Identification of QTLs Associated with Resistance to *Riptortus clavatus* Thunberg (Heteroptera: Alydidae) in Soybean (*Glycine max* L. Merr.)

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Abstract

The bean bug *Riptortus clavatus* Thunberg (Heteroptera: Alydidae) is an important pest, causing serious yield loss in soybean. But the information on mechanism of resistance to *R. clavatus* is limited. The objective of this study was to identify QTLs for *R. clavatus* resistance using simple sequence repeat (SSR) markers in a soybean population of recombinant inbred lines (RILs) developed from the cross PI 171451 × Hwaeomputkong. A genetic map from this population was constructed with a total of 136 SSR markers covering 1073.9 cM on 20 linkage groups (LGs). With 126 F_5 RILs, two independent QTLs for resistance to *R. clavatus* were mapped on LGs B1 and C2. The amount of phenotypic variation explained by these QTLs ranged from 12 to 16%. PI 171451 showed an escape response to *R. clavatus*. Under feeding conditions, 14.4% of RILs showed greater resistance to *R. clavatus* than the resistant parent. The resistance to *R. clavatus* in soybean from PI 171451 was incomplete and quantitatively inherited and the QTLs for resistance to *R. clavatus* detected in the RIL population were not significantly affected by epistatic interactions.

Introduction

Plant resistance to insects is considered to be an important component of integrated pest management which reduces the use of chemical pesticides (Boerma and Walker 2005; Youn and Jung 2008). Insect resistance is an important quantitative trait for soybean breeders to develop elite cultivars (Rector et al. 1998). Three plant introductions, PI 171451, PI 227687, and PI 229358, showed resistance to a lot of defoliating insect species, but with a low agronomic value (Van Duyn et al. 1971).

The bean bug *Riptortus clavatus* Thunberg (Heteroptera: Alydidae) is a serious pest of soybean causing a large amount of yield loss in Korea and Japan (Endo et al. 2005; Huh et al. 2006;

Jung et al. 2004; Son et al. 2000). *R. clavatus* completed more than two generations per year in Korea (Lee et al. 1997). As it showed the highest flight activities, pesticides were difficult for controlling *R. clavatus* (Choi et al. 2005; Youn and Jung 2008).

Recently, there has been an increasing interest in quantitative trait loci (QTLs) associated with insect resistance (Arahana et al. 2001; Rector et al. 1998; Walker et al. 2004; Winter et al. 2007; Zhu et al. 2008). One major QTL on linkage group (LG) M and two minor QTLs on linkage groups (LGs) H and D1 for resistance to corn earworm (*Helicoverpa zea Boddie*) were identified in a population derived from the cross between Cobb and PI 229358 (Rector et al. 1998). Subsequently, the major QTL for corn earworm from PI 229358 was finely mapped to a Satt220-Satt536 interval using recombinant substitution lines (Zhu et al. 2006). Antibiosis (toxicity) and antixenosis (non-preference) are two principle modes for resistance to defoliating insects in soy-

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bean (Lambert and Kilen 1984). An antibiosis QTL on LG M for resistance to corn earworm was discovered in both F_2 populations developed by crossing Cobb with PI 171451 and Cobb with PI 229358 (Rector et al. 2000). An antixenosis QTL was also significant in this location. Therefore, they primarily reported one insect resistance QTL that was detected for both modes (Rector et al. 2000; Zhu et al. 2006). However, genetic mode of resistance to *R. clavatus* has not been sufficiently investigated in soybean.

The objectives of this study were (1) to determine the resistance of soybean parents PI 171451 and Hwaeomputkong to *R. clavatus* and (2) to detect QTLs related to *R. clavatus* resistance in soybean using the RILs derived from the cross PI 171451 × Hwaeomputkong.

Materials and Methods

Plant materials

A total of 126 F_5 RILs derived from a cross between PI 171451 and Hwaeomputkong were used to construct a genetic linkage map and detect QTLs related with the resistance to bean bug *R. clavatus* Thunberg (Heteroptera: Alydidae). PI 171451 with late maturity was resistant to several insects (Boerma and Walker 2005) and Hwaeomputkong was a recommended variety for vegetable soybean with early maturity (Hong et al. 1995).

Field trials and insect treatments

The RILs and their parents were grown in the field at the experimental farm at Seoul National University in Suwon. The bean bug *R. clavatus* (Thunberg) was reared at 25 ± 1 °C under long-day conditions (16L: 8D). For greenhouse test, one seed of each parent with eight replicates and each RIL with two replications were planted in a plastic pot (diameter 20 cm, depth 30 cm). To prevent insects escaping, plastic pots were covered by gauze (180×260×190 cm). Six insects of third or fourth instars

were put into each pot covered with gauze cage. For greenhouse test and field studies, eight replications of each parent and two replications of each RIL were used to test insect resistance. Total number of pods, number of damaged pods, total number of seeds, and number of damaged seeds were measured in both conditions.

DNA extraction and SSR marker analysis

The procedure of DNA extraction was described in our previous study (Li et al. 2008). Briefly, genomic DNA was extracted from fresh leaves of young seedlings of each of two parents and 126 RILs grown in the greenhouse according to the procedure of Shure et al. (1983). Genetic mapping of this population was reported in the previous study (Li et al. 2008). A total of 322 SSR markers were selected from Soybase (http://www.soybase. com/resources/ssr.php). For SSR marker analysis, two different methods, a fluorescent-labelled primer as either forward- or reverse-primer and universal primer with M13 (-21) (18 bases, 5'- TGT AAA ACG ACG GCC AGT -3') at the 5' end, were applied in this study. The detailed procedure for SSR marker analysis, accurate characterization of the alleles, and automated data output was also described in our previous study (Li et al. 2008).

Genetic mapping and QTL identification

The genetic soybean linkage map was constructed by the protocol described by Li et al. (2008). MAPMAKER/EXP version 3.0b (Lincoln et al. 1993) was used for construction of the soybean genetic map with Kosambi mapping function, LOD greater than or equal to 3.0 and a maximum distance of 50 cM. Also, identification of QTLs for resistance to *R. clavatus* was followed by Li et al. (2008). Using QTLMapper version 1.6, composite interval analysis (Wang et al. 1999) was applied and the LOD score of 3.0 or higher than 10 as R^2 were used for the presence of a main-effect QTL on the total map distance.
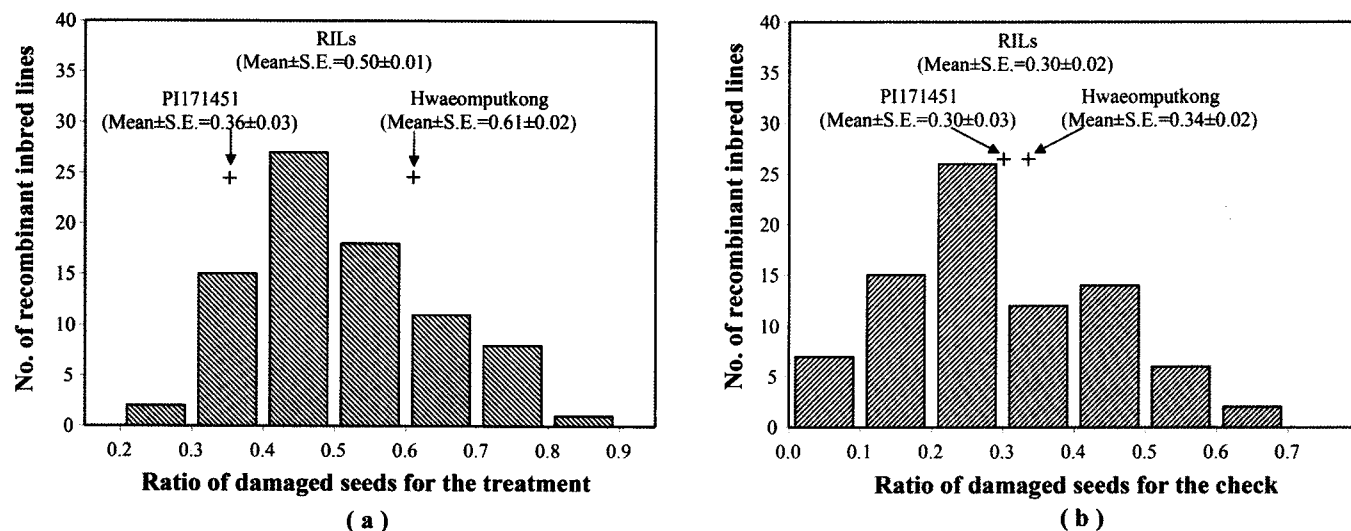

Fig. 1. Distribution of recombinant inbred lines (RILs) with various values of ratio of damaged seeds for the treatment (a) and the check (in the field condition) (b).

Table 1. Statistical descriptions of resistance to the *Riptortus clavatus* (Thunberg) (Heteroptera: Alydidae) in the treated and field conditions for two soybean parents.

	PI 171451				Hwaeomputkong			
	Treated[a]	Field condition (Check)[b]	Mean	Treated /Check	Treated[a]	Field condition (Check)[b]	Mean	Treated /Check
Total no. of pods	52.30	55.20	53.75	0.95	15.00	13.67	14.34	1.10
No. of damaged pods	16.10	16.80	16.45	0.96	9.00	3.83	6.42	2.35
Ratio of damaged pods	0.31	0.28	0.30	1.11	0.57	0.28	0.43	2.04
Total no. of seeds	97.80	96.20	97.00	1.02	26.00	23.17	24.59	1.12
No. of damaged seeds	51.30	29.00	40.15	1.77	19.50	8.00	13.75	2.44
Ratio of damaged seeds	0.36	0.30	0.33	1.20	0.61	0.34	0.48	1.79

[a] Treated with the *R. clavatus* (Thunberg) (Heteroptera: Alydidae).
[b] Naturally damaged by the *R. clavatus* (Thunberg) (Heteroptera: Alydidae) in the field condition.

Table 2. Simple correlations of ratio of the damaged seeds by artificially infected and ratio of the damaged seeds in the field with ratio of lodging plants, days to flowering and maturity, plant height, 100-seed weight, numbers of branches and seed-filling period.

	Ratio of damaged seeds With the treatment	Ratio of damaged seeds in the field
Ratio of lodging plants	-0.14	0.03
Days to flowering	-0.25*	0.08
Days to maturity	-0.17	-0.03
Plant height	-0.13	0.14
100-seed weight	0.18	-0.09
Numbers of branches	-0.08	0.21
Seed-filling period	-0.04	-0.12

* indicates significance level of correlations at $P < 0.05$.

Results

Statistical analysis of *Riptortus clavatus* resistance

Insect natural and feeding damage on pods and seeds of parents and RILs are shown in Table 1 and Fig. 1, respectively. These results show that the parents, PI 171451 and Hwaeomputkong, exhibited significant differences in resistance to *R. clavatus* under feeding conditions. For PI 171451, no significant difference in degrees of damage on pods and seeds were observed between feeding and natural conditions, whereas feeding damages on pods and seeds for Hwaeomputkong were significantly higher than natural damages under field condition. Ratios of damaged pods and seeds with treatments were 1.8 to 2 times as much as those in the field for Hwaeomputkong and 1.7 to 1.8 times as much as those under the feeding conditions for PI 171451. However, ratios of damaged pods and seeds in the field for two parents, PI 171451 and Hwaeomputkong, were not significantly different. In addition, the RILs revealed a wide range of resistance variations. This observation might be due to a mixture of different insect species in the field in addition to *R.*

clavatus. In the RIL population, large ranges of resistance variations were observed under both feeding and field conditions. Ratio of damaged seeds with the treatment varied from 0.24 to 0.84 with an average of 0.50 ± 0.01, whereas that in the field ranged from 0.06 to 0.63 with an average of 0.30 ± 0.02. In addition, transgressive segregations with greater or weaker resistances than parents were observed in the RIL population. Under the feeding conditions, 14.4% of RILs revealed greater resistance to *R. clavatus* with lower values of ratio of damaged seeds than the resistant parent, PI 171451. These RILs could be suggested as being the lines enhanced alleles related to *R. clavatus* resistance.

Correlations of insect resistance with seven major agronomic traits

Among the agronomic traits examined, days to flowering were correlated negatively with ratio of damaged seeds under the feeding condition ($r = -0.25$, $P < 0.05$) (Table 2 and Fig. 2). Except for the above, the correlations of the ratio of damaged seeds with other agronomic traits examined were not significant under both the feeding and field conditions ($P > 0.05$); the corre-
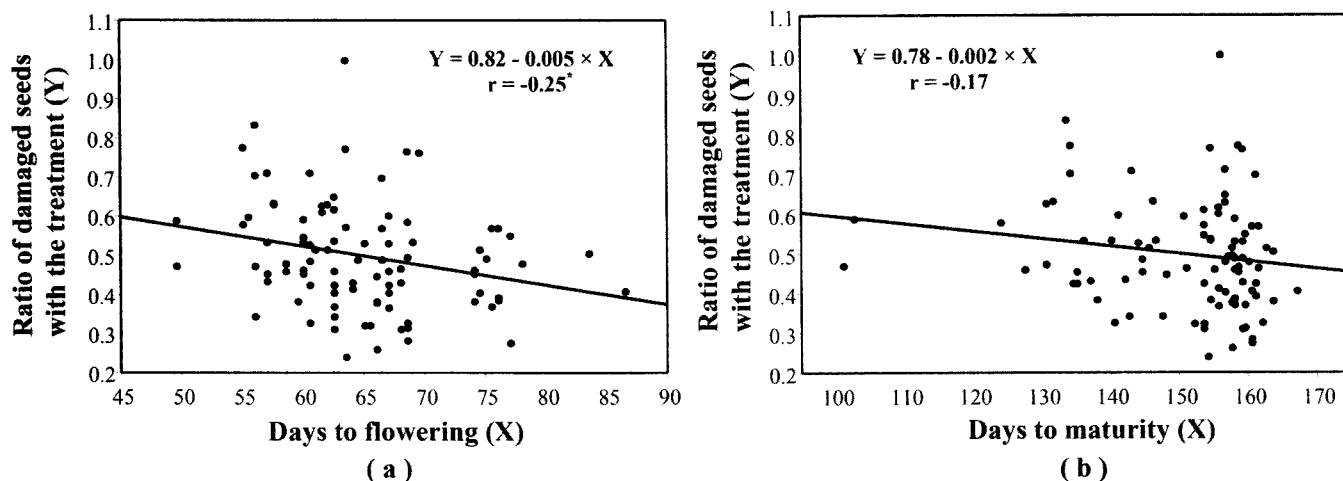

(a)

(b)

Fig. 2. Regression of ratio of damaged seeds with the treatment (Y) on days to flowering and days to maturity (X) for recombinant inbred lines (RILs) derived from the cross PI 171451 × Hwaeomputkong.

Table 3. QTL location, interval, length, position, likelihood value, additive effect and variance explained from the F₅ RIL population derived from the cross PI 171451 × Hwaeomputkong.

	Interval	LG	Length (cM)[a]	QTL POS[b]	LOD	Additive effect	R² (%)
Treatment[c]	Satt509-Satt197	B1	11.0	0	2.6	-0.06	12.3
	Satt227-Satt422	C2	10.7	0	3.4	-0.06	15.5

[a] Distance of the QTL on the linkage group (LG) given in centiMorgans (cM) from Mapmaker program.
[b] Distance of QTL detected in this study from the first marker on the corresponding linkage group.
[c] Ratio of damaged seeds with the treatment.

lation coefficients varied from -0.17 to 0.21. In addition, days to maturity and seed-filling period were negatively correlated with the ratio of damaged seeds under both treatment and field conditions ($r < -0.03$).

Molecular markers and QTLs for resistance to *Riptortus clavatus*

Among the SSRs showing polymorphisms between parents, PI 171451 and Hwaeomputkong, 136 out of 201 polymorphic SSR markers produced PCR products in all individuals of the RIL population. Of the 136 SSR makers, 73 markers were well segregated with a good fit to the expected ratio of 1:1 ($P > 0.05$). The segregating markers were distributed over all 20 LGs and covered 1073.9 cM.

Within the LGs constructed, two minor QTLs for resistance to R. clavatus were detected on LGs B1 and C2 (Table 3 and Fig. 3). One QTL with the LOD score of 2.6 was mapped between Satt509 and Satt197 on LG B1, and accounted for 12.3% of total phenotypic variance. The other QTL with LOD

Fig. 3. QTL detection for resistance to the *Riptortus clavatus* (Thunberg) (Heteroptera: Alydidae) based on the RIL population derived from the cross PI 171451 × Hwaeomputkong.

scores of 3.4 was located at the marker interval between Satt227 and Satt422 on LG C2, and accounted for 15.5% of total phenotypic variance. The QTL for resistance to R. clavatus at the marker interval between Satt227 and Satt422 on LG C2 overlapped with one QTL for days to maturity (Fig. 3), probably indicating that this marker interval was likely related to two or more traits in this soybean population.

Discussion

Three Japanese plant introductions, PI 171451, PI 227687, and PI 229358 (PIs) have been the primary sources of insect resistance alleles (Boerma and Walker 2005). However, a combination of quantitative inheritance of resistance and poor agronomic performance has hindered their progress. Boerma and Walker (2005) reported that PI 171451, PI 227687, and PI 229358 differed in their relative resistance to some pest species. Each of the PIs possessed at least one unique resistance gene (Kilen and Lambert 1986). The level of antibiosis resistance of the three PIs to four Asian insects varied, with PI 227687 most resistant to *Spodoptera exigua*, PI 171451 most resistant to *Porthesia taiwana* and *Orgyia sp.*, and PI 229358 most resistant to *Anomala cupripes* (Talekar et al. 1988).

In this study, PI 171451 showed an escape response to R. clavatus, although it had a high resistance to P. taiwana and O. sp. In the field, due to the existence of more than one pest and quite different days to maturity between two cultivars, natural pest damage on plants cannot exactly reflect the resistance of a parent to a specific pest. Here, natural pest damage on pods and seeds for PI 171451 was not obviously different from that for the susceptible parent, Hwaeomputkong. In the population of RILs derived from the cross PI 171451 × Hwaeomputkong, transgressive segregations with greater or weaker resistances than parents suggested that the resistance of PI 171451 was also quantitatively inherited. Under the feeding conditions, 14.4% of RILs having greater resistance to R. clavatus than the resistant parent, PI 171451, were segregated, but the resistance was still incomplete. In addition, the ratio of damaged seeds was negatively correlated with days to maturity and seed-filling period under both feeding and field conditions, and significant and negatively correlated with days to flowering under the feeding conditions. This suggested that late flowering and late maturity were
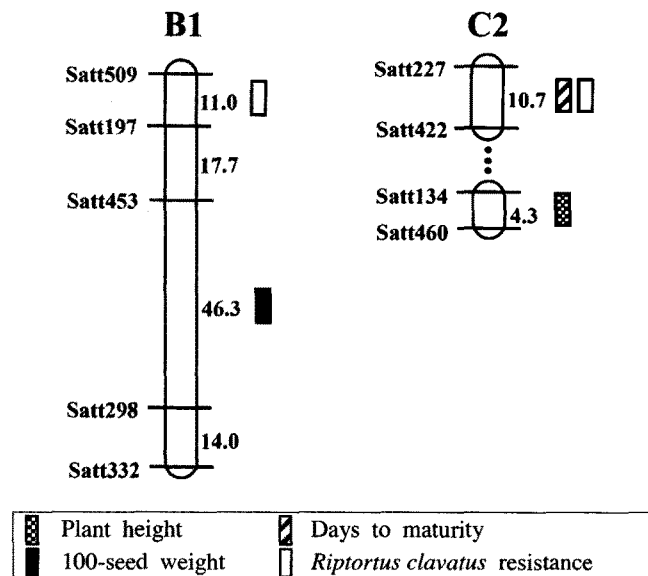
Table 4. Digenic epistatic effects for resistance to the *Riptortus clavatus* (Thunberg) (Heteroptera: Alydidae) in the F₅ RIL population derived from the cross PI 171451 × Hwaeomputkong identified using the QTLMapper version 1.6.

	LG	Interval i	LG	Interval j	LOD	A_i[a]	A_j[a]	AA_ij[b]	R²[c]
Treatment[d]	C2	Satt227-Satt422	D1a	Satt439-Sat_414	4.3	-0.05***	-0.02	0.03*	13.6
	L	Satt232-Sat_320	O	Satt173-Satt633	3.5	-0.08**	-0.05*	0.10**	12.5

[a] A_i and A_j represent the additive effect on interval i and interval j.
[b] AA_ij are the epistatic effects between interval i and interval j. (Mei et al. 2005).
[c] R² is the proportion of the total phenotypic variation explained by AA_ij.
[d] Ratio of damaged seeds with the treatment.
*, **, and *** mean significant at $P < 0.05$, $P < 0.001$, and $P < 0.0001$, respectively.

helpful to reduce pest damage on plants, consistent with Wada et al. (2006) where seed damage by pest in late-planting cultivars was lower than that in the normal planted cultivar. So in QTL mapping, the feeding damage data are more favorable than natural field damages.

Two QTLs for *R. clavatus* were newly identified from this study. One QTL located at the maker interval between Satt509 and Satt197 on LG B1 was associated with a putative QTL for resistance to *Sclerotinia* stem rot in soybean identified from a RIL population developed by a cross between Williams 82, a susceptible cultivar, and Dassel, a partial resistance cultivar (Arahana et al. 2001). This suggested that pest resistant QTLs were likely located around this region. However, there is high possibility of erroneous QTL positioning in this study due to the difficulties in collecting phenotypic data. The insect was not inoculated at the same growth stage of the RIL soybean lines, as RILs showed a greater range in maturity. The accurate QTLs for *R. clavatus* could be mapped on the soybean genetic linkage map with more advanced RILs and more tested plants per each replicate.

Shen et al. (2006) reported that epistasis influenced genetic variation in populations. There are three types of epistasis that affect complex traits: interactions between QTLs, between QTLs and background, and between complementary loci (Liao et al. 2001; Shen et al. 2006). Two digenic epistatic interactions were detected for *R. clavatus* resistances in the RIL population derived from the cross PI 171451 × Hwaeomputkong (Table 4). Epistatic interactions accounted for 13.6 and 12.5% of the total phenotypic variation, respectively. Among the marker intervals contributing to digenic epistatic interactions, only one marker interval Satt227-Satt422 on LG C2, where one QTL for days to maturity was also detected, was the QTL for resistance to *R. clavatus*. Nonetheless, no significant difference was deleted between the additive effect (15.5%, Table 3) and epistatic interaction effect (13.6%, Table 4) for this QTL related to *R. clavatus* resistance.

In summary, the resistance to *R. clavatus* in soybean from PI 171451 was incomplete and the RILs derived from the cross PI 171451 × Hwaeomputkong consequently exhibited the escape response to *R. clavatus*. Two minor QTLs for *R. clavatus* resistance were identified and mapped on LGs B1 and C2 in this RIL population. One of two QTLs for *R. clavatus* resistance and one QTL related to days to maturity were located at the same marker interval Satt227-Satt422 on LG C2. *R. clavatus* resistance in this RIL population was not significantly affected by epistasis interactions.

Acknowledgments

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