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ORIGINAL PAPER

QTLs for resistance to Phomopsis seed decay are associated with days to maturity in soybean (*Glycine max*)

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Abstract Phomopsis seed decay (PSD), primarily caused by *Phomopsis longicolla*, is a major contributor to poor soybean seed quality and significant yield loss, particularly in early maturing soybean genotypes. However, it is not yet known whether PSD resistance is associated with early maturity. This study was conducted to identify quantitative trait loci (QTLs) for resistance to PSD and days to maturity using a recombinant inbred line (RIL) population derived from a cross between the PSD-resistant Taekwangkong and the PSD-susceptible SS2-2. Based on a genetic linkage map incorporating 117 simple sequence repeat markers, QTL analysis revealed two and three QTLs conferring PSD resistance and days to maturity, respectively, in the RIL population. Two QTLs (*PSD-6-1* and *PSD-10-2*) for PSD resistance were identified in the intervals of Satt100–Satt460 and Sat_038–Satt243 on chromosomes 6 and 10, respectively. Two QTLs explained phenotypic variances in PSD resistance of 46.3 and 14.1 %, respectively. At the *PSD-6-1* QTL, the PSD-resistant cultivar Taekwangkong contributed the allele with negative effect decreasing the infection rate of PSD and this QTL does not overlap with any previously reported loci for PSD resistance in other soybean genotypes. Among the three QTLs for days to maturity, two (*Mat-6-2* and *Mat-10-3*) were located at positions similar to the PSD-resistance QTLs. The identification of the QTLs linked to both PSD resistance and days to maturity indicates a biological correlation between these two traits. The newly identified QTL for resistance to PSD associated with days to maturity in Taekwangkong will help improve soybean resistance to *P. longicolla*.

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Introduction

Phomopsis seed decay (PSD) is one of the most severe diseases in soybean seed production. PSD causes soybeans to be moldy or split, which results in reduced seed quality and decreased yield (Hepperly and Sinclair 1978; Sinclair 1999; Zhang et al. 1999). This disease has become a serious problem in soybean production areas with warm, wet weather, such as the USA (Zhang et al. 1998), Korea (Oh 1998), Argentina (Pioli et al. 2003), Italy (Riccioni et al. 2003), Yugoslavia (Medić-Pap et al. 2007), China (Cui et al. 2009) and other areas (Zimmerman and Minor 1993), especially during the pod filling and maturity stages. PSD is primarily caused by *Phomopsis longicolla* T. W. Hobbs

(teleomorph unknown), which accounts for more than 85 % of seeds infected with *Diaporthe/Phomopsis* spp. (Hobbs et al. 1985; Zimmerman and Minor 1993; Nevena et al. 1997; Zhang et al. 1999). Seriously infected seeds are shriveled, elongated, cracked and often chalky-white, with significantly low rates of germination or slow germination (Sinclair 1999). Moreover, a strong negative relationship between the incidence of *Phomopsis* seed infection and soybean germination, vigor and emergence has been reported in numerous studies (Kmetz et al. 1978; Tekrony et al. 1984; Mayhew and Caviness 1994; Mengistu et al. 2009).

Several agricultural practices, such as deep tillage, field sanitization, profitable crop rotation, fungicide treatment and weed management, are used to control PSD (Park 1991; Mengistu et al. 2010). However, the most useful and effective strategy for managing PSD is the development of resistant soybean cultivars. This strategy would help meet international challenges in food security and environmental conservation (Minor et al. 1995). So far, more than ten resistance resources for PSD have been reported, including plant introduction (PI) 82264, PI 181550, Delmar, PI 227687, PI 229358, PI 200510, PI 209908, Arksoy, PI 417479, PI 80837 and PI 360841 (Zimmerman and Minor 1993; Smith et al. 2008) and some breeding lines (SS93-6012 and SS93-6181) derived from PI genotypes (Pathan et al. 2009). These sources exhibit different levels of resistance to PSD when naturally occurring or artificially inoculated into the field. Of these, four PI accessions, PI 417479, PI 360841, PI 80837 and PI 562694 (MO/PSD-0259), have been intensively used for genetic studies of resistance to PSD (Zimmerman and Minor 1993; Berger and Minor 1999; Jackson et al. 2005; Smith et al. 2008; Jackson et al. 2009). Genetic data from these resistant genotypes indicate that one or two dominant genes confer resistance to PSD.

In Korea, the incidence of PSD caused by *P. longicolla* significantly increases in response to increasing rainfall levels and high relative humidity levels (Park 1991). In particular, early maturing soybean genotypes (e.g., vegetable soybeans) are highly susceptible to PSD because moist, warm conditions that occur during June through September (http://www.kma.go.kr/index.jsp) coincide with pod and seed development. It is not known if PSD resistance is associated with days to maturity. Furthermore, few early maturing soybean genotypes with high levels of PSD resistance have been reported. More recently, several Korean isolates of Diaporthe/Phomopsis fungi from soybean plants were identified in our previous studies (Sun et al. 2012a, c). In response to the isolate of *P. longicolla,* genotypic differences among Korean major elite cultivars were observed and Taekwangkong showed a higher level of resistance to PSD (Sun et al. 2012b). These are good biological materials for understanding the genetic basis of the correlation between resistance to PSD and days to maturity to improve PSD-resistant, early maturing soybean cultivars.

The present study was performed to identify quantitative trait loci (QTLs) conferring resistance to PSD and days to maturity using a recombinant inbred line (RIL) population derived from Taekwangkong (PSD-resistant) × SS2-2 (PSD-susceptible).

Materials and methods

Plant material and DNA extraction

This study involved an F_8 population of 124 RILs that were developed by single seed descent from a cross between PSD-resistant Taekwangkong (Kim et al. 1992) and PSD-susceptible SS2-2 (Lee et al. 1997). Genomic DNA was extracted from healthy young leaves of seedlings using the procedure described by Shure et al. (1983). The DNA concentration was determined using an ND-1000 NanoDrop (NanoDrop Technologies Inc., Wilmington, DE, USA). The DNA solution was diluted to a working concentration with Tris–EDTA buffer (pH 8.0) and stored at −20 °C until use.

Phomopsis longicolla inoculation and phenotypic evaluation

Experiments were carried out in the greenhouse of Seoul National University in Suwon, Republic of Korea during the summer of 2010. For phenotypic evaluation, parental cultivars with five replications and 124 RILs with three replications each were planted in blue rectangular plastic pots (width 15 cm, length 60 cm, depth 30 cm) on 28 May, which was the normal field planting time in Korea. Three previously reported resistant genotypes, PI 562694 (MO/PSD-0259), PI 080837 and PI 360841, and two susceptible genotypes, PI 091113 and PI 548507, obtained from the USDA Soybean Germplasm Collection (http://www.ars-grin.gov/npgs/), were also included in this study to confirm adequate disease pressure (Sun et al. 2012b).

The *P. longicolla* strain SSLP-3, which was found in a previous study to be the most virulent among the Korean isolates (Sun et al. 2012a, b), was used as an inoculum in the present study. The inoculum was prepared by flooding the α-conidia of a *P. longicolla* culture that was grown on acidified potato-dextrose agar (APDA, adjusted to pH 4.5 with lactic acid) under a 12 h photoperiod for 3 weeks. Conidial suspensions were adjusted to approximately 2.0×10^6 conidia/ml, as determined using a hemocytometer (Superior, Marienfeld, Germany). Before inoculation,

a minimum of 20 three-seed pods per replication that were at the R4 growth stage (Fehr et al. 1971) were marked so that individuals grown under consistent conditions could be used to accurately estimate the incidence of PSD. The plants with the marked pods were sprayed using the inoculation method described by Jackson et al. (2009). Then, scheduled overhead sprinkler irrigation and plastic tents were used to maintain warm temperatures (28 °C) and high humidity levels (about 100 %) to provide favorable conditions for infection with *P. longicolla*. To provide high disease pressure, artificial inoculation with the conidial suspensions was performed four times during the R4–R7 growth stages (Fehr et al. 1971). Negative controls of parents and PI genotypes were treated in a similar manner using sterile distilled water in place of the conidial suspension.

Soybean seeds (50 seeds per replication) of mapping parents, PI genotypes and 124 RILs were collected from the marked pods on the inoculated plants 10 days after reaching maturity (the R8 growth stage) (Fehr et al. 1971) and dried under forced air at 30 °C. The collected seeds were surface sterilized in 1 % sodium hypochlorite (NaOCl) for 1 min, rinsed three times for 30 s each in sterile distilled water and incubated (ten seeds per plate) on APDA (pH 4.5) plates at 25 °C under a 12 h light/dark regime. Four and 7 days later, the percentage of seeds infected by *P. longicolla* was recorded based on the recovery of *P. longicolla* from tested seeds (Hobbs et al. 1985).

The days to maturity for the parents and their RILs was recorded by measuring the number of days between planting and the appearance of mature (i.e., tan or brown) coloring on more than 95 % of the pods.

SSR marker analysis

SSR markers that are nearly evenly distributed on 20 soybean chromosomes (Chrs) were selected from SoyBase (http://soybase.org/resources/ssr.php). Using a total of 124 SSR markers showing polymorphism between the parents, their allelic variations were identified in the RIL population of Taekwangkong × SS2-2. PCR amplification was performed in a total volume of 5 μl containing 10 ng genomic DNA, 1× reaction buffer (w/$MgCl_2$), 160 μM dNTP mix, 0.4 units *Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA) and 5 μM of primer mixture. PCR reactions were conducted at 94 °C for 10 min followed by 30 cycles of 94 °C for 30 s, 46–60 °C (depending on the primer-specific annealing temperature) for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min, using an MJ Research PCT-225 Peltier Thermal Cycler (MJ Research, Watertown, MA, USA). The amplified PCR products were separated on a 1.0 % ethidium bromide-stained agarose gel. The PCR fragment that produced a single band was resolved using an ABI PRISM 3730xl automatic DNA sequencer (Applied Biosystems), and SSR genotypes of the parents and the RILs were analyzed using GeneMapper version 3.7 software (Applied Biosystems, Foster City, CA, USA).

Genetic map construction, QTL identification and statistical analysis

Genetic linkage analysis was performed using MAPMAKER/EXP version 3.0b (Lander et al. 1993), and the genetic distance was computed using the Kosambi mapping function (Kosambi 1944). A logarithm of the odds (LOD) score of 3.0 and a maximum distance of 50 cM were used as the thresholds for grouping markers into Chrs. The genetic linkage map was drawn using MapChart 2.1 (Voorrips 2002). The segregation of SSR markers in the 124 RILs was evaluated by Chi-square (χ^2) testing. The skewness of the phenotypic distribution of the resistance to *P. longicolla* and days to maturity in the RIL population was analyzed using SAS software (SAS ver. 9.1, SAS Institute Inc 2002). QTL analysis for PSD resistance and days to maturity was performed using WinQTL Cartographer (ver. 2.5) with a composite interval mapping (CIM) module (Wang et al. 2007). The threshold of LOD scores for evaluating the statistical significance of QTL effects was determined using 1,000 permutations. The LOD scores of 6.2 and 2.5 corresponding to an experiment-wide threshold ($P < 0.05$) were used to identify the presence of a major QTL for PSD and days to maturity, respectively.

Results

Genetic map construction

Using 124 SSR markers showing polymorphisms between the mapping parents, segregation analysis was performed for the RIL population of Taekwangkong × SS2-2 to construct a genetic linkage map. Based on the χ^2 testing, the seven SSR markers showing segregation distortion were not used for constructing genetic map and QTL analysis. Therefore, a total of 117 markers were incorporated into all 20 soybean Chrs, spanning a total distance of 1,506.4 cM, with an average adjacent marker distance of 12.9 cM. The remaining seven SSR markers were not linked to the genetic map and were not used for further QTL analysis for PSD resistance and days to maturity. The constructed map was basically consistent with the consensus USDA soybean genetic linkage map developed by Choi et al. (2007), with the exception of a few markers.

Phenotypic assessment of PSD resistance and days to maturity

Two parental cultivars, Taekwangkong and SS2-2, were different in terms of PSD symptoms, as revealed by bio-assaying seeds harvested from inoculated plants in the greenhouse (Fig. 1). An infection rate of 9.3 ± 0.7 % by *P. longicolla* strain SSLP-3 was observed in Taekwangkong. In SS2-2, however, most of the infected seeds were covered with white mycelia at an infection rate of 88 ± 4.3 %. Thus, Taekwangkong was shown to be PSD resistant, while SS2-2 was found to be susceptible to PSD. With examples of PSD severity on soybean seeds by infection rates (Fig. 2a), a wide range of phenotypic variation in the incidence of *P. longicolla* was observed in the 124 F_8 RILs from Taekwangkong × SS2-2 (Fig. 2b). The incidence of *P. longicolla* in the Taekwangkong × SS2-2 population showed bi-modal peaks, indicating the presence of a major QTL.

PSD-susceptible SS2-2 matured 7 days earlier in the greenhouse than PSD-resistant Taekwangkong (Fig. 2c). The RIL population of Taekwangkong × SS2-2 showed a wide phenotypic range in days to maturity (from 90 to 180 days) and the frequency of days to maturity displayed a normal distribution. Furthermore, transgressive variation occurred among the progenies. Days to maturity and PSD resistance were found to be negatively correlated in this population ($r = -0.75$, $P = 0.01$) (Fig. 2d). It showed that some RILs have both resistance and early maturity QTL alleles.

QTL analysis of PSD resistance and days to maturity

The map positions and characteristics of the QTLs detected are shown in Table 1. Two QTLs were detected for PSD

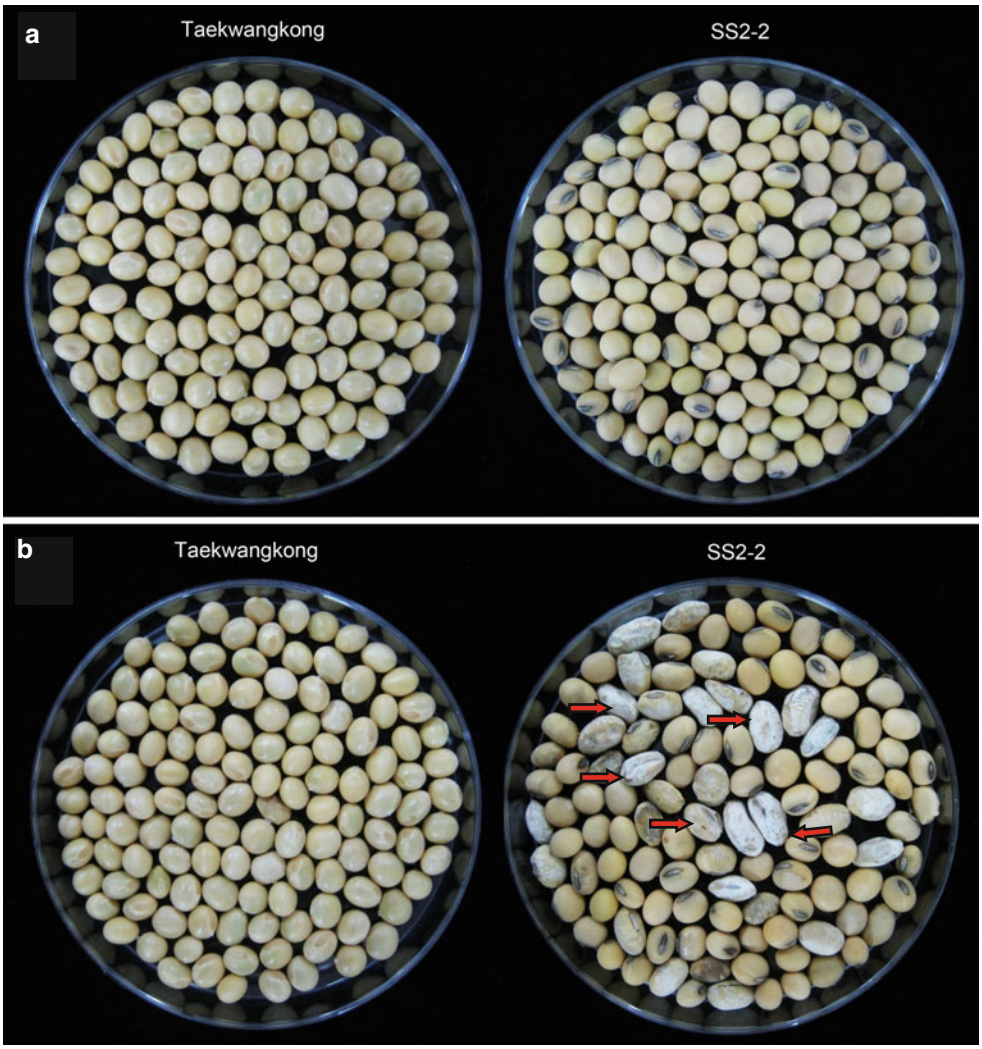

Fig. 1 Comparison of seed infection by *Phomopsis longicolla* between non-inoculated (**a**) and inoculated (**b**) plants of mapping parents, Taekwangkong and SS2-2. Severely infected seeds of SS2-2 were covered with white mycelia and showed typical symptoms of Phomopsis seed decay (PSD; marked with *red arrowheads*)

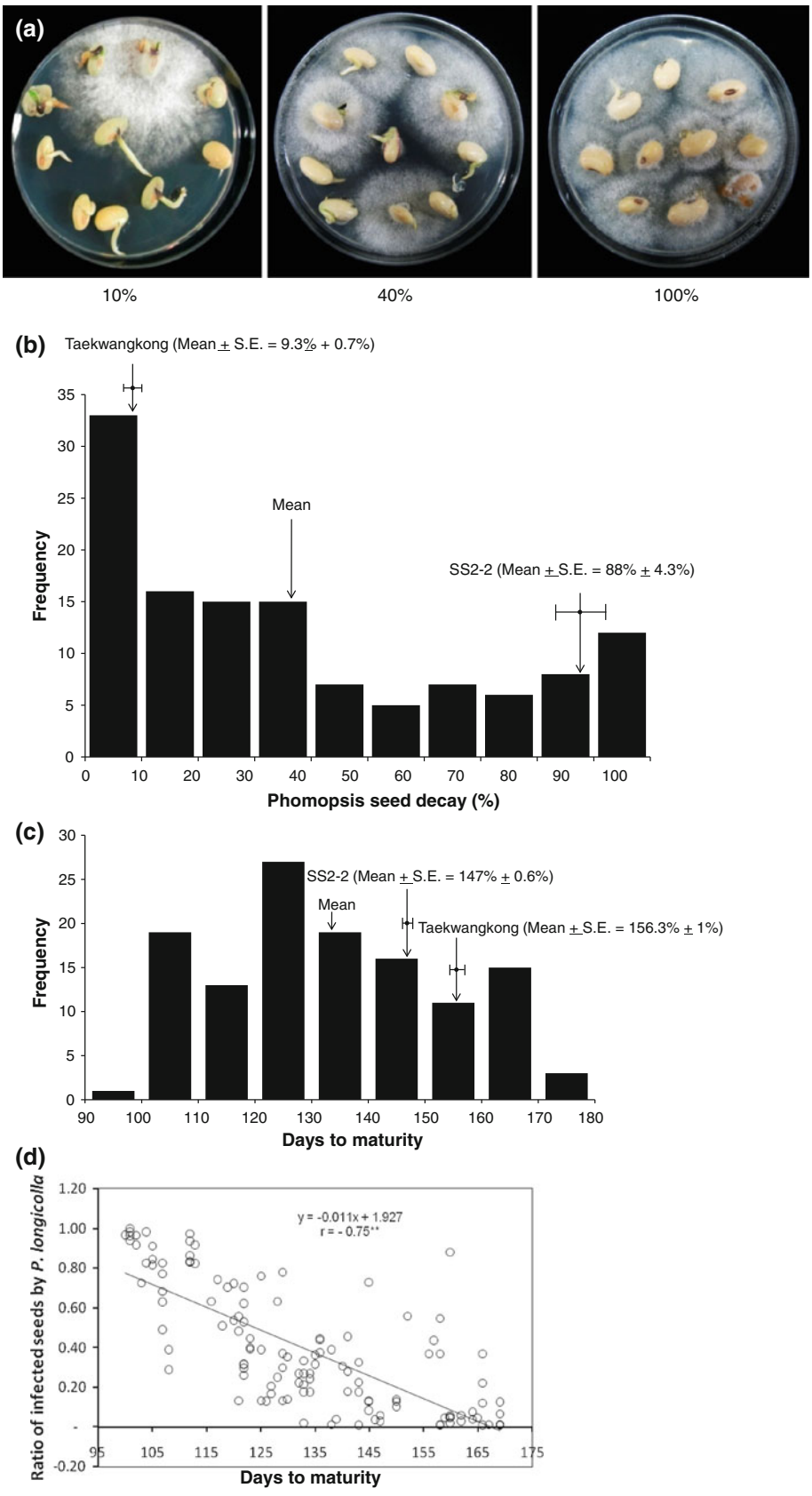

Fig. 2 Example of severity of Phomopsis seed decay (PSD) on soybean seeds by infection rates (**a**) and frequency distribution of PSD (**b**) and days to maturity (**c**) in the RIL population of Taekwangkong × SS2-2. A maturity–PSD scatter plot is also represented in (**d**)

Table 1 QTLs for Phomopsis seed decay (PSD) resistance and days to maturity in the RIL population of Taekwangkong (PSD-resistant, late maturity) × SS2-2 (PSD-susceptible, early maturity)

Trait	Chromosome	Locus	Marker interval[a]	Favorable allele	Position[b] (cM)	LOD[c]	Add[d]	R^{2e} (%)
PSD	6	*PSD 6-1*	Satt100–Satt460	Taekwangkong	110.8	24.7	−0.2	46.3
	10	*PSD-10-2*	Sat_038–Satt243	SS2-2	85.8	10.5	0.1	14.1
Days to maturity	4	*Mat-4-1*	Satt294–Sct_191	Taekwangkong	54.1	4.3	2.5	6.0
	6	*Mat-6-2*	Satt134–Satt100	Taekwangkong	108.8	20.2	12.56	32.0
	10	*Mat-10-3*	Sat_038–Satt243	SS2-2	85.8	13.9	−9.9	18.4

[a] Interval detected by CIM between markers (cM) flanking the QTL

[b] Peak position of QTL in the linkage map developed in the present study

[c] Maximum-likelihood LOD score for the individual QTL

[d] The allelic genetic effect

[e] Phenotypic variance explained by the QTL

resistance, with one on Chr 6 and one on Chr 10. The QTL (*PSD-6-1*) controlling resistance to PSD in Taekwangkong on Chr 6 was flanked by Satt100 and Satt460 and had an LOD score of 24.7. The interval of Sat_038 and Satt243 on Chr 10 carried the second QTL (*PSD-10-2*) for PSD resistance, with an LOD score of 10.5. These two QTL conferring PSD resistance accounted for 46.3 and 14.1 % of the phenotypic variance, respectively. At the *PSD-6-1* QTL, Taekwangkong allele decreased the infection rate of PSD.

Three QTLs that conferred days to maturity were observed on three Chrs, i.e., 4, 6 and 10 (Table 1). On Chr 4, a minor QTL for days to maturity (*Mat-4-1*) existed between Satt294 and Sct_191, representing the lowest R^2 value (6 %) compared with other QTLs. The QTL *Mat-6-2,* explaining the highest amount of the phenotypic variance (32 %), was located at the interval of Satt134–Satt100 on Chr 6. The LOD score of *Mat-6-2* was 20.2. The third QTL controlling days to maturity (*Mat-10-3*) was observed on Chr 10 at 85.8 cM, with an LOD score of 13.9, flanked by Sat_038 and Satt243. The individual effect of this QTL on the expression of days to maturity was 18.4 % and its favorable allele was contributed by SS2-2, which matured earlier than Taekwangkong (Table 1).

Discussion

This study employed a QTL mapping approach to examine the PSD-resistance trait in soybean. This study differed from previous investigations that examined the qualitative inheritance and mapping of the PSD-resistance trait in soybean. We used the PSD-resistant cultivar Taekwangkong to identify genetic elements responsible for PSD resistance. Taekwangkong was shown to be the cultivar that consistently displayed the lowest seed infection rate by *P. longicolla* in field tests at different locations in Korea performed in multiple years and in greenhouse tests (Sun et al. 2012b). Few genetic studies of PSD resistance in existing elite cultivars have been conducted, and PSD resistance in Taekwangkong has not been characterized. We also developed an $F_{2:8}$ RIL population of Taekwangkong × SS2-2 (PSD-susceptible) and observed a wide range of phenotypic variation of PSD resistance, with a frequency distribution toward the low incidence of PSD of the Taekwangkong phenotype in this population (Fig. 2b). These results indicate that there is quantitative regulation of resistance to PSD in Taekwangkong by two or more major genes. Thus, QTL mapping analysis was performed to identify the QTLs for PSD resistance in Taekwangkong.

The previous studies on the inheritance mode of PSD resistance were primarily conducted based on Mendelian genetic analysis using three PIs and MO/PSD-0259. Data concerning PSD resistance in the segregation populations agreed with the models of qualitative inheritance of one or two complementary dominant genes in PI 417479 and PI 80837 (Zimmerman and Minor 1993; Jackson et al. 2005). Data from an F_2 population of MO/PSD-0259 × AP 350 (PSD-susceptible) fit the model for single dominant gene resistance in MO/PSD-0259 (Jackson et al. 2005). Furthermore, phenotypic data concerning the incidence of PSD in the F_2 and F_3 populations of PI 80837 × MO/PSD-0259 revealed that two dominant genes conferring PSD resistance in PI 180837 and MO/PSD-0259 were different from each other. To characterize the resistance to PSD in PI 360841, crosses were made between PI 360841 and PSD-susceptible genotypes (AP 350 and PI 91113) as well as PSD-resistant genotypes (MO/PSD-0259 and PI 80837) (Smith et al. 2008). Chi-square (χ^2) analysis of segregation ratio for PSD seed infection exhibited good agreement with the model of two complementary dominant genes conferring PSD resistance in PI 360841. One of the resistance genes in PI 360841 was thought to be different from MO/PSD-0259 and identical to PI 80837.

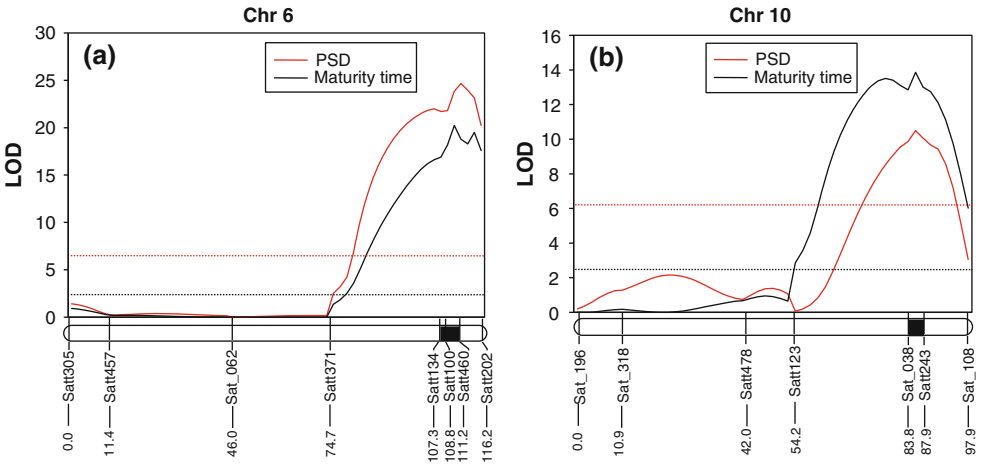

Fig. 3 Identifications of QTLs conferring PSD and days to maturity on Chrs 6 and 10. Positions of QTLs identified are filled as *black* on both chromosomes. The *red* and *black horizontal lines* represent LOD threshold at the 5 % significance level for PSD (6.2) and days to maturity (2.5) based on 1,000 permutations

Two QTLs regulating PSD resistance were identified in the RIL population of Taekwangkong × SS2-2. These QTLs are flanked by Satt100–Satt460 and Sat_038–Satt243 on Chrs 6 and 10, respectively (Table 1; Fig. 3). Genomic regions associated with PSD resistance in PI 417479 had been previously identified only by QTL mapping using RFLP markers (Berger and Minor 1999). In an F_2 population of AP 350 × PI 417479, two RFLP markers, A708 and A162, were associated with resistance to PSD on Chrs 13 (LG F) and 12 (LG H), respectively. Phenotypic variations of PSD infection explained by these markers were 62.2 and 4.5 %, respectively. Additionally, an F_2 population of PI 417479 × Williams 82 exhibited only the A708 marker associated with PSD resistance. Genetic mapping of resistance to PSD in other soybean genotypes, MO/PSD-0259 and PI 80837, was conducted based on qualitative inheritance analysis (Jackson et al. 2009). Using F_2 populations from each cross of AP 350 × PI 80837 and AP 350 × MO/PSD-0259, resistance to PSD in PI 80837 was linked to Sat_177 and Sat_342 on Chr 14 (LG B2), and PSD resistance of MO/PSD-0259 was associated with Sat_317 and Sat_120 on Chr 13 (LG F) (Jackson et al. 2009). Based on the integrated soybean genetic linkage map (Song et al. 2004), the markers Sat_317 and Sat_120 linked to PSD resistance in MO/PSD-0259 were 8.2 and 11.2 cM, respectively, away from the previously identified marker A708 that is located on the same chromosome and is associated with PSD resistance in PI 417479. Also, the resistance gene in MO/PSD-0259 was mapped to 16.8 cM from A708. According to the results from previous genetic mapping studies of PSD resistance, therefore, the QTL *PSD-6-1* on Chr 6 conferring PSD resistance in Taekwangkong does not overlap with other markers linked to PSD resistance, indicating that this is an new additional QTL. These results therefore suggest that Taekwangkong is a novel PSD-resistant resource and the genetic mechanism underlying resistance to PSD in soybean is complicated.

Many QTLs associated with disease resistance were found to be located in closely linked clusters, particularly on Chrs 6 and 10, which contain the PSD-resistance QTLs described on SoyBase website (http://soybase.org/), as shown in Fig. 4. QTLs associated with resistance to other fungal diseases appear to be located in proximity to the SSR markers associated with PSD resistance. In the genomic region of *PSD-6-1* in the interval of Satt100–Satt460 on Chr 6, six QTLs associated with resistance to soybean sudden-death syndrome (SDS) and four QTLs associated with Phytophthora root rot have been reported (Fig. 4) (Chang et al. 1996; Hnetkovsky et al. 1996; Iqbal et al. 2001; Njiti et al. 2002; Li et al. 2010; Xu et al. 2011). Five QTLs associated with soybean Sclerotinia stem rot and one QTL for resistance to Phytophthora root rot are located near the Sat_038–Satt243 interval (*PSD-10-2*) on Chr 10 (Arahana et al. 2001; Xu et al. 2011). The symptoms caused by three fungal diseases, PSD (occasionally called Phomopsis seed rot), Phytophthora root rot and Sclerotinia stem rot are related to rot, although infected target organs are different in soybean. Therefore, the QTL for PSD resistance in Taekwangkong may be part of a closely linked gene cluster involved in resistance to these diseases.

Interestingly, the RIL population of Taekwangkong × SS2-2 showed highly significant negative correlation between PSD incidence and days to maturity even in controlled-environment conditions, which could eliminate the confounding effect from seasonal variation on PSD. This was consistent with the previous study performed in the field, which indicated that seed infection with *Phomopsis* spp. is high when early maturity cultivars are grown in the early soybean production system (ESPS) (Mayhew and Caviness 1994). Our results combined with the previous report implies PSD resistance is related with maturity not only environmentally but also genetically. In this study, two QTLs associated with days to maturity were identified at almost the same positions as the PSD-resistance QTLs

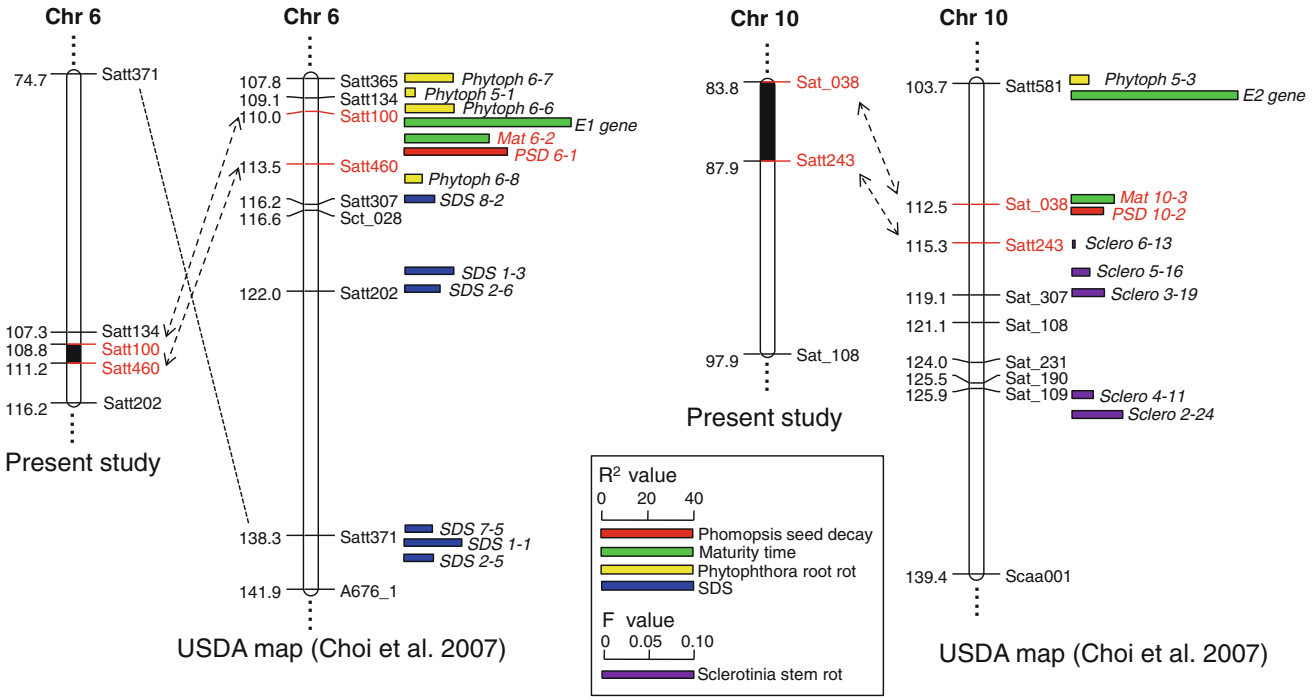

Fig. 4 Maps showing comparable regions between this study and the USDA soybean genetic map (Choi et al. 2007). Positions of QTLs and SSR markers identified in the present study are labeled as *red* on Chrs 6 and 10 for both maps. QTLs for PSD resistance and days to maturity identified by this present study or the previous study are indicated by *red* and *green* bars, respectively. The previously reported QTLs for resistance to Phytophthora root rot, sudden-death syndrome and Sclerotinia stem rot are indicated in *yellow*, *blue* and *purple*, respectively. The information related to all previous identified QTLs are obtained from SoyBase website (http://soybase.org)

(Table 1; Fig. 3). Satt100 and Sat_038, associated with PSD resistance and days to maturity on Chrs 6 and 10, respectively, have been reported to be closely linked to the QTLs or genes for flowering time and maturity, *E1* and *E2*, respectively (Fig. 4) (Yamanaka et al. 2005; Watanabe et al. 2011). The other markers, Satt294, accounting for maturity on Chr 4 was identified closeby, 2.8 cM from Satt136, which was associated with gene *E8* for flowering time and maturity (Cober et al. 2010). Our results showed that these three QTLs for days to maturity were located on Chrs 4, 6 and 10 (Table 1), which were corresponding positions with previous genes *E1*, *E2* and *E8*, respectively (Yamanaka et al. 2005; Choi et al. 2007; Watanabe et al. 2011). Generally, soybean varieties that mature early show a higher incidence of PSD than late-maturing varieties (Vaughan et al. 1989). The identification of the loci linked to both PSD resistance and days to maturity may provide direct evidence for the genetic correlation between the two traits. Genetic linkage of QTLs for resistance to blight and maturity or flowering time has been reported for several crops such as potato and chickpea (Visker et al. 2005; Lichtenzveig et al. 2006; Danan et al. 2011), although few studies have been performed on the genetic relationship between resistance to fungal diseases, including PSD and days to maturity in soybean. This present study reports the association of PSD resistance and maturity on inheritance in soybean. However, whether this genetic association is due to pleiotropy or tight genetic linkage still remains to be examined.

In conclusion, the selection of PSD-resistant Taekwangkong may offer opportunities for breeders to develop new resistant lines by exploring new resistance genes. Because *P. longicolla* infection is dependent on environmental conditions, gene pyramiding using multiple resistance genes will be an effective method for developing cultivars with stable resistance to PSD. The two QTLs that were identified, along with the linked markers, will be helpful for marker-assisted selection of PSD resistance. In addition, there is a need to confirm the QTL for PSD resistance in Taekwangkong using a high-resolution mapping population to isolate a resistance gene to *P. longicolla* and to understand the mechanisms underlying fungal disease resistance.

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