ORIGINAL ARTICLE

Fine-mapping and candidate gene analysis for the foxglove aphid resistance gene *Raso2* from wild soybean PI 366121

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Abstract

Key message **The foxglove aphid resistance gene *Raso2* from PI 366121 was fine-mapped to 77 Kb region, and one candidate gene was identified.**

Abstract The foxglove aphid (FA: *Aulacorthum solani* Kaltenbach) is an important insect pest that causes serious yield losses in soybean. The FA resistance gene *Raso2* from wild soybean PI 366121 was previously mapped to a 13 cM interval on soybean chromosome 7. However, fine-mapping of *Raso2* was needed to improve the effectiveness of marker-assisted selection (MAS) and to eventually clone it. The objectives of this study were to fine-map *Raso2* from PI 366121 using Axiom® 180 K SoyaSNP array, to confirm the resistance and inheritance of *Raso2* in a different background, and to identify candidate gene(s). The 105 $F_{4:8}$ recombinant inbred lines were used to fine-map the gene and to test antibiosis and antixenosis of *Raso2* to FA. These efforts resulted in the mapping of *Raso2* on 1 cM interval which corresponds to 77 Kb containing eight annotated genes based on the Williams 82 reference genome assembly (Wm82.a2.v1). Interestingly, all nonsynonymous substitutions were in *Glyma.07g077700* which encodes the disease resistance protein containing LRR domain and expression of the gene in PI 366121 was significantly higher than that in Williams 82. In addition, distinct SNPs within *Glyma.07g077700* that can distinguish PI 366121 and diverse FA-susceptible soybeans were identified. We also confirmed that *Raso2* presented the resistance to FA and the Mendelian inheritance for single dominant gene in a different background. The results of this study would provide fundamental information on MAS for development of FA-resistant cultivars as well as functional study and cloning of the candidate gene in soybean.

Introduction

As one of the major Hemipteran pests in soybean, foxglove aphid (FA, *Aulacorthum solani* Kaltenbach) is a highly polyphagous insect. It has a wide range of host plants on which it can feed 540 identified plant species from 33 orders and 82 families across the world (Jandricic et al. 2010). The FA can overwinter as eggs in the holocyclic or as nymphs/adults in

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the anholocyclic on various host plants (Leather 1992; Turl 1983; Lee et al. 2002). It can damage the plants it feeds upon by sucking plant sap and depriving the plant of nutrients, and by transmitting viruses. High FA populations directly reduce soybean yield when their feeding causes stunting, leaf distortion, and severe leaf yellowing. An additional threat posed by the FA is its ability to transmit about 40 kinds of viruses to soybean (Blackman and Eastop 2000). As a major insect pest of soybean in Korea (Kim et al. 1991) and Japan, yield loss of 90% was reported in Japan in 2000 (Nagano et al. 2001). In spite of significant potential of yield losses by FA, relatively little research on the genetic basis of resistance to the insect and breeding efforts for developing FA-resistant cultivars in soybean have been conducted.

Soybean [*Glycine max* (L.) Merr] is one of the most important crops for food and feed sources across the world. Wild soybean (*Glycine soja* Siebold & Zucc) has been known as a progenitor species of soybean (*Glycine max*). Wild soybeans have been considered as a gene bank for soybean breeding programs because it contains positive genetic variations on seed compositions (Pham et al. 2010; Jun et al. 2008; Zhang et al. 2016) and tolerances to abiotic stresses (Ji et al. 2010; Kao et al. 2006; Li et al. 2017). Wild soybean has been also used as sources of resistances to biotic stresses including insect pests in soybean. For example, *Rhg1* from PI 88788 for SCN resistance, *Raso2* from PI 366121 for FA resistance (Lee et al. 2015a), and two quantitative trait loci (QTLs) from *G. soja* 85–32 for soybean aphid (SA; *Aphis glycines* Matsumura) resistance (Zhang et al. 2017a) were derived from wild soybeans. Because it has been reported that cultivated soybean has narrow genetic base, continuous supply of genetic diversity/variations in target traits would be fundamental component for successful breeding programs in the future. However, genetic diversity from wild species could often result in a barrier to progress due to their unfavorable agronomic characteristics such as small less seed yield/size, pod shattering, and hard seed (Smalley et al. 2004).

Host plant resistance can provide an effective and economical way to control insect pests. In addition, host plant resistance is regarded as the most important component of an integrated pest management program for insect control (Harrewijn and Minks 1989). Three kinds of host plant resistance to insect pests have been reported (Painter 1951; Kogan and Ortman 1978), and antibiosis and antixenosis have been reported as resistant responses to FA in soybean (Lee et al. 2015a; Koh et al. 2018). Antibiosis is the ability of host plants to reduce the survival, growth, or reproduction of insects and is often caused by the production of toxic chemicals or the secondary metabolites by the plants (Kim et al. 2008). Antixenosis is the ability of host plants to repel insects. Therefore, feeding or oviposition on the plants could be reduced. The third type of host plant resistance is

tolerance. Although it does not associated with ability to produce of toxic chemicals or to repel insects, genotypes with tolerance do not present a significant yield loss by equal levels of colonization that occur on susceptible genotypes.

Till now, several insect resistance genes have been mapped on soybean chromosome 7 (https://www.soybase.org). In the case of SA, a single dominant resistance gene *Rag1* from Jackson (PI 548657) and Dowling (PI 548663) were mapped on chromosome 7 (Li et al. 2007; Kim et al. 2010a, b). One SA resistance gene in PI 587732 (Kim et al. 2014) and one QTL in PI 567541B (Zhang et al. 2009) were also mapped on chromosome 7. In the case of FA, no resistance gene or QTL has been mapped on chromosome 7, while Ohnishi et al. (2012) mapped *Raso1* from Adams (PI 548502) on chromosome 3. For other insect pests, two QTLs in Himeshirazu (PI 594177) providing antibiosis type of resistance to common cutworm (CCW; *Spodoptera litura* Fabricius) were mapped on chromosome 7. One was mapped between Satt220 and Satt175 and another was positioned between Satt567 and Satt463 (Komatsu et al. 2005). Oki et al. (2012) suggested that the antixenosis in Himeshirazu to CCW controlled by previously identified QTLs might be associated with pubescence characteristics. Rector et al. (2000) and Narvel et al. (2001) reported that a QTL in PI 229358 with antibiosis to corn earworm was flanked by Satt220 and Satt463 on chromosome 7.

The dominant FA resistance gene *Raso2* from PI 366121 was previously mapped to a 13 cM interval by Lee et al. (2015a). However, fine-mapping of *Raso2* would be needed to improve the effectiveness of marker-assisted selection (MAS) and to eventually clone it. Therefore, the objectives of this study were (1) to fine-map FA resistance gene *Raso2* from PI 366121 with 180 K Axiom® SoyaSNP assay, (2) to identify and evaluate expression levels of candidate gene(s), (3) to validate resistance and inheritance of *Raso2* in a different background, and (4) to investigate sequence variations of the candidate gene(s) for *Raso2* in diverse soybean germplasm.

Materials and methods

Plant materials

One recombinant inbred line (RIL) and one F₃ population were used in the present study. The first population of 105 F₄:₈ RILs from the cross between Williams 82 and PI 366121 which was previously used to map *Raso2* (Lee et al. 2015a) was used to fine-map *Raso2*. PI 366121 is a maturity group (MG) IV wild soybean accession originating from Fukusima, Japan (USDA-ARS Germplasm Resource Information Network, http://www.ars-grin.gpv/npgs/; accessed 31 Oct. 2020). It has been reported that PI 366121 presented

resistance to FA (Lee et al. 2015a; Koh et al. 2018), a purple flower color, and black colors of pod, seed coat, and hilum. Williams 82 (PI 518671) is a MG III soybean cultivar (Bernard and Cremeens 1988) that is susceptible to SA (Kim et al. 2008) and FA (Lee et al. 2015a). Williams 82 was selected for the parent of the first population due to its susceptibility to FA and it has been used a source of reference genome for soybean (Schmutz et al. 2010).

The second population was used to validate a resistance and an inheritance of *Raso2* to FA in a different background. The population consisted of 41 F_3 individuals derived from a cross between PI 483463 and PI 366121. PI 483463 is a FA-susceptible wild soybean accession which was originally collected from Shanxi Sheng, China (USDA-ARS Germplasm Resource Information Network, accessed 31 Oct. 2020). We hypothesized that the resistance to FA in the second population would be controlled by *Raso2* and presents the Mendelian inheritance for a single gene in the F_3 generation.

Evaluation of foxglove aphid resistance

The FA was collected at the Crop Environmental Research Division of National Institute of Crop Science, Suwon, Korea during the summer of 2008 by collecting aphids from nearby soybean fields. The FA was maintained on a continuous supply of plants of FA-susceptible Korean soybean germplasm (Sowon) (Park et al. 2000) in a growth chamber at 22–25 ℃, 15/9 day/night photoperiod at 370 µmol m^{-2} s^{-1} photosynthetically active radiation irradiation, and 60–80% of relative humidity.

Both the antixenosis (choice test) and antibiosis (nonchoice test) to FA were evaluated as described by Lee et al. (2015a). The tests were conducted in a growth chamber under the conditions as described above. For both tests, four adult FAs were placed on the upper side leaf of each plant at the V1 growth stage (Fehr et al. 1971) with a paint brush. For the tests, resistance was evaluated by scoring plants with primary leaf damage (PLD) and total plant damage (TPD). Jandricic et al. (2010) have reported that the number of FAs was not highly correlated with the total plant damage grade unlike soybean aphids. Thus, PLD and TPD were used for our previous and current studies. The PLD and TPD were graded by assigning scores 1 (no damage to the inoculated leaf), 2 (< 5% damage), 3 (5–30% damage), 5 (31–50% damage), 7 (51–70% damage), or 9 (> 70% damage) (Lee et al. 2015a). For antixenosis test, RILs and the parents of the first population were planted in 10 × 5 trays (550L × 270 W × 120H mm) with Sowon in the center column. The plants were arranged in a complete randomized design (CRD) with three replications. Resistance was evaluated at fourteen days after infestation by scoring plants with the PLD and TPD. For antibiosis test, RILs and the

parents were planted in the same tray without Sowon. After infestation, the plants were isolated with a 120-mesh cage to restrict aphid movement among plants. Seven days after inoculation, the PLD and TPD were scored for each plant.

To validate resistance to FA and inheritance of *Raso2* in the second population, antibiosis was tested as described above. Five plants from each line were inoculated with five adult FAs, and then, lines were determined as homozygous resistant (when all plants have scores less than 2), heterozygous (when plants have scores between 2 and 3), or homozygous susceptible (when all plants have scores greater than 3) to FA based on responses of the five plants of each line.

DNA extraction and SNP genotyping

Genomic DNA from each line and the parents in the first population was extracted using young trifoliate leaves with the CTAB (hexadecyltrimethylammonium bromide) method described by Porebski et al. (1997) with the following modifications: an incubation time of 90 min, re-suspension of the DNA pellet in 500 µL 1 × TE, and no RNase A treatment. All DNA was firstly quantified by ND-1000 Spectrophotometer and diluted to 100 ng µL^{-1} for further study. For the SNP the genotyping, Axiom® 180 K (180,961) SoyaSNP array (Affymetrix, CA, USA) was used (Lee et al. 2015b). Genomic DNA from the lines and parents were hybridized to Affymetrix GeneTitan array system and then scanned with GeneTitan® Scanner (Affymetrix, CA, USA) according to the manufacturer's protocol. SNP genotype analysis was conducted based on Axiom® Genotyping Solution Data Analysis User Guide (http://www.affymetrix.com). Of the 180,961 SNPs, 28,752 high-quality SNPs with the following parameters: (1) missing value < 10% and (2) minor allele frequency (MAF) < 0.001 were used to fine-genetic map *Raso2*.

Construction of Genetic Linkage Map and QTL analysis

A linkage mapping of the first population was conducted using QTL IciMapping software (version 4.1) with the following parameters: (1) a logarithm of odds (LOD) of 3.0 to group markers into linkage groups, (2) a ordering algorithm of nnTwoOpt for tour construction and two-opt for tour improvement, and (3) rippling by sum of adjacent recombination fractions (Li et al. 2008).

Associations between the two phenotypes, PLD and TPD, and SNP markers were first tested by a single-factor analysis of variance using IciMapping software with 3.0 of LOD threshold. Multiple-regression analysis with all significant markers on single linkage group was conducted using PROC REG function in SAS 9.3 (SAS Institute, NC, USA) with $\alpha = 0.05$ to determine the total phenotypic variance explained (R^2) by QTL. In addition, the inclusive composite

interval mapping (ICIM) was performed using a 1.0 cM walk speed and 3.0 of LOD threshold (Li et al. 2008).

Evaluation of expression level of the candidate gene

To evaluate expression levels of the candidate gene, ten FAs were first inoculated to the trifoliate of Williams82 and PI 366121, parents of the first population. And then leaf samples were collected after 0, 24, and 48 h after inoculation with three biological replications. Total RNA was isolated using Trizol reagent (Invitrogen, CA, USA), 10 µg of the total RNA was purified via magnetic beads (Thermo Fischer Scientific, USA) and cDNA was synthesized using a reverse transcription reaction (EcoDry cDNA Synthesis Premix) following the manufacturer's instructions (Takara Bio Inc., Japan). The expression levels were determined by quantitative real-time polymerase chain reaction (qRT-PCR) using an ABI StepOnePlus system (Applied Biosystems, CA, USA). All experiments were performed with three replications and the results were analyzed using StepOne software V2.1 (Applied Biosystems, CA, USA). Primers for target gene were designed using Primer3.0 version 4.1.0 (http://primer3.ut.ee/). Primer sequences for the target candidate gene (*Glyma.07g077700*) were 5'GTTTGACTCTTAGCT CGTTACCAA3' for forward and 5'CAAGGTTTGCTG AACGACAT3' for reverse. Primers for housekeeping gene (Cons6; *Glyma.12G051100*) were 5'AGATAGGGAAAT GGTGCAGGT3' for forward and 5'CTAATGGCAATTGCA GCTCTC3' for reverse (Libault et al. 2008). T-test in SAS 9.3 (SAS Institute, NC, USA) was conducted to compare relative expressions of the gene between Williams 82 and PI 366121.

Sequence comparison of the candidate gene between PI 366121 and diverse soybean germplasm

To confirm sequence variations within the candidate gene identified from the first population, sequences of eight exon regions in the *Glyma.07g077700* between PI 366121 and 26 parental soybean germplasms for the Korean soybean nested association mapping (NAM) populations were compared (Kim et al. 2021). Out of 26 germplasm, one Korean soybean cultivar, Cheongja, presents resistance to Korean FA biotype but resistant gene(s) in the germplasm has not been identified. Other 25 germplasm were susceptible to Korean FA biotype (Kang Lab, unpublished data).

Results

To narrow down the genetic interval containing the *Raso2* from PI 366121, Axiom® 180 K SoyaSNP array was employed to 105 $F_{4:8}$ RIL from the first population. Among

the high-quality SNPs, 28,752 SNPs after eliminating multi-collinearity and/or redundant markers were finally used for construction of genetic linkage map. The high-density genetic linkage map of 105 RILs consisted of 20 chromosomes which spanned about 4300 cM. Compared to the previous study using 414 SNP markers from GoldenGate® assay (Lee et al. 2015a), average distance between SNPs in the present study was about 0.15 cM increased as 45 times than the previous study (Fig. 1; Table 1).

The QTL analysis revealed that the major QTL on chromosome 7 was highly associated with the tested four phenotypes (PLD and TPD from the choice and nonchoice test). The QTL was positioned in the interval previously identified by Lee et al. (2015a) and was mapped between two SNP markers, AX-90462843 and AX-90334585 (Figs. 2, 3). Therefore, it could be assured that the mapped QTL in the present study was *Raso2*. This effort resulted in narrowing the genetic interval containing the gene from 13 to 1 cM in genetic length using the same population. Based on the ICIM, *Raso2* accounted for 35.3 and 28.5% of the phenotypic variations for PLD and TPD in the choice test, respectively (Table 2). In the nonchoice test, 21.5 and 20.2% of variances for PLD and TPD was explained by *Raso2*, respectively (Table 2). Additive effect of *Raso2* region ranged from 0.9 to 1.5 according to the test and traits (Table 2).

Based on the Williams 82 genome assembly (Wm82. a2.v1), *Raso2* was located within 76 Kb region defined by the above SNP markers and the current gene annotation of the region predicted the presence of eight gene models (Fig. 3). Of these genes, six annotated gene models encode putative protein with function (Table 3). Through re-sequencings of exon regions in the eight annotated genes, sixteen SNPs between Williams 82 and PI 366121 were identified from three annotated genes (Table 3). Two, two, and twelve SNPs between Williams 82 and PI 366121 were detected in *Glyma.07g077000*, *Glyma.07g077400*, and *Glyma.07g077700*, respectively. Among the SNPs, ten SNPs were nonsynonymous substitutions and all of them were located within one gene, *Glyma.07g077700*, which is putative *R* gene encodes the disease resistance protein (PTHR23155) containing leucine-rich repeat (LRR) domain (Table 3).

To validate the *Raso2* resistance to FA and inheritance of the gene in a different background, 41 F_3 families derived from PI 366121 and PI 483,463 were tested with FAs. The population was segregated as 12 (homozygous resistant):21 (heterozygous; segregating):8 (homozygous susceptible) based on the responses of the five plants from each line and the ratio was fit to the Mendelian inheritance for a single dominant gene, 1:2:1 ($\chi^2 = 0.805$, $P = 0.67$, Table 4).

A relative expression level of the candidate gene between Williams 82 and PI 366121 was analyzed by qRT-PCR (Fig. 4). No statistical difference in the expression levels
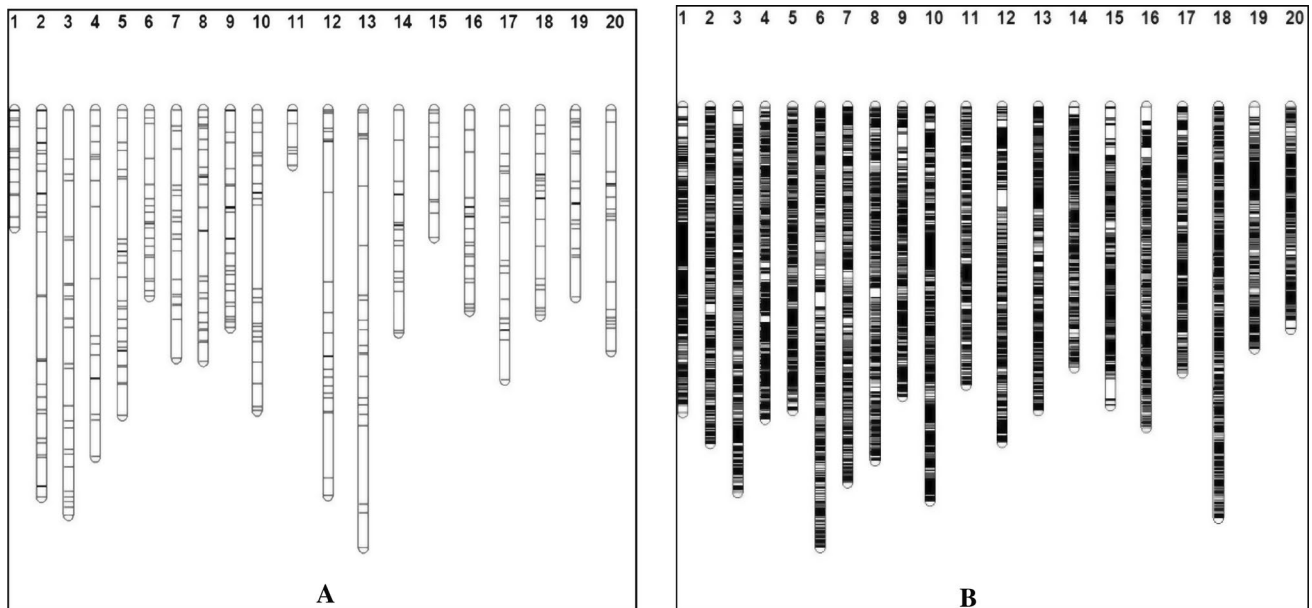
Fig. 1 Genetic linkage maps of 105 $F_{4:8}$ recombinant inbred lines from Williams 82 and PI 366121. **a** Genetic linkage map using the GoldenGate® assay (Lee et al. 2015a), **b** genetic linkage map using the 180 K Axiom® SoyaSNP assay (current study)

Table 1 Comparison of genetic maps by the Illumina GoldenGate SoyOPA-4 (Lee et al. 2015a) and 180 K Axiom® SoyaSNP Assay in the $F_{4:8}$ RIL population

| Chr[a]	Illumina GoldenGate SoyOPA-4			180 K Axiom® SoyaSNP Assay				
	Length (cM)	No. of mapped markers	Average distance between SNPs (cM)	Length (cM)	No. of total SNPs	No. of polymorphic SNPs	No. of mapped SNPs	Average distance between SNPs (cM)
1	66	15	4.4	180	8379	1453	1442	0.12
2	215	32	6.7	257	9514	1480	1467	0.18
3	225	26	8.7	205	7673	1501	1475	0.14
4	193	17	11.4	240	8399	1191	1169	0.21
5	170	28	6.1	212	7577	1564	1552	0.14
6	104	22	4.7	278	9392	1840	1822	0.15
7	138	20	6.9	322	8126	1462	1455	0.22
8	140	26	5.4	226	10,475	1505	1493	0.15
9	121	25	4.8	171	8621	1345	1326	0.13
10	167	23	7.3	235	8820	1666	1650	0.14
11	31	7	4.4	170	7481	1073	1020	0.17
12	214	24	8.9	215	7222	1410	1406	0.15
13	243	22	11.1	323	10,338	1700	1685	0.19
14	124	17	7.3	209	7195	1291	1286	0.16
15	71	11	6.5	212	9423	1568	1561	0.14
16	112	20	5.6	191	6980	1450	1425	0.13
17	150	21	7.1	225	8347	1187	1150	0.20
18	114	20	5.7	194	9103	2073	2062	0.09
19	104	20	5.2	159	8166	2063	1251	0.13
20	134	18	7.4	156	7797	1076	1055	0.15
Average	142	20.7	6.8	219	8451	1495	1438	0.15
Total	2,837	414		4,380	169,028	29,898	28,752	

[a]Chromosome
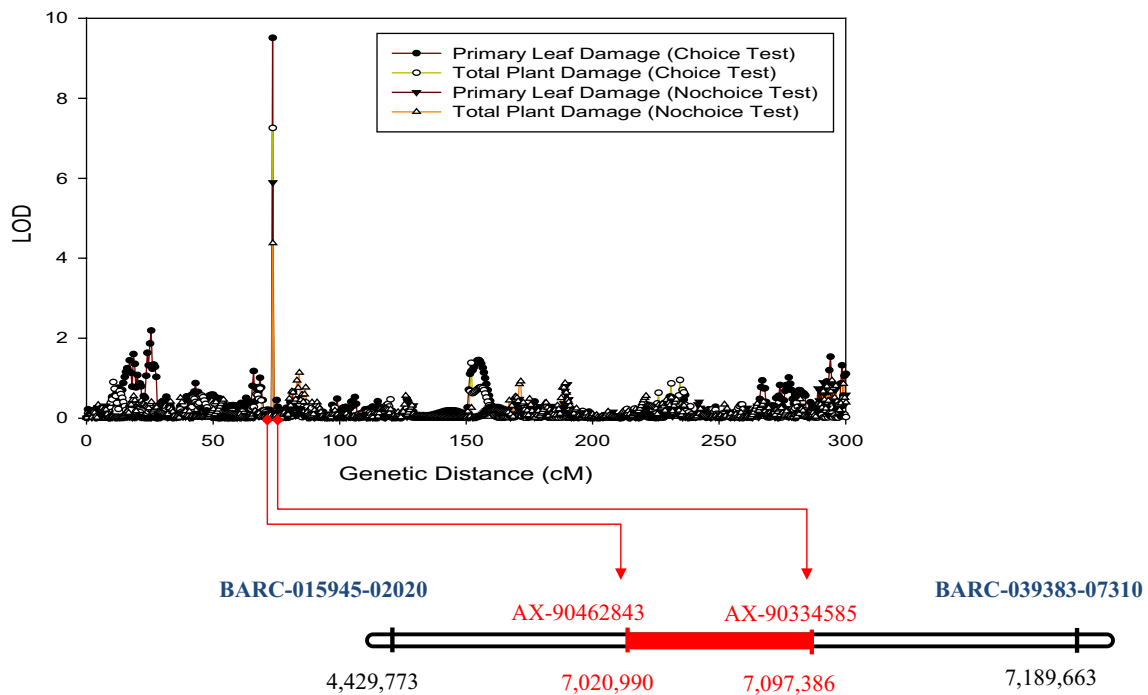
Fig. 2 Inclusive composite interval mapping for primary leaf damage (PLD) and total plant damage (TPD) in the choice and nonchoice test in the first population. BARC markers from GoldenGate® assay (Lee et al. 2015a) and AX markers from Axiom® 180 k SoyaSNP array (present study) on soybean chromosome 7
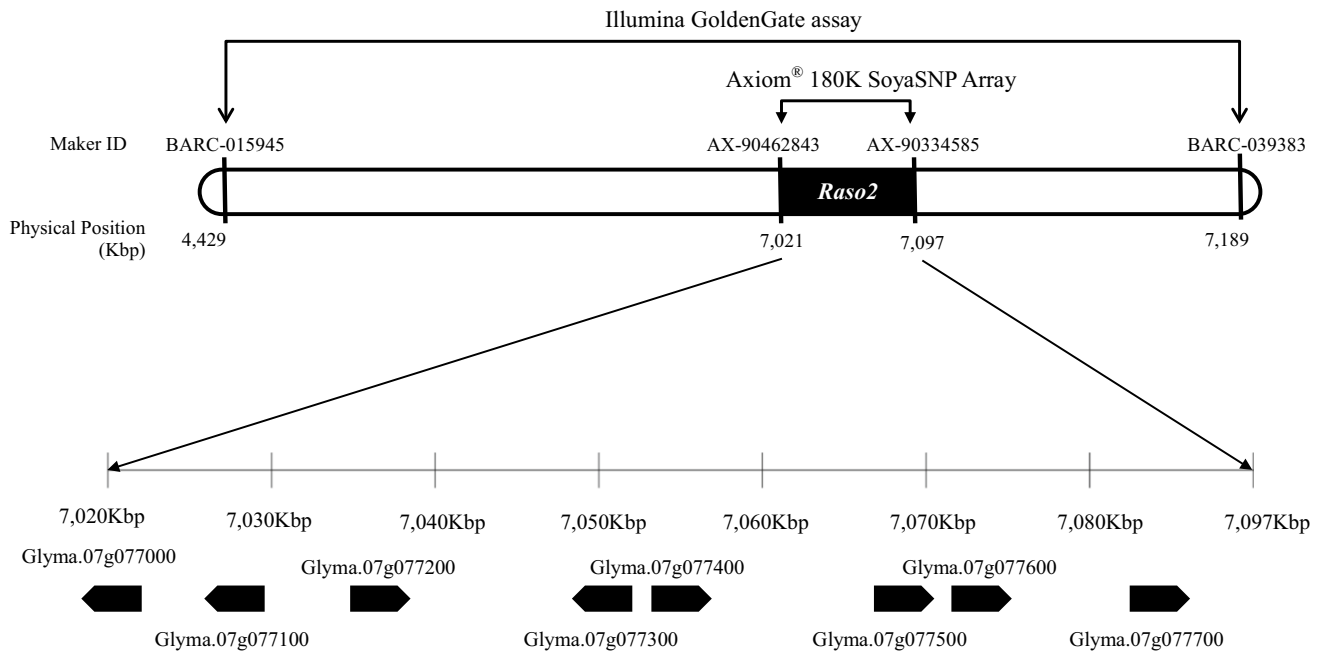

Fig. 3 Annotated gene models within 77 kb of *Raso2 region* based on the Williams 82 genome assembly (Wm82.a2.v1). BARC markers from GoldenGate® assay (Lee et al. 2015a, b) and AX markers from Axiom® 180 k SoyaSNP array (present study) on soybean chromosome 7

of *Glyma.07g077700* between Williams 82 and PI 366121 was detected at 0 and 24 h after inoculation. However, the expression level of the gene was dramatically increased 48 h after inoculation in PI 366121 and the expression level between Williams 82 and PI 366121 was significantly different (Fig. 4; $P < 0.0001$).

Table 2 Significant region for the four types of resistances to foxglove aphid in the first population using inclusive composite interval mapping

	Trait	Chr[c]	Position (cM)	Left marker	Right marker	LOD[d]	PVE (%)[e]	Add[f]
Choice test	PLD[a]	7	73.5	AX-90334585	AX-90462843	9.5	35.3	0.9
	TPD[b]	7	73.5	AX-90334585	AX-90462843	7.2	28.5	1.5
Nonchoice test	PLD	7	73.5	AX-90334585	AX-90462843	5.9	21.5	1.1
	TPD	7	73.5	AX-90334585	AX-90462843	4.3	20.2	1.1

[a]Primary leaf damage

[b]Total plant damage

[c]Chromosome

[d]Log of odds

[e]Phenotypic variance explained

[f]Additive effect

Table 3 Eight anotated gene models in *Raso2* region and SNPs in the exon of the gene models between Williams 82 and PI 366121

ID	Gene	Protein function	SNP information	Type of amino acid substitution	AA change
SNP1-1	*Glyma.07g077000*	Methyltransferase	TCCA[C/T]GTGG	Synonymous	–
SNP1-2			AGGC[C/G]GGTC	Synonymous	–
	Glyma.07g077100	Signalosome	–	–	–
	Glyma.07g077200	FAS-Associated protein	–	–	–
	Glyma.07g077300	ARA4-INTERACTING protein	–	–	–
SNP2-1	*Glyma.07g077400*	Methyltransferase	ACCT[T/G]CCTC	Synonymous	–
SNP2-2			TTCC[C/A]CGTG	Synonymous	–
	Glyma.07g077500	–	–	–	–
	Glyma.07g077600	Phloemprotein	–	–	–
SNP3-1	*Glyma.07g077700*	NB-ARC domain	CATC[T/A]GTTC	Nonsynonymous	Leu/Gln
SNP3-2			GTAG[T/C]TTGA	Synonymous	–
SNP3-3			ATTA[T/G]ATCT	Nonsynonymous	Ileu /Arg
SNP3-4			AGAT[A/C]AATT	Nonsynonymous	Lys/Gln
SNP3-5			ATTA[C/T]ATGT	Nonsynonymous	His/Tyr
SNP3-6			TGTG[C/G]TAGA	Nonsynonymous	Leu/Val
SNP3-7			TTGC[C/T]CAAA	Nonsynonymous	Pro/Ser
SNP3-8			CAGA[C/A]AACC	Nonsynonymous	Gly/Lys
SNP3-9			ACTT[G/A]TCAA	Synonymous	–
SNP3-10			GAAG[T/G]GTCG	Nonsynonymous	Ser/Arg
SNP3-11			CTTC[G/C]ACGA	Nonsynonymous	Asp/His
SNP3-12			TGCA[G/T]AGTC	Nonsynonymous	Gln/His

Table 4 Chi-square analysis of the segregation of foxglove aphid resistance in 41 F_3 recombinant inbred lines from the second population

Observed			Expected ratio			χ^2	P
R[a]	H	S	R	H	S		
12	21	8	1	2	1	0.805	0.67

[a]'R' when all tested plants from the line presented resistance to FA; homozygous for the allele of the resistant parent (PI 366121), 'H' when tested plants from the line presented both resistance and susceptibility to FA; heterozygous, and 'S' when all tested plants from the line presented susceptibility to FA; homozygous for the allele of the susceptible parent (PI 483463)
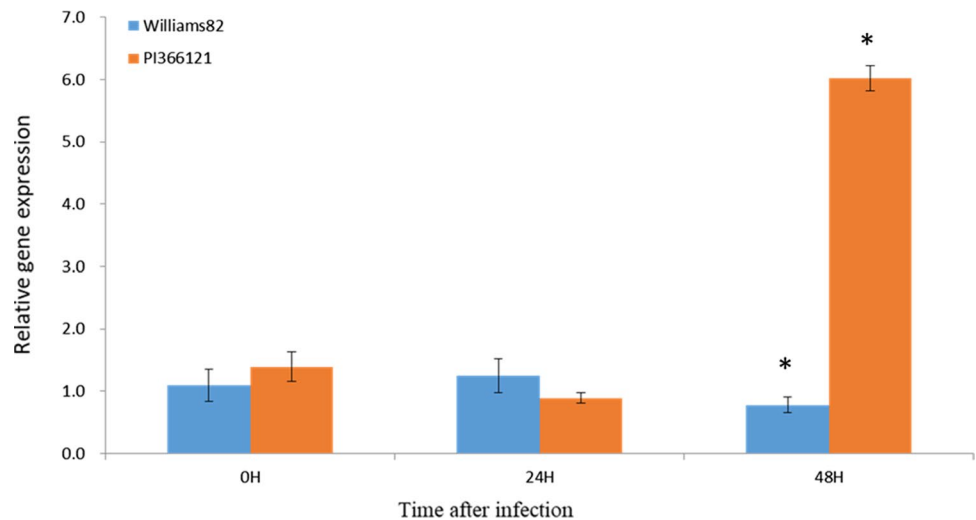
To confirm the sequence variations within the candidate gene, *Glyma.07g077700*, identified from the first population, exon regions of the candidate gene were re-sequenced and compared between PI 366121 and 26 soybean germplasm. Several FA-susceptible germplasms had the same alleles as PI 366121 in the SNP 3–1, 3–9, 3–10, 3–11, and 3–12 (Table 5). Therefore, it could be assumed that the allele substitutions in above SNPs might be not associated with the FA resistance from PI 366121. In remaining SNPs, all susceptible germplasm had a different allele from PI 366121. The SNPs 3–2, 3–3, 3–4, 3–5, 3–6, 3–7, and 3–8 located between 7,093,145 and 7,096,564 bp on soybean chromosome 7 presented a distinct allele between the FA-resistant source and the FA-susceptible germplasm. All FA-susceptible germplasm had the different alleles from PI 366121 at the seven loci. Expected amino acid replacements by the SNPs were listed in Table 5. However, the SNP 3–2 was synonymous type and could not cause an amino acid replacement. Therefore, six SNPs within exon 5 only presented unique variation for PI 366121 and amino acid replacements. Based on the SNPs, Cheongja had the same alleles as other FA-susceptible germplasm. Therefore, it is likely that resistant gene(s) from PI 366121 and Cheongja are different.

Discussion

The FA resistance gene *Raso2* from PI 366121 was fine-mapped on chromosome 7 in this study. By the high-density genetic linkage map using the 180 K Axiom® SoyaSNP assay, the *Raso2* interval was narrow down from 13 cM (2.2 Mbp) with 275 annotated genes to 1 cM (76 Kb) with eight annotated gene models. Ohnishi et al. (2012) previously mapped the *Raso1* from Adams on chromosome 3. Kim et al. (2010a, b) previously fine-mapped the SA resistance gene *Rag1* from Dowling on

chromosome 7 and one SA resistance gene in PI 587,732 was also mapped on chromosome 7 (Kim et al. 2014). Firstly, we confirmed that *Raso1* from Adams and *Raso2* from PI 366121 were different FA resistance genes because two genes were mapped on different chromosome as well as Adams presented susceptible reaction to the Korean FA biotype (Lee et al. 2015a). In the case of SA resistance genes mapped on chromosome 7, physical positions of the SA resistance genes and *Raso2* were quite different. The SA resistance genes from Dowling and PI 587,732 were mapped on 43 Mb regions, while *Raso2* from PI 366121 was positioned on 7 Mb regions on chromosome 7 (https://soybase.org/). Other reported insect resistance genes were also physically far from *Raso2*. Considered quite distances in the physical locations on the Williams 82 genome assembly (Wm82.a2.v1), it is likely that the *Raso2* is different gene from previously reported insect resistance genes although they were mapped on the same chromosome.

Our mapping efforts were greatly accelerated by the availability of the high-density SNP array and the public sequence of the soybean genome. Lee et al. (2015a) previously mapped *Raso2* region using the GoldenGate® assay containing 1,536 SNP loci but only 414 SNPs were actually used to map the gene. In the present study, the 180 K Axiom® SoyaSNP array was used to genotype the same population and 28,752 SNPs were used to fine-map *Raso2* region. The average distance between SNP markers mapped in the present study was approximately 0.15 cM as increased 45 times than the previous study (6.8 cM) and they sufficiently covered all genomic regions of soybean (Fig. 1, Table 1). In addition to high-density SNP markers, the use of precisely tested phenotypic data from the choice and non-choice tests could also play pivotal role in fine-mapping the gene and evaluating effects of the gene to the tested phenotypes. High-depth re-sequencing information on PI 366121

Table 5 Single-nucleotide polymorphisms within *Glyma.07g077700* in diverse soybean germplasm

Location	Exon 1	Exon 4	Exon 5						Exon 6		Exon 7	
Name	SNP3-1	SNP3-2	SNP3-3	SNP3-4	SNP3-5	SNP3-6	SNP3-7	SNP3-8	SNP3-9	SNP3-10	SNP3-11	SNP3-12
Position (bp)	7,086,216	7,093,145	7,096,343	7,096,510	7,096,516	7,096,522	7,096,531	7,096,564	7,097,322	7,097,487	7,097,497	7,100,004
AA change	Leu/Gln	–	Ileu /Arg	Lys/Gln	His/Tyr	Leu/Val	Pro/Ser	Gly/Lys	–	Ser/Arg	Asp/His	Gln/His
PI 366121[a]	A	C	G	C	T	G	T	A	A	G	C	T
Williams82[b]	T	T	T	A	C	C	C	C	G	T	G	G
PI 96983[b]	T	T	T	A	C	C	C	C	A	G	C	T
Galchae[b]	T	T	T	A	C	C	C	C	G	T	G	T
Danbaegkong[b]	T	T	T	A	C	C	C	C	G	T	G	G
Daewonkong[b]	T	T	T	A	C	C	C	C	G	T	G	T
Daepung[b]	T	T	T	A	C	C	C	C	G	T	G	G
Dae-heug[b]	T	T	T	A	C	C	C	C	A	G	C	T
Bangsa[b]	T	T	T	A	C	C	C	C	G	T	G	G
Saedanbaek[b]	A	T	T	A	C	C	C	C	G	G	C	T
Somyeongkong[b]	T	T	T	A	C	C	C	C	G	T	G	G
Sohokong[b]	T	T	T	A	C	C	C	C	G	T	G	G
Shinhwa[b]	T	T	T	A	C	C	C	C	G	T	G	G
Yongpoong[b]	T	T	T	A	C	C	C	C	A	G	C	T
Uram[b]	A	T	T	A	C	C	C	C	G	G	C	T
Ilpumgeomjeong[b]	T	T	T	A	C	C	C	C	A	G	C	T
Josaengseori[b]	T	T	T	A	C	C	C	C	G	T	G	G
Chunal[b]	T	T	T	A	C	C	C	C	G	T	G	G
Cheongjakong[b]	T	T	T	A	C	C	C	C	G	T	G	G
Cheongja[a]	T	T	T	A	C	C	C	C	G	T	G	G
Taekwangkong[b]	A	T	T	A	C	C	C	C	A	G	C	T
Pureunkong[b]	T	T	T	A	C	C	C	C	G	T	G	G
Pungwon[b]	T	T	T	A	C	C	C	C	G	T	G	T
Hannamkong[b]	T	T	T	A	C	C	C	C	G	T	G	G
Haman[b]	T	T	T	A	C	C	C	C	G	T	G	G
Hwangkeum[b]	A	T	T	A	C	C	C	C	G	G	C	T
Heugcheong[b]	T	T	T	A	C	C	C	C	G	T	G	G

[a] Resistant to FA

[b] Susceptible to FA

and diverse soybean germplasm also play important role in identifying sequence variations within the candidate gene which might be associated with the resistance to FA. Therefore, for successful fine-mapping identifying candidate gene(s) of target gene, use of accurately evaluated diverse phenotypes, high-density genetic map, and diverse soybean germplasm would be essential.

Based on the Williams 82 genome assembly (Wm82. a2.v1) which was the FA-susceptible parent in the first population, the current gene annotation of the 76 Kb region containing the *Raso2* predicts the presence of eight annotated genes. Of these genes, *Glyma.07g077700* is the only gene encoding disease resistance protein with leucine-rich repeat (LRR) domain. *Glyma.07g077700* is a homolog gene of *AT4G26090.1* in Arabidopsis which is a member of the *RPS2* gene family encoding a nucleotide-binding domain shared with *APAF1*, R gene products and *CED4* (NB-ARC) protein that confers to disease resistance (https://soybase.org/, https://www.arabidopsis.org/). Based on the legume information system (https://legumeinfo.org/), the gene has significant homology with *Vradi0292s00010* encoding disease resistance protein [coiled-coil (CC)–nucleotide-binding site (NBS)–LRR class] in mung bean (*Vigna radiate*). The majority of cloned resistance genes are members of the NBS-LRR gene family. Cloned NBS-LRR genes that confer aphid resistances include the Mi for resistance to potato aphid (*Macrosiphum euphorbiae*) in tomato (*Lycopersion esculentum* Mill.) (Rossi et al. 1998) and the Vat for resistance to *A. gossypii* in melon (*Cucumis melo* L.) (Dogimont et al. 2009). In the case of soybean aphid resistance, *Rag1* (Kim et al. 2010a) and *Rag2* (Kim et al. 2010b), *Rag6* and *Rag3c* (Zhang et al. 2017b) were fine-mapped to a NBS-LRR cluster region, respectively. In *Medicago truncatula*, resistance genes to bluegreen aphid (*Acyrthosiphon kondoi* S.) and pea aphid (*Acyrthosiphon pisum* H.) were also mapped in a NBS-LRR cluster on different chromosomes, respectively (Klingler et al. 2005; Kamphuis et al. 2016). Resistance genes to green peach aphid (*Myzus persicae* S.) in pepper (Capsicum spp.) and rosy apple aphid (*Dysaphis plantaginea* P.) in apple (*Malus* x *domestica*) were fine-mapped a locus containing NBS-LRR genes (Sun et al. 2020; Pagliarani et al. 2016). Most R proteins contain leucine-rich repeats (LRRs), a central NBS, and a variable amino-terminal domain (Takken et al. 2006). The LRRs are mainly involved in recognition, whereas the amino-terminal domain determines signaling specificity. It has been reported that the NBS forms part of a nucleotide-binding (NB)–ARC domain that presumably functions as a molecular switch. The distinctly expression of *Glyma.07g077700* in PI 366121 at 48 h after FA inoculation might indicate that the high expression of the gene was associated with the resistance to FA. Further studies would be needed to ensure the relationship between the FA resistance and expression levels of *Glyma.07g077700* in diverse soybean germplasm or transgenic plants.

The high-resolution genetic map and SNP markers identified in the present study will facilitate MAS of *Raso2* and pyramiding of *Raso2* with other target genes on chromosome 7 because of their very close proximity to the gene. In addition, the identification of the precise physical position of *Raso2* region and the candidate gene (*Glyma.07g077700)* on the soybean chromosome 7 could greatly facilitate the cloning and functional characterization of the gene. The cloning of *Raso2* might improve understanding of FA defense mechanism in soybean as well as other host species of FA. This information could be applied to compare the function of this gene to other aphid resistance genes such as *Raso1, Rag1, Rag2,* or cloned insect resistance genes in other species. We are conducting ongoing studies to clone *Raso2*. Transformation of *Glyma.07g077700* from PI 366121 to Willams82 or other transformation recipient lines as well as genome editing of *Glyma.07g077700* in Williams 82 according to the SNP information within the candidate gene might be needed to investigate functions of the candidate gene and interaction of the gene with other chromosome regions.

Author contribution statement KSK and JMK contributed equally to this research. KSK outlined, wrote the first draft of the manuscript, revised, and finalized the manuscript; JMK performed evaluation of FA resistance, analyzed the data, and wrote M&M, figures, and tables in the first manuscript; JJ, IS, and SP performed experiments; SCJ and JSL analyzed the genotypic data; JDL provided the second soybean population; JKJ provided the Foxglove aphid; BKH performed the gene expression study; and SK acquired funding and supervised the study and finalized the manuscript. All authors read and approved the final manuscript.

Declarations

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

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