlm.nih.gov/blast).

Table 1 General information of primer sequences for SSR and SNP markers

Marker type	Primer name	Location[a]	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	T_m (°C)	Amplicon length (bp)	Miscellaneous[b]
SSR	SNUSSR17_1	6,817,439–6,817,739	GGTGGACAATCATCCAACT	TTAGTCATCTCTAGGTTACCTACG	48	300	AT
	SNUSSR17_3	7,064,132–7,064,465	CCTCAGATATCTCTATTCG	TCATATTCAGCTCACTACTTAG	44	333	AT
	SNUSSR17_9	7,303,694–7,303,940	ATGTGTATCAAAGTATGGACG	TTCAGATAAGCATCTGGGA	47	246	AT
	SNUSSR17_12	7,368,501–7,368,886	TGATTTAGTGATCGGAAGAG	AGTTGCTAATGAGATCTCACT	44	385	AT
	SNUSSR17_13	7,431,730–7,431,971	AAATGCTTGAAAACCCATTCA	AATACCCTTTATGCACATACCC	51	241	AT
	SNUSSR17_14	7,471,253–7,471,550	TGCATATTCAGATACGGAAG	AGTTGTGTGACTGCAAAGTT	46	297	AT
	SNUSSR17_15	7,515,826–7,516,019	CAAGACGTTGAGTATAAGCA	CGGAAATAATGTAATGACAA	45	193	AT
	SNUSSR17_17	7,654,172–7,654,477	TGGCCCACGGTAGACTTTTA	TTCACATGGTTGATGGTTGTC	52	305	AT
SNP	SNUSNP17_2	7,098,190–7,099,018	CAGGGCCTCCATGAAGGTAT	TCCTTCCTAGCCTTCAACCTC	55	828	C/G (7,098,492)
	→ SNUHRM17_2	7,098,320–7,098,680	TCAGGAGGGAGAAAGATAGA	GGTGTGAGAGAACGAAGACT	64	360	C/G (7,098,492)
	SNUSNP17_12	7,270,139–7,270,984	GGCAAAATAGAGAAGCCTCTACC	CAAAGAAAAGCGTCACACCA	54	845	G/A (7,270,471)
	→ SNUHRM17_12	7,270,259–7,270,570	AGCCAATGAGTGATAGTTGC	CTCCAAAGGACATAAAATGG	64	311	G/A (7,270,471)

[a] Location: physical location in soybean genome reference sequence (http://www.phytozome.net/soybean.php)

[b] Miscellaneous: repeat motif in SSR marker, SNP sequence of Taekwangkong/Danbaekkong and its location in Phytozome

Results

Evaluation of disease

Danbaekkong belonged to scale 1 and Taekwangkong to scale 3 (Fig. 1). Danbaekkong showed strong resistance to BLP, but Taekwangkong was not severely susceptible. Among RILs, a total of 33, 67 and 57 plants were scaled as 0, 1 and 2, respectively, which were considered as resistant to BLP. For susceptible RILs, 25, 40 and 8 plants were in scale 3, 4 and 5, respectively (Fig. 1). So, the progenies showed various phenotypes, some plants showed more resistance than Danbaekkong and some lines had more severe damage by *Xag* than Taekwangkong (Fig. 1).

Development of SSR and SNP markers

For the fine mapping of *rxp*, sequence data available at Phytozome and the BAC clones sequenced in our previous study (Van et al. 2008) were used for designing SSR and SNP markers. Three SNP markers, BARC-021191-04000, BARC-022037-04263 and BARC-040963-07870 were between Satt372 and Satt486 in the recent soybean transcript map (Choi et al. 2007). However, the genetic distance between BARC-021191-04000 and BARC-022037-04263 was 0.06 cM and they were monomorphic in our mapping population. BARC-040963-07870 was physically located on the outer range of Satt372 and Satt486. Therefore, new genetic markers in this region should be developed for fine mapping of *rxp*.

Within this region, putative 70 SSR loci were identified by Sputnik. A total of 17 putative SSR loci having more than 40 bp repeat motifs were selected for designing primers to survey polymorphisms between Taekwangkong and Danbaekkong. Out of the 17 SSR markers, 8 markers having 'AT' as a repeat motif showed polymorphisms between the parents of the RILs (Table 1).

To identify SNPs for fine mapping, five regions were selected based on two criteria, low similarity with duplicated regions in other chromosomes and longer than 3 kb in size. Two SNP markers, SNUSNP17_2 and SNUSNP17_12, were developed between SNUSSR17_3 and SNUSSR17_9 (Table 1). In SNUSNP17_2, a transversion SNP (C: Taekwangkong/G: Danbaekkong) was observed at 7,098,492 bp on chromosome 17. SNUSNP17_12 showed transition substitution (G: Taekwangkong/A: Danbaekkong) at 7,270,471 bp on chromosome 17. And, both of these SNPs were located in introns. Thus, eight new SSR markers and two new SNP markers were developed in this region spanning of about 820 kb.

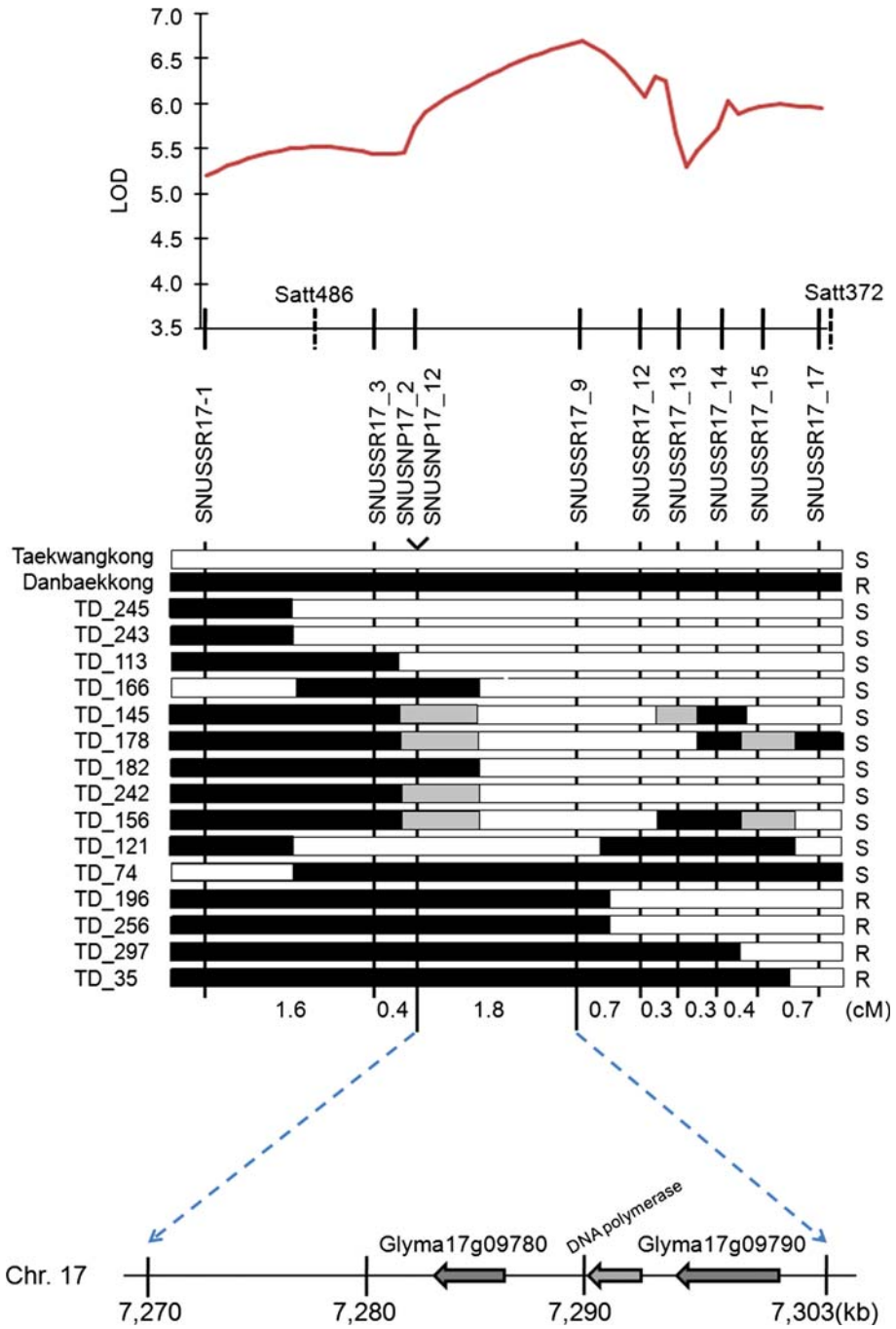

Fig. 2 Fine mapping of *rxp* and graphical genotypes of RILs. QTL map of *rxp* in the middle of chromosome 17 was constructed with 8 SSR markers and 2 SNP markers. Two SNP markers (SNUSNP17_2 and SNUSNP17_12) showed the same genotypes. *Black bars*, *white bars* and *grey bars* represent Danbaekkong segments, Taekwangkong segments and heterozygous region, respectively. Susceptible and resistant phenotypes are represented by *S* and *R*, respectively. The *black arrows* represent predicted genes

Fine mapping and QTL identification

We genotyped the RILs with eight SSR and two SNP newly developed markers and a genetic map was constructed within 6.2 cM of genetic distance between SNUSSR17_1 and SNUSSR17_17. The average distance between markers in this region was 0.68 cM, and the largest marker interval was 1.8 cM between SNUSNP17_12 and SNUSSR17_9. Around Satt372 and Satt486, ten newly developed markers were added, thus increasing the genetic resolution of the previous map (Choi et al. 2007). Two SNP markers were positioned at 0.4 cM from SNUSSR17_3 (Fig. 2) and their physical distance was 170 kb (Table 1). But the genetic distance between the two SNP markers was 0 cM because the RILs showed the same genotype in these SNP markers. On this map, the genetic order of these markers was completely consistent with their physical location in soybean genome sequences.

QTL analysis using composite interval mapping was performed by comparison of the BLP phenotyping data

with the marker genotyping data. Out of 227 F_7 RILs, 15 RILs were identified as recombinants between Taekwangkong and Danbaekkong (Fig. 2). The maximum LOD score was on SNUSSR17_9. The genotypes of RILs at SNUSSR17_9 were identical with their phenotypes except TD_74. TD_74 showed resistant genotype in SNUSNP17_2 and SNUSSR17_9, even though it had susceptible phenotype.

Confirmation of linkage map with NILs

To confirm newly developed markers linked to *rxp*, two different set of NILs having resistant phenotypes were used to genotype with four markers, SNUSSR17_3, SNUSSR17_9, SNUSNP17_2 and SNUSSR17_12 (Kim et al. 2008). Hwangkeumkong followed susceptible Taekwangkong genotypes and SS2-2 was the same as Danbaekkong. Taekwangkong-resistant (TR) lines showed resistant genotypes at both SNUSSR17_9 and SNUSNP17_2 whereas Hwangkeumkong-resistant (HR) lines had susceptible genotype at SNUSSR17_9 (Fig. 3). Therefore, according to linkage mapping of both RILs and NILs, *rxp* could be located between SNUSNP17_12 and SNUSSR17_9.

Candidate gene prediction

Analysis of the 33 kb sequences flanked by two markers, SNUSSR17_9 and SNUSNP17_12, revealed that three open reading frames were predicted; one was DNA polymerase and the other two genes could be considered as putative candidate genes after each predicted gene was subjected to a BLASTP query of the UniRef database (Table 2). First, membrane protein (Glyma17g09780, 6E-43) has three exons and is 768 bp long. The second candidate gene is similar to zinc finger family protein from *Arabidopsis* (Glyma17g09790, 2E-97), having six exons and 759 bp long. The expression of these genes was supported by EST data (CD398246 and CO984038 at http://www.ncbi.nlm.nih.gov/).

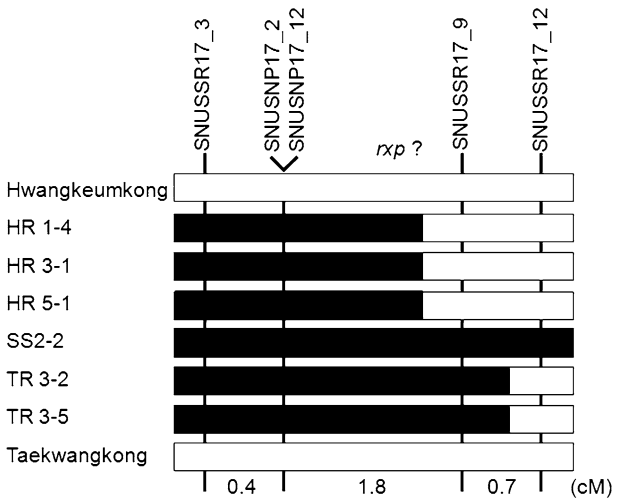

Fig. 3 The *rxp* genetic mapping with NILs. *Black bars* and *white bars* represent resistant genotype and susceptible genotype, respectively. Hwangkeumkong-resistant lines (HR 1-4, HR 3-1 and HR 5-1) are developed by backcrossing *rxp* donor parent SS2-2 with Hwangkeumkong as recurrent parent. Taekwangkong-resistant lines (TR 3-2 and TR 3-5) are developed by backcrossing SS2-2 with Taekwangkong as recurrent parent. HR lines and TR line showed resistant phenotype but HR lines had susceptible genotype in SNUSSR17_9, TS lines had resistant genotype in SNUSSR17_9

Discussion

A total of ten markers including eight SSRs and two SNPs were developed and *rxp* mapped between SNUSNP17_12 and SNUSSR17_9. The region between Satt486 and Satt372 showed high similarity with the duplicated region (chromosomes 4, 5, 6, 10 and 17) (Kim et al. 2009; Van et al. 2008) and six QTLs for resistance to BLP were also detected in chromosomes 4, 5, 10, 13, 17 and 19 (Van et al. 2004). It suggested that the candidate gene of *rxp* was duplicated and each duplicated gene might have its own function for the resistance to BLP. So, six regions contained SSR markers for BLP resistance QTLs were annotated and compared by BLASTN for candidate genes (Table 3). The membrane protein showed high similarity with five genes, which were located on the duplicated

Table 2 BLAST results of candidate genes positioned between SNUSNP17_12 and SNUSSR17_9

Candidate name[a]	Uniref ID[b]	Identity[c] (%)	Length (amino acid)	*e* value	Description
Glyma17g09780	UniRef100_Q84WP5	48.44	256	6.00E-43	Membrane protein At2g36330; *Arabidopsis thaliana*
Glyma17g09790	UniRef100_UPI0000196F93	69.96	253	2.00E-97	Zinc finger (C3HC4-type RING finger) family protein; *Arabidopsis thaliana*

[a] Candidate name: annotation by Phytozome (http://www.phytozome.net/soybean.php)

[b] Uniref ID: BLASTP results in UniRef database (http://www.ebi.ac.uk/uniref)

[c] Identity: percentage of amino acid homology

Table 3 Percentage in nucleotide similarity between candidate genes and their homologs

Candidate gene	Membrane protein	Zinc finger family protein
Chromosome 17	100 (Glyma17g09780)	100 (Glyma17g09790)
Chromosome 5	93 (Glyma05g02140)	94 (Glyma05g02130)
Chromosome 4	83 (Glyma04g35320)	89 (Glyma04g35340)
Chromosome 6	82 (Glyma06g19450)	86 (Glyma06g19470)
Chromosome 19	80 (Glyma19g23460)	–
Chromosome 10	80 (Glyma10g15610)	–

regions with BLP resistance QTLs. For zinc finger family protein, only three paralogous genes (chromosomes 4, 5 and 6) were identified instead of five paralogous genes. However, all annotated names by Phytozome, which contained chromosome number and numerical index of genes, indicated gene synteny. Also, their gene order and orientation were conserved among paralogous genes in chromosomes 4, 5 and 6.

The SSR and SNP markers developed in this study were much closer than any marker published. Until now, Satt372 and Satt486 were used for MAS of BLP resistance (Kim et al. 2008). Among the 15 NILs selected based on Satt486 and Satt372, 4 did not show any resistant phenotype. However, in SNUSSR17_9, genotypes of the RILs were identical with their phenotypes except a TD-74 RIL and SNUSNP17_12 had three exceptions (TD-74, 166 and 182 RILs) among 227 RILs. SNUSSR17_9 and SNUSNP17_12 were found to be more tightly linked to *rxp* than Satt372 and Satt486. Thus, these newly developed markers were highly useful for MAS.

The fine mapping allowed us to narrow down the location of *rxp* within 33 kb between SNUSSR17_9 and SNUSNP17_12. Among three annotated ORFs, one was DNA polymerase and the rest were considered as potential candidate genes. These two candidate genes are a membrane protein and a zinc finger (C3H4-type RING finger) family protein. The membrane protein is one of the largest protein superfamily. Although at least 12 functional categories were identified in *Arabidopsis*, each of their functions was not fully characterized (Ward 2001). Usually, their members have predicted transmembrane regions like Mildew resistance Locus O (MLO) (Kim et al. 2002). The zinc finger family protein has RING finger domain, a specialized type of zinc finger with 40–60 residues bound to two atoms of zinc. It was probably involved in mediation of protein–protein interaction. The RING finger protein is known as having a wide range of functions such as viral replication, signal transduction and development. The zinc proteins are also considered as contributing towards protection against environmental stresses (Davletova et al. 2005). Two genes were the most likely candidates for the observed resistance because some well-known genes with similar structures were involved in resistance processes.

Until now, little about the mechanisms for recessive resistance are known because few recessive resistance genes have been characterized (Iyer-Pascuzzi and McCouch 2007). Since recessive resistance genes have various structures, the functions of each gene might be different. *Xa5*, bacterial blight resistance gene in rice, encoded the gamma subunit of transcription factor IIA, but only two nucleotide substitutions resulted in amino acid change from valine to glutamic acid leading to resistance phenotype (Jiang et al. 2006). MLO has seven transmembrane domains and its function seems to be a negative regulator of the defense against powdery mildew fungus in barley (Kim et al. 2002). The *RRS1-R* gene from *Arabidopsis* gives resistance to bacterial wilt and encodes a nucleotide binding site-leucine-rich repeat (NBS-LRR) protein (Deslandes et al. 2002).

Two hypotheses for recessive resistance in plants have been suggested. First, recessive resistance genes may be required for the pathogen growth or reproduction. For example, translation initiation factors, eIF4E and eIF4G, responsible for resistance to *Rice yellow mottle virus* are recessive (Albar et al. 2006). The recessive allele prevented the formation of complexes involved in the fixation of mRNA cap and ribosome recruitment. Therefore, mutation in plant gene products, which are essential for pathogen's activities, can give resistance to plants because the virus does not have its own system for transcription or translation. Secondly, recessive resistance genes may be a negative regulator in plant defense pathways. For example, recessive mutations in the barley gene, *mlo*, give resistance to the powdery mildew pathogen. MLO encodes plant-specific integral membrane protein and has calmodulin (CaM)-binding domain in C-terminal. CaM binding enhanced MLO activity and mutations in CaM-binding domain prevented MLO with negative defense regulation against powdery mildew (Kim et al. 2002). Since genes with binding domains are related to the signal pathways, BLP candidate genes with similar structures might be involved in the signal pathway for disease resistance. Therefore, the second hypothesis might be more positive, if one of these candidate genes is the BLP resistance gene.

Our results showed that *rxp* is between SNUSNP17_12 and SNUSSR17_9. Two candidate genes were selected and

their potentials as a resistance gene were described. High-resolution map in *rxp* region with eight SSR and two SNP markers could help breeders for MAS of resistant cultivars to BLP because the markers identified in this study are tightly linked to *rxp*.

Acknowledgments This work was supported by the Agricultural R&D Promotion Center, Ministry for Food, Agriculture, Forestry, and Fisheries, Republic of Korea (grant no. 305005–4), by the Crop Functional Genomics Center of the 21st Century Frontier R&D Program funded by the Ministry of Education, Science, and Technology, Republic of Korea (grant no. CG3121), by the BioGreen 21 Project, Rural Development Administration, Republic of Korea (grant no. 20080401034011 for DNA sequencing).

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