Genetic Mapping of a Resistance Locus to *Phytophthora sojae* in the Korean Soybean Cultivar Daewon

Ik-Hyun Jang¹, In Jeong Kang², Ji-Min Kim³, Sung-Taeg Kang³, Young Eun Jang¹, and Sungwoo Lee ^{1*}

¹Department of Crop Science, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 34134, Korea

²Department of Central Area Crop Science, National Institute of Crop Science, Suwon 16613, Korea

³Department of Crop Science and Biotechnology, College of Bioresource Science, Dankook University, Cheonan 31116, Korea

(Received on September 7, 2020; Revised on October 16, 2020; Accepted on October 26, 2020)

Phytophthora root and stem rot reduce soybean yields worldwide. The use of *R*-gene type resistance is currently crucial for protecting soybean production. The present study aimed to identify the genomic location of a gene conferring resistance to *Phytophthora sojae* isolate 2457 in the recombinant inbred line population developed by a cross of Daepung × Daewon. Single-marker analysis identified 20 single nucleotide polymorphisms associated with resistance to the *P. sojae* isolate 2457, which explained ~67% of phenotypic variance. Daewon contributed a resistance allele for the locus. This region is a well-known location for *Rps1* and *Rps7*. The present study is the first, however, to identify an *Rps* gene locus from a major soybean variety cultivated in South Korea. Linkage analysis also identified a 573 kb region on chromosome 3 with high significance (logarithm of odds = 13.7). This genomic region was not further narrowed down due to lack of recombinants within the interval. Based on the latest soybean genome, ten leucine-rich repeat coding genes and four serine/threonine protein kinase-coding genes are annotated in this region, which all are well-known types of genes

for conferring disease resistance in crops. These genes would be candidates for molecular characterization of the resistance in further studies. The identified *R*-gene locus would be useful in developing *P. sojae* resistant varieties in the future. The results of the present study provide foundational knowledge for researchers who are interested in soybean–*P. sojae* interaction.

Keywords : high-resolution genetic mapping, nucleotide-binding site-leucine rich repeat (NBS-LRR), *Phytophthora sojae*, plant resistance, soybean

Handling Editor : Inhwa Yeam

Phytophthora sojae Kauffmann and Gerdemann is an oomycete pathogen that causes destructive root and stem rot in susceptible soybean plants [*Glycine max* (L.) Merr.]. From 1996 to 2014, about 25 million tons were lost annually due to *Phytophthora* root and stem rot (PRR), with an average annual loss of more than 1.1 million tons (Allen et al., 2017; Wrather and Koenning, 2006, 2009; Wrather et al., 2001).

In wet soil, zoospores of *P. sojae* are attracted by exudates released from soybean roots. After reaching a root surface, the zoospores attach and transform into a cyst that germinates to produce a hypha that penetrates the plant within a few hours of inoculation (Morris and Ward, 1992). *P. sojae* colonizes root and stem tissues as reproducing oospores. Seedling damping-off occurs at early stages of susceptible varieties, whereas root and stem rot, leaf yellowing, and wilting typically develop at later stages of growth followed by plant death (Schmitthenner, 1985).

Soybean varieties carrying an effective resistance (*R*)

*Corresponding author.

Phone) +82-42-821-5727, FAX) +82-42-822-2631

E-mail) sungwoolee@cnu.ac.kr

ORCID

Sungwoo Lee

https://orcid.org/0000-0003-3564-236

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

gene for defending against *P. sojae* isolates carrying an avirulence (*Avr*) gene rapidly develop a hypersensitive response within hours after infection (Staskawicz et al., 1995). *R*-gene resistance can provide effective protection from *P. sojae* and is widely used in developing resistant varieties in several major soybean growing countries such as the US, China, Brazil, and Japan (Dorrance, 2018). Jang and Lee summarized a comprehensive list of *Rps* (resistance to *P. sojae*) documented in previous studies. To date, 34 allele have been mapped to nine loci: *Rps1a*, *1b*, *1c*, *1d*, *1k*, *7*, *9*, *UN1*, *YU25*, *YD29*, *HN*, *Q*, *WY*, *HC18*, *X*, and *Rps* gene in the Waseshiroge soybean cultivar on chromosome 3 (Cheng et al., 2017; Demirbas et al., 2001; Li et al., 2017; Lin et al., 2013; Niu et al., 2017; Sugimoto et al., 2007, 2011; Sun et al., 2011; Weng et al., 2001; Wu et al., 2011a; Zhang et al., 2013b; Zhong et al., 2017, 2019); *Rps3a*, *3b*, *3c*, *8*, and *SN10* on chromosome 13 (Burnham et al., 2003; Gordon et al., 2006, 2007; Yu et al., 2010); *Rps4*, *5*, *6*, *12*, and *JS* on chromosome 18 (Gordon et al., 2007; Sahoo et al., 2017; Sandhu et al., 2004; Sun et al., 2014); *Rps2* and *UN2* on chromosome 16 (Demirbas et al., 2001; Huang et al., 2016; Lin et al., 2013); *RpsZS18* on chromosome 2 (Yao et al., 2010); *Rps11* on chromosome 7 (Ping et al., 2015); *RpsSU* on chromosome 10 (Wu et al., 2011b); *Rps10* on chromosome 17 (Zhang et al., 2013a); and *RpsYB30* on chromosome 19 (Zhu et al., 2007).

PRR was first identified two decades ago in South Korea (Jee et al., 1998). PRR began to be seriously considered as an issue as soybean cultivation in paddy soil increased (Ministry of Agriculture, Food and Rural Affairs, 2019). Paddy soils are usually wet because of poor drainage. This condition usually facilitates infection by soil-borne pathogens such as *P. sojae* or *Fusarium* spp. (Dorrance, 2018; Schmitthenner, 1985). Especially, a few-week period after soybean planting, there is heavy and frequent rain in June to July; 163 and 289 mm in 30-year average (1981-2010) according to the Annual Climatological Report of Korea (available at http://book.kma.go.kr/ebook/ebView.asp?eb_no=202006301610443121&r=R), which would make favorable condition for the plant pathogens. As the use of paddy fields for soybean production is increasing year by year, PRR might occur more frequent and severe near future.

PRR has been little studied in S. Korea and interaction between soybean and *P. sojae* in domestic soybean varieties is largely unknown. Kang et al. (2019) examined the presence of *Rps* resistance against four isolates in 20 universal soybean cultivars widely planted in South Korea. Surprisingly, susceptibility was observed in 14-19

of the 20 cultivars. About 50% of the evaluated cultivars were susceptible. Only a few cultivars, such as Daewon, showed resistance to isolates, implying that most cultivars are vulnerable to *P. sojae*. Still, few reports on interactions between domestic soybean varieties and *P. sojae* isolates in S. Korea are available. In the present study, the universal cultivar, Daewon, is first characterized for resistance to *P. sojae* via linkage analysis using a recombinant inbred line (RIL) population that segregates in reactions to *P. sojae* isolate 2457.

Materials and Methods

Plant materials. The mapping population was developed from crosses between female parent Daepung and male parent Daewon (Kim, 2018). Briefly, the first cross for F₁ seeds occurred in 2012. Eleven F₁ seeds were planted in the winter of 2012, and 103 F₂ seeds were collected. Generations of seeds were produced by single-seed descent from F₂ to F₅ by 2015. Seeds of F₅-derived RILs were increased at the university farm of Chungnam National University in 2018. The two parents, Daepung and Daewon, have white flowers, determinate growth habit, and yellow seeds. Daepung and Daewon were recently reported as susceptible and resistant to the *P. sojae* isolate 2457, respectively (Kang et al., 2019). The isolate 2457 was isolated from the cultivar Uram in Gyeongbuk province in 2016 (Kang et al., 2019).

Evaluation of resistance to *P. sojae*. The two parents and RILs of the mapping population were examined for resistance to *P. sojae* using hypocotyl inoculation (Dorrance et al., 2004). Briefly, 10 to 15 seedlings were grown in a 13 cm pot filled with soil. An inoculum was prepared on a clarified V8 medium about 10 days before inoculation. V8 medium fully covered by *P. sojae* mycelia was macerated twice using a 50 ml syringe and transferred into a 10 ml syringe. Seven days after sowing, a 1 cm slit was made on hypocotyl of the seedlings, and 0.2 to 0.4 ml of a mycelial slurry was placed in the slit. The inoculated seedlings were placed in dark and humid condition for 16 h. Seedlings were then incubated in a growth chamber (14 h [light]/10 h [dark], temperature 25°C, and humidity >70%) and their reactions were observed 7 days after inoculation. The reaction of each RIL was determined as a percentage of the number of killed to the number of seedlings. Seedlings were resistant (R) if <20% died, susceptible (S) if >80% died, or intermediate (I) if 20 to 80% died. This experiment was replicated three times.

DNA extraction and single nucleotide polymorphism (SNP) genotyping.

Genomic DNA of parents and RILs was extracted using cetyl trimethyl ammonium bromide as described by Doyle and Doyle (1987). Samples were genotyped with Axiom 180 K SoyaSNP arrays (Affymetrix, Santa Clara, CA, USA) (Lee et al., 2015). SNP data were preprocessed as follows. Beginning with 169,028 SNPs obtained from the array, 143,083 undefined or non-polymorphic SNPs between the two parents were removed. Additional 184 and 335 SNPs were eliminated because of $\geq 10\%$ missing values and $\geq 10\%$ heterozygosity, respectively. Two RILs were also excluded because of $\geq 10\%$ missing values.

Goodness-of-fit test and single-marker analysis of variance. A goodness-of-fit to Mendelian segregation ratios was calculated using chi-square (χ^2) analysis to examine patterns of the phenotypes in mapping populations.

Marker-trait association was analyzed via a single-marker analysis of variance (ANOVA) using the lme4 package (Bates et al., 2015) in R (R Core Team, 2019). Bonferroni correction ($P < 1.00E-6$) was applied to determine the most stringent threshold to minimize false positiveness in ANOVA results.

Construction of genetic map and linkage analysis.

A genetic map of Daepung $\times$ Daewon population was constructed using a software IciMapping version 4.1 (Meng et al., 2015). First, the function BIN (i.e., Binning) was used to remove redundant markers. Remaining markers were grouped and ordered using the function MAP (i.e., mapping). Linkage analysis then conducted using the function BIP (i.e., Biparental mapping), to confirm marker-trait associations identified by single-marker ANOVA. For quantitative analysis, disease score (i.e., R or S) of an RIL was converted to quantitative form as an average percentage of dead seedlings over three replicates. Additivity was calculated as an allelic effect of Daepung relative to Daewon.

Results

Phenotypic assay for resistance to *P. sojae*. Two parents and 71 RILs of the Daewon $\times$ Daepung population were evaluated for *R*-gene type resistance to *P. sojae* isolate 2457. Daewon and 35 RILs were resistant, whereas Daepung and 24 RILs were susceptible (Table 1, Fig. 1). Four RILs exhibited intermediate resistance with 41-59% dead, and eight RILs were indeterminant because of inconsistent results; consequently, 12 RILs were excluded from analyses.

A goodness-of-fit test demonstrated that segregation ratios were consistent with the Mendelian segregation, suggesting that the trait is qualitatively controlled by a single gene ($\chi^2 = 1.49$, $P = 0.36$). Distributions of phenotypes were discrete with high peaks at both ends, typical of single-gene inheritance.

Fig. 1. Results from parents, Daepung (left) and Daewon (right), observed 7 days after the inoculation of *Phytophthora sojae* isolate 2457.

Table 1. Goodness-of-fit test for segregation ratios of recombinant inbred lines in the Daepung $\times$ Daewon population following inoculation with *Phytophthora sojae* isolate 2457

Parents and RILs	Observed numbers		Expected numbers		Goodness-of-fit	
	R	S	R	S	χ^2	<i>P</i> -value
Daepung (P1)		50				
Daewon (P2)	50					
Daepung $\times$ Daewon (RILs)	35	24	29.5	29.5	1.49	0.36

RIL, recombinant inbred line; R, resistant reaction; S, susceptible reaction.

Table 2. Twenty SNP significantly associated with resistance to *Phytophthora sojae* isolate 2457

Chr ^a	Position (bp) ^a	SNP	Daepung allele (S)	Daewon allele (R)	Adjusted <i>P</i> -value ^b	<i>R</i> ^{2c}
3	3,893,390	AX-90498561	GG	AA	6.53E-08	0.57
3	3,904,817	AX-90375813	GG	AA	6.53E-08	0.57
3	3,964,789	AX-90397660	CC	TT	1.54E-07	0.55
3	3,990,383	AX-90360753	GG	AA	6.53E-08	0.57
3	4,200,520	AX-90503215	AA	GG	8.76E-11	0.65
3	4,325,664	AX-90337768	GG	AA	4.42E-11	0.66
3	4,328,363	AX-90385376	GG	AA	4.16E-11	0.66
3	4,348,187	AX-90349616	CC	TT	1.68E-11	0.67
3	4,355,566	AX-90402524	CC	TT	1.68E-11	0.67
3	4,405,356	AX-90504759	AA	TT	1.09E-10	0.65
3	4,414,031	AX-90306896	CC	TT	1.09E-10	0.65
3	4,443,110	AX-90508997	TT	CC	1.10E-10	0.65
3	4,454,572	AX-90326515	AA	CC	4.42E-11	0.66
3	4,466,635	AX-90456732	AA	CC	1.09E-10	0.65
3	4,639,308	AX-90314290	AA	TT	5.21E-08	0.57
3	4,642,708	AX-90351666	GG	AA	1.01E-07	0.56
3	4,642,893	AX-90393523	CC	TT	5.21E-08	0.57
3	4,717,222	AX-90509372	CC	TT	2.60E-08	0.58
3	4,752,915	AX-90511516	TT	CC	2.60E-08	0.58
3	4,752,969	AX-90501677	AA	GG	5.22E-08	0.57

SNP, single nucleotide polymorphism; Chr, chromosome; R, resistant reaction; S, susceptible reaction.

^aPhysical positions are based on soybean genome Glyma2 (<http://soybase.org>).

^bAdjusted *P*-value was obtained using the Bonferroni correction ($P < 1.00E-7$).

^cPhenotypic variance explained by the marker.

Single-marker ANOVA. A total of 25,761 SNPs was analyzed to find significant marker–trait associations. ANOVA identified 20 highly significant SNPs located in an 859 kb region on chromosome 3, for which *P*-values ranged from 1.01E-7 to 1.68E-11 (Table 2). The estimated *R*² (%) ranged from 57 to 67, indicating that the locus would be a major gene controlling the resistance (Table 2).

Genetic mapping of a locus associated with resistance to *P. sojae*. An alternative analysis was used to confirm strong associations of the genomic region with resistance to *P. sojae*. Over 25,000 SNPs were reduced to 6,364 SNPs by binning, which subsequently were used to construct genetic maps (Table 3). A total of 263 SNPs were integrated on chromosome 3 with the total genetic length of 87.3 cM (Table 3).

Linkage analysis narrowed down to a 573 kb region (3,893,390 to 4,752,969 bp) from the 859 kb interval identified from the single-marker ANOVA, where 46 genes are annotated based on the latest soybean reference genome Glyma2 (<http://soybase.org>). The 573 kb region on chro-

sosome 3 was detected with high significance (logarithm of odds = 13.7), explaining 66.4% of phenotypic variance (Table 4, Fig. 2). The positive additive effect indicates that the percent of dead seedlings was higher in RILs with the Daepung allele than in those with the Daewon allele for identified SNPs, indicating that Daepung provides susceptibility alleles (Table 4).

Discussion

In the present study, Phenotypic variation among RILs in the Daepung × Daewon RIL population was evaluated for *R*-gene mediated resistance to *P. sojae* isolate 2457. A genomic region significantly associated with the resistance, containing multiple *Rps* genes, was identified via single-marker ANOVA and linkage analysis. This report is the first to characterize a major soybean variety cultivated in S. Korea for the resistance to *P. sojae*.

Single-marker ANOVA and linkage analysis identified a locus with high levels of statistical significance on chromosome 3 (Table 3, Fig. 2). Since no other region was

Table 3. Information of the genetic map of Daepung × Daewon population

Chr	Total length (cM) (a)	No. of total SNP markers	No. of unique loci (b) ^a	Avg. marker interval (cM) (a/b) ^b
1	112.1	336	77	1.5
2	34.5	215	37	0.9
3	87.3	263	62	1.4
4	90.2	338	54	1.7
5	97.7	348	78	1.3
6	122.1	419	80	1.5
7	83.3	421	68	1.2
8	130.8	325	86	1.5
9	85.2	422	77	1.1
10	90.8	274	43	2.1
11	82.5	251	56	1.5
12	19.1	95	14	1.4
13	112.5	381	77	1.5
14	87.7	341	73	1.2
15	100.5	374	61	1.6
16	92.3	378	60	1.5
17	127.3	269	72	1.8
18	71.6	394	66	1.1
19	59.2	262	40	1.5
20	88.9	258	66	1.3

Chr, chromosome; SNP, single nucleotide polymorphism

^aActual number of loci where markers are positioned in the genetic map.^bAverage interval between loci.

identified from ANOVA and linkage analysis, resistance is apparently conditioned on the expression of a gene or a few in this single locus. The identified region partially overlaps or is in close proximity to genomic regions where over ten *Rps* alleles were reported, including five *RpsI* alleles—*RpsIa*, *RpsIb*, *RpsIc*, *RpsId*, and *RpsIk*—and *Rps7*, *Rps9*, *RpsHC18*, *RpsHN*, and *Rps* of cv. Waseshiroge, *RpsQ*, *RpsUNI*, *RpsYD29*, *RpsYU25*, *RpsWY*, and *RpsX* (Fig. 3) (Cheng et al., 2017; Demirbas et al., 2001; Li et al., 2017; Lin et al., 2013; Niu et al., 2017; Sugimoto et al., 2007, 2011; Sun et al., 2011; Weng et al., 2001;

Wu et al., 2011a; Zhang et al., 2013b; Zhong et al., 2017, 2019). Since no RILs display recombination within the ~573 kb marker interval, the target resistance locus could not be further narrowed down in the genome. The *Rps* of Waseshiroge, *RpsYD29*, *RpsHN*, and *RpsHC18* mostly overlap in the identified interval of the present study (Fig. 3). An *Rps* gene locus of the cv. Waseshiroge mapped between Satt009 and T0003044871 (3,931,955–4,486,048 bp) (Sugimoto et al., 2011) that largely overlaps the interval identified in the present study, whereas *RpsYD29* (Zhang et al., 2013b) and *RpsHC18* (Zhong et al., 2017) are located at the ends of the interval, flanked between SattWM82-50 and Satt1k4b (3,857,715–4,062,474 bp) and between BARCSOYSSR_03_0269 and BARCSOYSSR_03_0272 (4,514,938–4,611,259 bp), respectively.

The gene encoding nucleotide-binding site-leucine rich repeat (NBS-LRR) protein is a well-known category of resistance gene in plants. In soybean–*P. sojae* interactions, *RpsIk* is best characterized and codes for a type of NBS-LRR protein (Gao and Bhattacharyya, 2008). Additionally, previous studies of soybean–*P. sojae* interactions report genes encoding NBS-LRR protein as a candidate gene (Li et al., 2017; Niu et al., 2017; Zhang et al., 2013b; Zhong et al., 2017). The identified locus was further examined with the latest gene annotation, i.e., Glyma.Wm82.a2.v1, at Soybase (<http://soybase.org>) (accessed September 1, 2020). A total of ten LRR type genes were found in the genomic region (3,893,390–4,642,893 bp), including Glyma.03g034200, Glyma.03g034400, Glyma.03g034500, Glyma.03g034800, Glyma.03g034900, Glyma.03g035300, Glyma.03g037000, Glyma.03g037100, Glyma.03g037300, and Glyma.03g037400. These genes would be primary candidate genes for future studies of resistance to *P. sojae* isolate 2457 in Daewon.

Serine/threonine protein kinase (STK) gene is another type of plant resistance gene (Zhong et al., 2019). Several STK genes are responsible for disease resistance in plants, including *Xa21*, *Xa21D*, and *Xa3/Xa26* for bacterial blight in rice; *Lr10* for wheat leaf rust; and *Rvi12_Cd5* for apple scab (Feuillet et al., 1997; Padmarasu et al., 2018; Song et al., 1995; Sun et al., 2004; Wang et al., 1998). In soy-

Table 4. A genomic region identified for resistance to *Phytophthora sojae* isolate 2457 in the Daepung × Daewon RIL population

Chr	Position (bp) ^a	Flanking markers	LOD ^b	PVE (%) ^b	<i>a</i> ^c
3	3,893,390 to 4,466,635	AX-90498561 to AX-90456732	13.7	66.4	40.1

RIL, recombinant inbred line; Chr, chromosome; LOD, logarithm of odds.

^aPhysical position in bp based on the genome version Glyma2.^bPhenotypic variance (%) explained (PVE) by this locus.^cAdditive effect. The positive value indicates that Daewon provides the resistance allele.

Fig. 2. Graphical presentation of *Rps* locus associated with resistance to *Phytophthora sojae* isolate 2457 in the Daepung × Daewon recombinant inbred line population. The genetic map is presented with a plot of logarithm of odds (LOD) score from linkage analysis with quantitative scales. Hatched lines on the LOD plots indicate the LOD threshold. The 1- and 2-LOD intervals are displayed as black bars and solid lines, respectively.

Fig. 3. A schematic diagram of the identified *Rps* region and annotated gene models (Glyma.Wm82.a2.v1). Selective known *Rps* genes overlapping this locus are presented by arrows, indicating marker intervals of them as previously reported. The dashed part of the interval presents outside of 6.0 Mbp. Ten leucine rich repeat- and four serine/threonine protein kinase-coding genes annotated in this genomic region (3.8 to 4.6 Mbp) are displayed at the bottom.

bean-*P. sojae* interactions, Zhang et al. (2013a) mapped *Rps10* on chromosome 17 and reported Glyma17g28950 and Glyma17g28970 (i.e., Glyma.17g196200 and Glyma.17g192300 in Glyma.Wm82.a2.v1, respectively) as candidate genes for *Rps10*. These two genes were predicted to encode plant-type Ser/Thr protein kinase, suggesting that the mechanism of resistance mediated by *Rps10* might differ from *Rps1*, *Rps2*, and *Rps4* that represent NBS-LRR genes. Four STK-coding genes are annotated in the identified genomic region of the present study, namely, Glyma.03g036000, Glyma.03g036500, Glyma.03g036900, and Glyma.03g037200. These are also strong candidate genes for resistance to *P. sojae* isolate 2457 and should be further examined.

In summary, Daewon was confirmed as a genetic source to *P. sojae* isolate 2457 and the 573 kb region of chromosome 3 was identified via linkage analysis. There were at least 14 annotated genes associated with disease resistance found in this interval, thus these genes in Daewon will need to be investigated to advance our understanding in further study. It is also unknown whether the resistance allele of Daewon is a novel gene or an alternative form of *Rps1*, *Rps7*, or other *Rps* genes previously reported in the several resistance sources. Investigation of allelic variation or relationship in this locus among the resistance sources

will be of interest in further work. This genomic region can be used to improve resistance to *P. sojae* of major soybean varieties in breeding programs in S. Korea and the understanding from the present study will provide fundamental knowledges for future studies of molecular interaction between soybean and *P. sojae*.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

The present study was funded by the Next-Generation Bio-Green 21 Program (Project No. PJ01333701; Title: Identification of resistance to *Phytophthora sojae* in the Korean soybean core collection and NAM population), Rural Development Administration, South Korea.

References

- Allen, T. W., Bradley, C. A., Sisson, A. J., Byamukama, E., Chilvers, M. I., Coker, C. M., Collins, A. A., Damiconi, J. P., Dorrance, A. E., Dufault, N. S., Esker, P. D., Faske, T. R., Giesler, L. J., Grybauskas, A. P., Hershman, D. E., Hollier, C. A., Isakeit, T., Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koenning, S. R., Kurle, J. E., Malvick, D. K., Markell, S. G., Mehl, H. L., Mueller, D. S., Mueller, J. D., Mulrooney, R. P., Nelson, B. D., Newman, M. A., Osborne, L., Overstreet, C., Padgett, G. B., Phipps, P. M., Price, P. P., Sikora, E. J., Smith, D. L., Spurlock, T. N., Tande, C. A., Tenuta, A. U., Wise, K. A. and Wrather, J. A. 2017. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2010 to 2014. *Plant Health Prog.* 18:19-27.
- Bates, D., Mächler, M., Bolker, B. and Walker, S. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67:1-48.
- Burnham, K. D., Dorrance, A. E., Francis, D. M., Fioritto, R. J. and St Martin, S. K. 2003. *Rps 8*, a new locus in soybean for resistance to *Phytophthora sojae*. *Crop Sci.* 43:101-105.
- Cheng, Y., Ma, Q., Ren, H., Xia, Q., Song, E., Tan, Z., Li, S., Zhang, G. and Nian, H. 2017. Fine mapping of a *Phytophthora*-resistance gene *RpsWY* in soybean (*Glycine max* L.) by high-throughput genome-wide sequencing. *Theor. Appl. Genet.* 130:1041-1051.
- Demirbas, A., Rector, B. G., Lohnes, D. G., Fioritto, R. J., Graef, G. L., Cregan, P. B., Shoemaker, R. C. and Specht, J. E. 2001. Simple sequence repeat markers linked to the soybean *Rps* genes for *Phytophthora* resistance. *Crop Sci.* 41:1220-1227.
- Dorrance, A. E. 2018. Management of *Phytophthora sojae* of soybean: a review and future perspectives. *Can. J. Plant Pathol.* 40:210-219.

- Dorrance, A. E., Jia, H. and Abney, T. S. 2004. Evaluation of soybean differentials for their interaction with *Phytophthora sojae*. *Plant Health Prog.* 5:9.
- Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11-15.
- Feuillet, C., Schachermayr, G. and Keller, B. 1997. Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. *Plant J.* 11:45-52.
- Gao, H. and Bhattacharyya, M. K. 2008. The soybean-*Phytophthora* resistance locus *Rps1-k* encompasses coiled coil-nucleotide binding-leucine rich repeat-like genes and repetitive sequences. *BMC Plant Biol.* 8:29.
- Gordon, S. G., Kowitwanich, K., Pipatpongpinoy, W., St. Martin, S. K. and Dorrance, A. E. 2007. Molecular marker analysis of soybean plant introductions with resistance to *Phytophthora sojae*. *Phytopathology* 97:113-118.
- Gordon, S., St. Martin, S. K. and Dorrance, A. E. 2006. *Rps 8* maps to a resistance gene rich region on soybean molecular linkage group F. *Crop Sci.* 46:168-173.
- Huang, J., Guo, N., Li, Y., Sun, J., Hu, G., Zhang, H., Li, Y., Zhang, X., Zhao, J., Xing, H. and Qiu, L. 2016. Phenotypic evaluation and genetic dissection of resistance to *Phytophthora sojae* in the Chinese soybean mini core collection. *BMC Genet.* 17:85.
- Jang, I.-K. and Lee, S. 2020. A review and perspective on soybean (*Glycine max* L.) breeding for the resistance to *Phytophthora sojae* in Korea. *Plant Breed. Biotechnol.* 8:114-130.
- Jee, H., Kim, W. and Cho, W. 1998. Occurrence of *Phytophthora* root rot on soybean (*Glycine max*) and identification of the causal fungus. *RDA J. Crop Prot.* 40:16-22.
- Kang, I. J., Kang, S., Jang, I. H., Jang, Y. W., Shim, H. K., Heu, S. and Lee, S. 2019. Identification of new isolates of *Phytophthora sojae* and the reactions of Korean soybean cultivars following hypocotyl inoculation. *Plant Pathol. J.* 35:698-704.
- Kim, K. H. 2018. Genome-wide association analysis of flowering time genes with nested association mapping (NAM) population in soybean. M.S. thesis. DanKook University, Cheonan, Korea.
- Lee, Y.-G., Jeong, N., Kim, J. H., Lee, K., Kim, K. H., Pirani, A., Ha, B.-K., Kang, S.-T., Park, B.-S. and Moon, J.-K. 2015. Development, validation and genetic analysis of a large soybean SNP genotyping array. *Plant J.* 81:625-636.
- Li, Y., Sun, S., Zhong, C., Wang, X., Wu, X. and Zhu, Z. 2017. Genetic mapping and development of co-segregating markers of *RpsQ*, which provides resistance to *Phytophthora sojae* in soybean. *Theor. Appl. Genet.* 130:1223-1233.
- Lin, F., Zhao, M., Ping, J., Johnson, A., Zhang, B., Abney, T. S., Hughes, T. J. and Ma, J. 2013. Molecular mapping of two genes conferring resistance to *Phytophthora sojae* in a soybean landrace PI 567139B. *Theor. Appl. Genet.* 126:2177-2185.
- Meng, L., Li, H., Zhang, L. and Wang, J. 2015. QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop J.* 3:269-283.
- Ministry of Agriculture, Food and Rural Affairs. 2019. Crop production area. In: *Agriculture, food and rural affairs statistics yearbook*, ed. by Director Information & Statistics & Policy Division, pp. 64-88. Director-General for Policy Planning Bureau, Sejong, Korea.
- Morris, P. F. and Ward, E. W. B. 1992. Chemoattraction of zoospores of the soybean pathogen, *Phytophthora sojae*, by isoflavones. *Physiol. Mol. Plant Pathol.* 40:17-22.
- Niu, J., Guo, N., Sun, J., Li, L., Cao, Y., Li, S., Huang, J., Zhao, J., Zhao, T. and Xing, H. 2017. Fine mapping of a resistance gene *RpsHN* that controls *Phytophthora sojae* using recombinant inbred lines and secondary populations. *Front. Plant Sci.* 8:538.
- Padmarasu, S., Sargent, D. J., Patocchi, A., Troggo, M., Baldi, P., Linsmith, G., Poles, L., Jansch, M., Kellerhals, M., Tartarini, S. and Velasco, R. 2018. Identification of a leucine-rich repeat receptor-like serine/threonine-protein kinase as a candidate gene for *Rvi12* (Vb)-based apple scab resistance. *Mol. Breed.* 38:73.
- Ping, J., Fitzgerald, J. C., Zhang, C., Lin, F., Bai, Y., Wang, D., Aggarwal, R., Rehman, M., Crasta, O. and Ma, J. 2015. Identification and molecular mapping of *Rps11*, a novel gene conferring resistance to *Phytophthora sojae* in soybean. *Theor. Appl. Genet.* 129:445-451.
- R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Sahoo, D. K., Abeysekara, N. S., Cianzio, S. R., Robertson, A. E. and Bhattacharyya, M. K. 2017. A novel *Phytophthora sojae* resistance *Rps12* gene mapped to a genomic region that contains several *Rps* genes. *PLoS ONE* 12:e0169950.
- Sandhu, D., Gao, H., Cianzio, S. and Bhattacharyya, M. K. 2004. Deletion of a disease resistance nucleotide-binding-site leucine-rich-repeat-like sequence is associated with the loss of the *Phytophthora* resistance gene *Rps4* in soybean. *Genetics* 168:2157-2167.
- Schmitthenner, A. 1985. Problems and progress in control of *Phytophthora* root rot of soybean. *Plant Dis.* 69:362-368.
- Song, W.-Y., Wang, G.-L., Chen, L.-L., Kim, H.-S., Pi, L.-Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.-X., Zhu, L.-H., Fauquet, C. and Ronald, P. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science* 270:1804-1806.
- Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G. and Jones, J. 1995. Molecular genetics of plant disease resistance. *Science* 268:661-667.
- Sugimoto, T., Yoshida, S., Kaga, A., Hajika, M., Watanabe, K., Aino, M., Tatsuda, K., Yamamoto, R., Matoh, T., Walker, D. R., Biggs, A. R. and Ishimoto, M. 2011. Genetic analysis and identification of DNA markers linked to a novel *Phytophthora sojae* resistance gene in the Japanese soybean cultivar Waseshiroge. *Euphytica* 182:133.

- Sugimoto, T., Yoshida, S., Watanabe, K., Aino, M., Kanto, T., Maekawa, K. and Irie, K. 2007. Identification of SSR markers linked to the *Phytophthora* resistance gene *Rps1-d* in soybean. *Plant Breed.* 127:154-159.
- Sun, J., Li, L., Zhao, J., Huang, J., Yan, Q., Xing, H. and Guo, N. 2014. Genetic analysis and fine mapping of *RpsJS*, a novel resistance gene to *Phytophthora sojae* in soybean [*Glycine max* (L.) Merr.]. *Theor. Appl. Genet.* 127:913-919.
- Sun, S., Wu, X. L., Zhao, J. M., Wang, Y. C., Tang, Q. H., Yu, D. Y., Gai, J. Y. and Xing, H. 2011. Characterization and mapping of *RpsYu25*, a novel resistance gene to *Phytophthora sojae*. *Plant Breed.* 130:139-143.
- Sun, X., Cao, Y., Yang, Z., Xu, C., Li, X., Wang, S. and Zhang, Q. 2004. *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. *Plant J.* 37:517-527.
- Wang, G.-L., Ruan, D.-L., Song, W.-Y., Sideris, S., Chen, L., Pi, L.-Y., Zhang, S., Zhang, Z., Fauquet, C., Gaut, B. S., Whalen, M. C. and Ronald, P. C. 1998. *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. *Plant Cell* 10:765-779.
- Weng, C., Yu, K., Anderson, T. R. and Poysa, V. 2001. Mapping genes conferring resistance to *Phytophthora* root rot of soybean, *Rps1a* and *Rps7*. *J. Hered.* 92:442-446.
- Wrather, J. A. and Koenning, S. R. 2006. Estimates of disease effects on soybean yields in the United States 2003 to 2005. *J. Nematol.* 38:173-180.
- Wrather, A. and Koenning, S. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. *Plant Health Prog.* 10:24.
- Wrather, J. A., Stienstra, W. C. and Koenning, S. R. 2001. Soybean disease loss estimates for the United States from 1996 to 1998. *Can. J. Plant Pathol.* 23:122-131.
- Wu, X.-L., Zhang, B.-Q., Sun, S., Zhao, J.-M., Feng, Y., Guo, N., Gai, J.-Y. and Xing, H. 2011a. Identification, genetic analysis and mapping of resistance to *Phytophthora sojae* of Pm28 in soybean. *Agric. Sci. China* 10:1506-1511.
- Wu, X., Zhou, B., Sun, S., Zhao, J., Chen, S., Gai, J. and Xing, H. 2011b. Genetic analysis and mapping of resistance to *Phytophthora sojae* of Pm14 in soybean. *Sci. Agric. Sin.* 44:456-460.
- Yao, H.-Y., Wang, X.-M., Wu, X.-F., Xiao, Y.-N. and Zhu, Z.-D. 2010. Molecular mapping of *Phytophthora* resistance gene in soybean cultivar Zaoshu18. *J. Plant Genet. Resour.* 11:213-217.
- Yu, A., Xu, P., Wang, J., Zhang, S., Wu, J., Li, W., Chen, W., Li, N., Fan, S., Wang, X. and Jiang, L. 2010. Genetic analysis and SSR mapping of gene resistance to *Phytophthora sojae* race 1 in soybean cv Suinong 10. *Chin. J. Oil Crop Sci.* 32:462-466.
- Zhang, J., Xia, C., Duan, C., Sun, S., Wang, X., Wu, X. and Zhu, Z. 2013a. Identification and candidate gene analysis of a novel *Phytophthora* resistance gene *Rps10* in a Chinese soybean cultivar. *PLoS ONE* 8:e69799.
- Zhang, J., Xia, C., Wang, X., Duan, C., Sun, S., Wu, X. and Zhu, Z. 2013b. Genetic characterization and fine mapping of the novel *Phytophthora* resistance gene in a Chinese soybean cultivar. *Theor. Appl. Genet.* 126:1555-1561.
- Zhong, C., Li, Y., Sun, S., Duan, C. and Zhu, Z. 2019. Genetic mapping and molecular characterization of a broad-spectrum *Phytophthora sojae* resistance gene in Chinese soybean. *Int. J. Mol. Sci.* 20:1809.
- Zhong, C., Sun, S., Li, Y., Duan, C. and Zhu, Z. 2017. Next-generation sequencing to identify candidate genes and develop diagnostic markers for a novel *Phytophthora* resistance gene, *RpsHC18*, in soybean. *Theor. Appl. Genet.* 131:525-538.
- Zhu, Z., Huo, Y., Wang, X., Huang, J. and Wu, X. 2007. Molecular identification of a novel *Phytophthora* resistance gene in soybean. *Acta Agron. Sin.* 33:154-157.