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ORIGINAL PAPER

Genetic components and major QTL confer resistance to bean pyralid (*Lamprosema indicata* Fabricius) under multiple environments in four RIL populations of soybean

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Abstract Bean pyralid (BP; *Lamprosema indicata* Fabricius) is one of the major leaf-feeding insects that affect soybean crops in central and southern China. Four recombinant inbred line populations (KY, WT, XG and SX) were tested during 2004–2006 in Nanjing, China, to identify quantitative trait loci (QTL) for resistance to BP on the basis of data for rolled leaflet percentage under field infestation conditions. The mapping was performed using QTL Network V2.0 and checked with Windows QTL Cartographer V2.5 and IciMapping V2.2. The results showed that 81–92 % of the phenotypic variation was accounted for by additive QTL (27–43 %), epistatic QTL pairs (5–13 %), and collective unmapped minor QTL (38–58 %). In total, 17 QTL were detected on 11 linkage groups, of which two had additive effects, six had both additive and epistatic effects, and nine had only epistatic effects. Eight epistatic QTL pairs were observed, of which three pairs involved two QTL with additive effects, one involved one QTL with additive effect, and four involved no QTL with additive effects. Different genetic structures for BP resistance were found among the populations. Eight QTL (five additive and three epistatic pairs) were detected in KY, ten QTL (four additive and five epistatic pairs) were detected in WT, and only one additive QTL was detected in both the XG and the SX populations. *BP12-1* and *BP1-1* are major QTL, with the former accounting for 15, 31, and 50 % of the total genetic variation (including epistasis) in KY, WT, and XG, respectively, and the latter accounting for 13 and 32 % of the total genetic variation in KY and SX, respectively. The additive × year and epistasis × year interaction effects were negligible, indicating that the QTL were stable over the years. Because 41–68 % of the total genetic variation could not be accounted for by these QTL, the use of both identified QTL and unmapped minor QTL in breeding for BP resistance should be considered.

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Introduction

Soybean (*Glycine max* (L.) Merr.) is the leading oilseed crop in the world, accounting for approximately 56 % of global oilseed production (Wilson 2008). Its production is often limited by a number of insect pests. Velvetbean caterpillar (*Anticarsia gemmatalis* Hübner), soybean looper (*Pseudoplusia includens* Walker), bean leaf beetle (*Cerotoma trifurcate* Forster), southern green stink bug (*Nezara viridula* L.), green stink bug (*Acrosternum hilare* Say), and corn earworm (*Helicoverpa zea* Boddie) are the major insects that affect soybean crops in the US (Boethel 2004). The major pests in Chinese soybean production are different from those in the US, and these include the bean pyralid (BP; *Lamprosema indicata* Fabricius), mugwort looper (*Ascotis selenaria* Schiffermuller et Denis), common cutworm (*Spodoptera litura* Fabricius), and globular stink bug (*Megacota cribraria* Fabricius), which are the most destructive insects in southern China (Cui et al.

1997a; Sun et al. 2001; Wu and Xu 2002; Xing et al. 2008a). BP is widely distributed throughout the world and is found in Korea, Japan, China, India, the Americas, and Africa (Choi et al. 2008). Choi et al. (2008) reported an increased incidence of BP in the Jeon-buk province of Korea that required management. The most destructive infestation occurs during the flowering and pod-formation stages (Xing et al. 2008a). When BP infestations are high, entire fields can be defoliated.

The use of resistant soybean cultivars would reduce both crop losses and the cost of insecticide application, so elite soybean cultivars with resistance to leaf-feeding insects would have both environmental and economic benefits (Komatsu et al. 2005). Van Duyn et al. (1971) evaluated maturity group VII and VIII plant introductions (PIs) from the USDA Soybean Germplasm Collection for resistance to the Mexican bean beetle (*Epilachna varivestis* Mulsant) and found that PI 171451, PI 227687 and PI 229358 were highly resistant. The resistance of soybean to BP has been reported to be significantly different among varieties and accessions (Cui et al. 1997b; Sun et al. 2005). Rolled leaflet percentage (RLP), rolled leaflet number (RLN), and seed yield are generally used as resistance (damage) indicators (Xing et al. 2008a). In field tests, 'NN1138-2', 'TSBPHDJ', 'Gantai-2-2', 'Su 88-M21', and PI 227687 were identified as highly resistant, while 'Kefeng No. 1', 'Wan 82-178', 'XYXHD', 'Morsoy', 'Bethol', PI 171451, and PI 229358 were highly susceptible to BP (Cui et al. 1997b; Xing et al. 2008a). Xing et al. (2008a) conducted a genetic study of these resistance sources using segregation analysis and found that inheritance of resistance to BP was controlled by two major genes plus polygenes in three recombinant inbred line (RIL) populations.

The complex genetic and phenotypic nature of plant resistance is one of the obstacles to the increased development and use of resistant cultivars in integrated pest management (Stout and Davis 2009). Yencho et al. (2000) examined the potential utility and benefits of molecular markers that are used by entomologists, breeders, biochemists, and molecular biologists to develop insect-resistant crops. The identification of genes/QTL and the development of higher-density molecular genetic maps have facilitated the study of complex quantitative traits and the dissection of the genes that influence such traits into individual Mendelian factors. Integrated genetic linkage maps of soybean have been constructed by Cregan et al. (1999), Song et al. (2004), Choi et al. (2007), and Wang (2009). Using these or earlier soybean genetic linkage maps, QTL associated with soybean resistance to corn earworm (*H. zea*; Rector et al. 1998, 1999, 2000; Terry et al. 2000; Narvel et al. 2001), common cutworm (*S. litura*; Komatsu et al. 2005; Fu et al. 2007), pod borer (*Leguminivora glycinivorella* (Mats.) Obraztsov; Zhao et al. 2008), globular stink bug (*M. cribraria* Fabricius; Xing et al. 2008b), soybean aphid (*Aphis glycines* Matsumura; Li et al. 2007b; Mian et al. 2008; Hill et al. 2009; Zhang et al. 2009), Japanese beetle (*Popillia japonica* Newman; Yesudas et al. 2010), and whitefly (*Bemisia tabaci* Gennadius; Perez-Sackett et al. 2011) have been found. The genetic basis for resistance in PI 171451, PI 227687, and PI 229358 has been dissected in detail since DNA markers were identified in the 1990s (Rector et al. 1998, 1999, 2000; Boerma and Walker 2005). Therefore, molecular marker studies have helped to elucidate the number, effects, and interactions of insect resistance QTL, and markers are now being used in breeding programs to facilitate the transfer of resistance alleles while minimizing linkage drag (Boerma and Walker 2005).

For an effective utilization of important QTL, suitable mapping procedures for detecting QTL with various genetic effects are needed. The use of RIL populations for QTL mapping is powerful because each line is nearly homozygous, so genotyping and phenotyping of many traits under various environmental conditions can be performed on the same material (Simon et al. 2008). Different procedures use different genetic models and algorithms that may not always be appropriate for a specific population and trait because the resulting inference about the identified QTL is only a probability statement, and is not necessarily appropriate. Su et al. (2010) suggested that for practical experimental data with an unknown genetic model, a multiple model mapping strategy should be adopted, followed by verification with another procedure corresponding to the results from the original mapping procedure.

There have been very few reports on mapping soybean QTL associated with BP resistance. With the objective of conducting a thorough dissection of the genetic architecture of BP resistance in soybean, the following points were considered when designing this study: multiple RIL populations should be examined for a wide range of detection of QTL for BP resistance, the study should be conducted over multiple years to compensate for environmental fluctuations, and multiple mapping procedures should be utilized for a thorough detection of QTL with various genetic effects. Therefore, in the present study, four RIL populations were tested for 3 years using multiple mapping procedures to (1) determine the genetic components of resistance to BP, (2) detect and evaluate the relative importance of different types of resistance QTL, (3) identify major QTL, (4) analyze QTL stability across infestation dates and years (or environments), and (5) understand the implications of these QTL (and unmapped minor QTL) in breeding for BP resistance.

Materials and methods

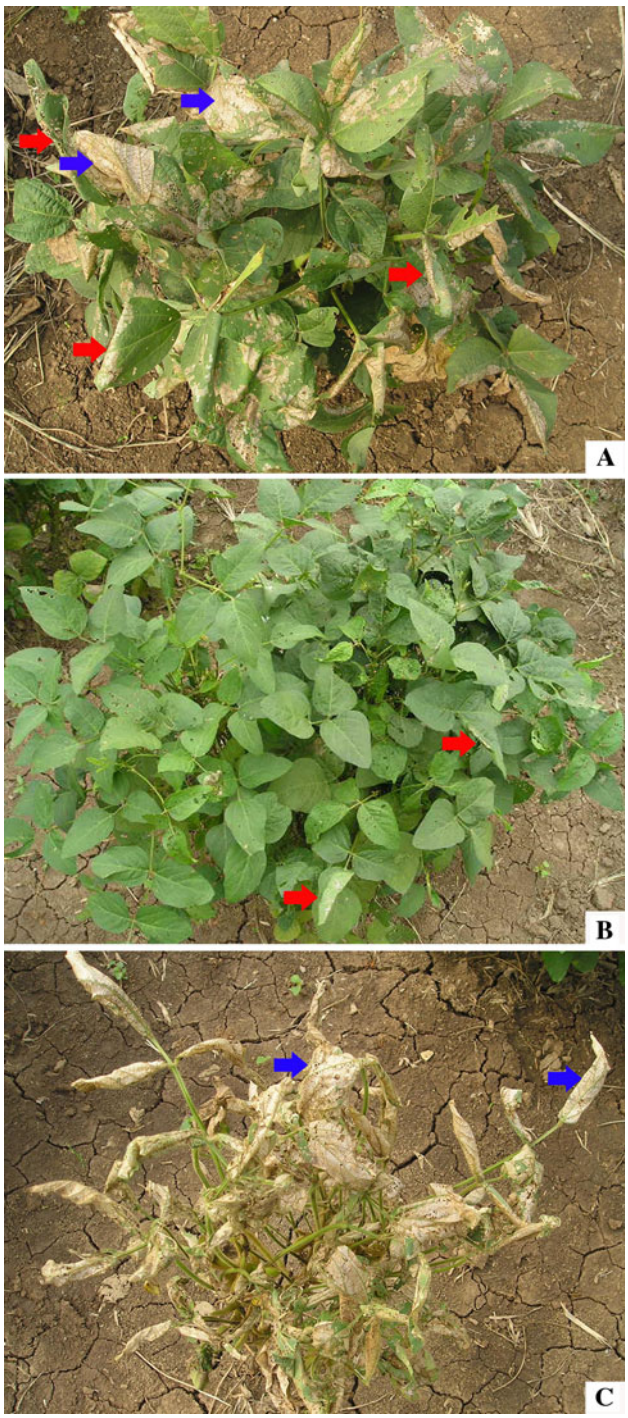
Mapping populations

To achieve a thorough dissection of the genetic architecture of BP resistance, four soybean RIL populations derived from the crosses Kefeng No. 1 × NN1138-2 (KY), Wan 82-178 × TSBPHDJ (WT), 'Xianjin 2' × Gantai-2-2 (XG), and Su 88-M21 × XYXHD (SX) were used to construct genetic linkage maps and evaluate resistance to BP. The eight parental accessions are from different eco-regions covering a wide genetic variation. Among them, Kefeng No. 1, Wan 82-178, Xianjin 2, and XYXHD are susceptible to BP, while NN1138-2, TSBPHDJ, Gantai-2-2, and Su 88-M21 are resistant to BP according to Cui et al. (1997b) and Xing et al. (2008a). The populations of KY, WT, XG, and SX consisted of 184, 142, 147, and 176 RILs, respectively, which were F_6-derived lines developed using single-seed descent from F_2 to F_5. All of the RIL populations were developed at the National Center for Soybean Improvement in Nanjing, China. Among the parents, Kefeng No. 1 is a selection from a black-seeded landrace in Maturity Group II (MG II) in Beijing that was released by the Institute of Genetics, Chinese Academy of Sciences, and that exhibits resistance to soybean mosaic virus (SMV) and tolerance to aluminum; and NN1138-2 (MG V) is a high-yielding selection from a landrace in Jiangsu that was released by Nanjing Agricultural University. The KY population has been used for QTL identification of a number of traits, including resistance to leaf-feeding insects (such as the common cutworm, *S. litura*) and SMV, tolerance to aluminum, and various agronomic traits (Zhang et al. 2004; Fu et al. 2007; Xing et al. 2008b; Korir et al. 2011). The other three pairs of parents were selected for the construction of RIL populations according to their differences in resistance to leaf-feeding insects and various agronomic traits (Cui et al. 1997b; Sun and Gai 2000; Xing et al. 2008a, b). Among these, Wan 82-178 and Xianjin 2 (both in MG III) are selections from landraces in Anhui province, XYXHD (MG III) is a landrace in Jiangsu province, TSBPHDJ (MG VI) is a landrace from Hubei province with a high protein content, and Gantai-2-2 (MG II) and Su 88-M21 (MG V) are two breeding lines from Jiangsu province. The WT and XG populations have been used to study the inheritance of resistance to *S. litura* (Sun and Gai 2000). In addition, the KY, WT, and SX populations have been used to investigate the mixed major gene and polygene inheritance of soybean resistance to BP (Xing et al. 2008a), and the former two have been used to map QTL for resistance to the globular stink bug, *M. cribraria* (Xing et al. 2008b).

Field experiments and resistance indicators

In 2004, the KY population and its parents were tested in a randomized complete block design (RCB) experiment with three 2.0-m rows per plot, a 0.5-m row spacing, and three replications. The WT population and its parents were planted in a RCB experiment with single-row plots that were 4 m long, with a 0.5-m spacing, in two replications. In 2005, the KY, WT, and XG populations and their parents were tested in RCB hill plot experiments with six replications; in 2006, the KY, WT, SX populations and their parents were planted in RCB hill plot experiments with three replications. The hill plots were planted with a 0.7 m × 0.8 m spacing, and each plot was thinned to a final stand of six plants per plot in 2005–2006. The plot design was modified each year to adjust the block size to achieve even natural infestation within a block and to minimize the whole experiment size to reduce experimental errors and expenses. All of the tests for each population were separated, and the planting dates were on 13 June 2004, 28 June 2005, and 19 June 2006 at the Jiangpu Experimental Station, Nanjing Agricultural University, Nanjing, China. To achieve an effective natural BP infestation, no insecticide was applied to the soybeans in the experimental blocks and surrounding fields.

The feeding habits of BP are quite different from those of other leaf-feeding insects in soybean. The BP larvae usually roll the leaflets, with a single insect making a single roll on a leaflet; occasionally, one insect will make a roll with two leaflets or two insects will make two rolls on the same leaflet. The insects feed on the mesophyll part of the leaflet, which results in rolled leaflets with a white surface. Thus, the leaflet rolls can be easily observed, and the percentage of rolled leaflets to total leaflets in a plot can be visually estimated (Fig. 1). Both RLN and RLP were considered damage or resistance indicators. However, in the present study, the latter was used to estimate BP damage (resistance) because it is a relative measure and is not affected by RIL plant size (or leaflet number per plant). For an accurate and consistent estimation of the RLP value, the observer first practiced collecting data from several trials to estimate the total number of leaflets and the number of rolled leaflets in order to obtain the RLP estimates. The observer then made the estimations of RLP directly from the plot without estimating the number of leaflets. In the present study, RLP was visually observed in late August, early September, and middle September. The dates of these observations shifted among populations and years. According to Xing et al. (2008a), observations made in early September did not vary much across environments; therefore, that set of data was the primary one that was analyzed in the present study, while the other data sets



◀**Fig. 1** The damage symptoms of bean pyralid on a hill plot in the middle of September. The *red arrows* indicate the leaflets rolled by BP. The larvae usually roll the leaflets, mostly with a single insect making a single roll on a leaflet, although sometimes one insect makes a roll with two leaflets or two insects make two rolls on the same leaflet. The *blue arrows* indicate leaflets that have turned white due to mesophyll damage caused by insects hidden in the leaflet rolls. **a** For this hill plot, the total number of leaflets is visually estimated as 110 and the rolled leaflets as 95; therefore, the RLP is estimated as 86 %. This line is highly susceptible to BP. **b** For this hill plot, the total number of leaflets is estimated as 200 and the rolled leaflets as 15. The RLP is estimated as 7 %. This line is highly resistant to BP. **c** This picture shows a line with almost entirely white leaflets, indicating possible severe symptoms after long-term damage. This is a highly susceptible line. For an accurate and stable estimation of the RLP value, the observer should be well practiced and experienced before estimating the total number of leaflets and the number of rolled leaflets to obtain RLP estimates. A skilled, experienced observer can make an estimation of RLP directly from the plot without estimating the numbers of leaflets (color figure online)

served as a reference. The rolled leaflet densities among lines observed in early September varied from 4 to 158 /m^2 in 2004, 5 to 264 /m^2 in 2005 and 13 to 304 /m^2 in 2006, which indicated an effective natural infestation for the study. In addition to the RLP measure, the plot yield was recorded for the evaluation of resistance (or susceptibility) allele effects on yield increases (or reductions) in populations.

Data analyses

The RLP data were arcsine-transformed prior to statistical analysis. For these data, descriptive statistics, including parent mean, difference between parents, RIL frequency distribution, mean of RIL population, coefficient of variation (CV), genotypic coefficient of variation (GCV) and heritability (h^2), and analysis of variance (ANOVA) under the random effect statistical model were performed using the SAS program (SAS Institute, Cary, NC). The expected mean squares were estimated to partition the total variance into genetic, environmental and related components. The heritability in a single environment was estimated using $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/r)$, where h^2 = heritability, σ_g^2 = genotypic variance, σ_e^2 = error variance, and r = number of replications for the trait. The heritability over environments was estimated using $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{gy}^2/n + \sigma_e^2/nr)$, where σ_g^2, σ_{gy}^2 and σ_e^2 are the genotype, genotype × environment (or year), and error components of variance, respectively, n is the number of environments, and r is the number of replications (Hanson et al. 1956). For the estimation of expected mean squares in the present study, n and r were estimated from SAS because replication numbers were different among years.

Construction of genetic linkage maps

The construction of genetic linkage maps for the four populations has been previously described by Zhang et al. (2004) and Xing et al. (2008b) for KY, Xing et al. (2008b) for WT, Wang (2009) for XG, and Zhang et al. (2008) for SX. Mapmaker 3.0 (Lander and Green 1987) or JoinMap (Van Ooijen and Voorrips 2001) was used for map construction. The KY map consists of 460 markers, including 300 simple sequence repeats (SSRs), 133 restricted fragment length

polymorphisms (RFLPs), 22 expressed sequence tags (ESTs), three SMV resistance gene loci, one sequence-characterized amplified region (SCAR), and one morphological trait locus, covering a total of 3,395.1 cM. The WT map consists of 85 markers, including 84 SSRs and one morphological trait locus, covering 1,110.3 cM. The XG map consists of 400 SSRs covering 1,412.9 cM, and the SX map consists of 134 markers, including 132 SSRs and two morphological trait loci, covering 2,097.0 cM. The markers on the constructed maps were of complete and nearly complete sets of genotypic data (without missing value or with missing values less than 2 % for most markers and less than 10 % for only a few markers), while a small number of markers with missing value more than 20 % (RFLP markers in KY population) were excluded before map construction. The nomenclature used for the linkage group (LG) was based on the common mapped SSR markers according to Cregan et al. (1999). The maps generated in this paper are nearly consistent with the consensus genetic map presented by Song et al. (2004) in SSR marker order. Some linkage groups were separated into two because no polymorphic markers were found in some regions.

QTL mapping

Following the mapping strategy of Su et al. (2010), the mixed-model-based composite interval mapping (MCIM) of QTL Network V2.0 (Yang et al. 2007, 2008) was used to detect additive QTL, additive × additive epistatic QTL pairs, additive QTL × year, and epistatic QTL pair × year interactions individually for respective populations. The arcsine-transformed RLP data from multiple years and/or a single year were analyzed for mapping QTL. The general model of MCIM incorporated the fixed terms of additive effects (A) and additive × additive epistatic effects (AA), as well as random terms for environment, additive × environment interaction, and epistasis × environment interaction effects, while the critical F value of MCIM was calculated with 1,000 permutation tests. The QTL effects were estimated using the Monte Carlo Markov Chain method with 20,000 Gibbs sampler iterations and candidate interval selection, putative QTL detection and QTL effects were calculated with an experiment-wise type I error under $\alpha = 0.05$ (Wang et al. 1994; Yang et al. 2007). A QTL was denoted as *BP1-1* where "BP" represents the abbreviation for bean pyralid resistance and 1-1 represents QTL 1 on chromosome 1 starting from the top of the chromosome. Because the genetic linkage maps of different populations might be different with respect to marker order and the corresponding distance in the same linkage group, the shared common markers anchored on the consensus genetic map developed by Song et al. (2004) were used for recognizing the same QTL within and among populations. Thus, two QTL with shared marker(s) or with position(s) close (less than 5 cM apart) to those on the consensus genetic map (Song et al. 2004) were considered to be the same QTL. The graphical linkage maps were generated with MapChart 2.1 (Voorrips 2002). To verify the MCIM results for additive QTL, composite interval mapping (CIM) and the modified algorithm of inclusive composite interval mapping (ICIM) were performed using Windows QTL Cartographer V2.5 (additive model, Wang et al. 2006) and QTL IciMapping V2.2 (additive model, Li et al. 2007a), respectively, with the LOD significance threshold determined empirically using 1,000 permutation tests.

Results

Phenotypic variation of RLP in RIL populations

In a previous study by Xing et al. (2008a), RLP evaluated in early September was chosen as the best indicator of resistance to BP because of its higher genetic variation, heritability value, stability throughout environments, and negative correlation with seed yield. Accordingly, in this study, we mainly analyzed the early September RLP data. A significant difference in resistance to BP between the parents was observed (Table 1). The RLP phenotypic performance of the RIL populations was continuously distributed and relatively consistent across the 3 years, with a large variation (more than 23 % of the genetic coefficient of variation [GCV]), but no large transgressive segregation was observed (Table 1). The ANOVA results (data omitted) for the RLP data showed a significant difference among lines and blocks in both KY and WT. A significant difference was observed for line × year in WT ($F = 1.94^{**}$, $P < 0.0001$), although it was not significant in KY ($F = 1.10$, $P = 0.12$). However, the estimated variances of line × year in WT and KY were both much smaller than what was observed among the RILs (σ^2_{gy}:σ^2_g = 31.6:264.5 in WT and 3.5:118.8 in KY, respectively), indicating that the responses of RILs to BP in 3 years were not very different in the two populations. Based on this information, the results for XG and SX, which were tested only in 1 year, with significant differences observed among lines and blocks, should also be relevant. The heritability values on an entry-mean basis in a single year estimated from ANOVA for RLP ranged from 76 to 93 % in different populations (Table 1), which suggests that genetic variation accounts for a major part of the phenotypic variance in all four populations.

Genetic constitution of resistance to BP

Table 2 shows the MCIM results for QTL Network-detected additive QTL in the four populations and

Table 1 Frequency distribution of RLP and descriptive statistics of four RIL populations

Population	Year	Class midpoint									Mean	$R - S$	Range	CV (%)	GCV (%)	h^2 (%)
		5	15	25	35	45	55	65	75	85						
KY	2004		21, Y	61	57	30	9, K	3	1		32.6	37.2	12.5–77.7	22.6	31.7	85.5
	2005	2	26, Y	63	46	29	14	3, K	1		32.3	58.7	9.1–70.9	39.3	35.8	83.2
	2006		18, Y	60	52	31	12	6, K	5		35.0	46.5	14.9–79.5	33.7	34.1	75.5
	M		17, Y	66	52	31	13	5, K			33.3	47.5	13.8–69.7	34.9	33.1	89.8
WT	2004		10	24, T	24	32	16	15	10, W	11	46.5	52.2	11.4–90.0	20.4	41.0	89.0
	2005	7	40, T	35	28	14, W	12	6			29.4	38.7	6.8–64.3	34.7	49.7	92.5
	2006	3	25, T	38	23	14	19	11, W	7	2	36.9	46.6	6.5–82.6	30.6	47.4	87.8
	M		26, T	29	32	20	15	14, W	6		37.6	45.8	10.8–76.3	30.1	42.1	92.1
XG	2005		19	89, G	30, X2	8	1				26.8	7.2	15.3–52.1	27.4	23.1	81.0
SX	2006		21	46, S	43	32	22, X1	9	3		36.4	33.2	11.9–75.4	26.4	37.0	85.4

The RLP data are arcsine-transformed in this and all other tables and figures

Class midpoint, the midpoint of each class in the RLP frequency distribution

M the distribution of average data over 3 years, *K* Kefeng No. 1, *Y* NN1138-2, *W* Wan 82-178, *T* TSBPHDJ, *X2* Xianjin 2, *G* Gantai-2-2, *S* Su 88-M21, *X1* XYXHD, $R - S$ the difference between the resistant and susceptible parent, *CV* coefficient of variation, *GCV* genetic coefficient of variation, h^2 the heritability value was obtained from ANOVA

Table 2 Contributions of QTL and their interactions to phenotypic variation for resistance to BP in soybean (%)

Population	Genetic contribution				Add. QTL × year	Epistatic QTL × year	Environment[c]	Total
	Additive QTL	Epistatic QTL	Minor QTL[a]	Total[b]				
KY	42.8 (47.6) (5)	5.0 (5.6) (3)	42.0 (46.8)	89.8	1.3	0.3	8.6	100
WT	41.8 (45.4) (4)	12.6 (13.7) (5)	37.7 (40.9)	92.1	1.3	1.1	5.5	100
XG	40.8 (50.4) (1)		40.2 (49.6)	81.0			19.0	100
SX	27.0 (31.6) (1)		58.4 (68.4)	85.4			14.6	100

The numbers in the first pair of parentheses in the columns of "Additive QTL" and "Epistatic QTL" are the contributions of the QTL to the total genetic variation, while those in second pair of parentheses are the numbers of QTL or QTL pairs, respectively

[a] Minor QTL represents the genetic contribution of a collection of unmapped minor QTL, Minor QTL = total genetic contribution − variation explained by all detected additive QTL and epistatic QTL. The numbers in parentheses are the contributions of minor QTL to the total genetic variation

[b] The total genetic contribution in KY and WT was obtained from ANOVA based on 3-year data, and those in XG and SX were obtained from ANOVA with 1-year data

[c] Environment = total phenotypic variation − total genetic contribution − variation explained by all detected additive QTL × year and epistatic QTL × year

additive × additive epistasis QTL pairs in KY and WT, with the former accounting for 27–43 % of the phenotypic variation (PV) and the latter accounting for 5–13 % of PV. In total, the two parts account for 27–54 % of the PV; however, the genetic contribution to the PV estimated from ANOVA over the years was 81–92 %, and a difference of 38–58 % was observed between the total genetic variation and that of the detected QTL. This result is most likely due to the differences in the genetic models between the two procedures because the same set of data was used (Korir et al. 2011). Here, the total genetic variance among the lines estimated from ANOVA should include all types of QTL with genetic effects of additive and additive-by-additive interaction in RIL populations, while the genetic variation estimated from MCIM includes only the detected additive QTL and the additive-by-additive epistatic QTL pairs. Therefore, the difference of genetic variation between the two procedures should be the genetic variation due to some additive QTL and additive-by-additive epistatic QTL pairs with smaller effects that could not be detected individually and significantly under the experiment precision conditions by MCIM. Here, we propose this part of the genetic constitution as a collection of unmapped minor QTL that have been previously identified as polygenes based on biometrical genetics. This part of genetic variation may be subject to fluctuation because some QTL with large effects might be included under an inaccurate mapping or might be excluded under an accurate study.

This finding is very meaningful for understanding the relative importance of mapped QTL in a genetic system of the population using mapping procedures combined with ANOVA. Therefore, Table 2 shows a dissection of the phenotypic variation into genetic contribution, additive QTL × year, epistatic QTL × year, and environmental variation. The amounts of additive QTL × year and epistatic QTL × year were relatively small, while among the genetic components, the additive QTL were the most important, followed by the collective minor QTL and the epistatic QTL, the latter of which accounted for only a small part of the genetic variation. It is obvious that the genetic components were different from each other among the populations (Table 2).

QTL detection in the populations

Table 3 and Fig. 2 show that the QTL on chromosome (Chr) 12 (or LG H) in the KY, WT, and XG populations can be considered the same (*BP12-1*) because there was a commonly linked marker between KY and WT (Satt434) and between KY and XG (Sat_218). The QTL on Chr 1 (LG D1a) detected in the KY and SX populations was also considered the same (*BP1-1*) because the marker Satt531 was common to both KY and SX and because Sat_346 in SX was close to Satt321 in KY on the consensus genetic maps. A QTL with additive effect and a QTL involved in epistasis sharing commonly linked markers in a population were also considered the same. Thus, the additive QTL and epistatic QTL that shared the same marker region, Satt482–KNBS22I, were recognized as the same QTL *BP1-1* in the KY population: in other words, *BP1-1* performed as a QTL with both additive and epistasis effects; similarly, *BP4-1* and *BP12-1* were recognized to perform as QTL with both additive and epistasis effects due to sharing the same marker regions, respectively, in the KY population, and so for *BP2-1*, *BP10-2,* and *BP20-1* in the WT population (Tables 3, 4, 5). It should be noted that *BP2-1* of WT and *BP2-2* of KY are recognized as different QTL because they are located in different marker regions with a distance of more than 10 cM between their nearest support interval boundaries on D1b of the consensus genetic map (Song et al. 2004), even though their positions on respective maps are similar (Table 3; Fig. 2).

All the detected QTL were compiled in Table 5; a total of 17 QTL were detected on 11 chromosomes (linkage

Table 3 Additive QTL of resistance to BP in the KY, WT, XG, and SX RIL populations

LG	QTL	Marker region	Position	Support interval	A	*P* value	AE1	AE2	AE3	$h^2_{(a)}$(%)	$h^2_{(ae)}$ (%)	Carto-grapher[a]	IciMapping[a]
					KY								
D1a	*BP1-1*	**Satt482**–KNBS22I	12.0	8.4–15.0	4.6	0.0000				10.3	0.4	1, 2, 3, m	1, 2, 3, m
D1b	*BP2-2*	Satt157–Satt558	5.4	2.4–8.4	3.9	0.0000				7.8	0.4	1, 2, 3, m	1, 2, m
C1	*BP4-1*	LE39T–Satt607	100.6	86.1–110.6	−2.0	0.0001				2.2	0.3	m	m
C2	*BP6-2*	A748V–A397I	85.2	78.2–91.6	3.5	0.0000				10.0	0.0	1, 2, 3, m	1, 2, 3, m
H	*BP12-1*	**Sat_218–Satt434**	34.7	30.7–43.0	4.0	0.0000				12.5	0.3	1, 2, 3, m	1, 2, 3, m
					WT								
D1b	*BP2-1*	Sat_351–BE475343	4.7	0.7–14.7	4.5	0.0000				5.7	0.0	2	2
O	*BP10-2*	BE801128–Satt331	101.0	90.0–110.5	3.3	0.0000				3.1	0.4	1, 2	
H	*BP12-1*	**Satt181–Satt434**	68.2	64.2–72.2	10.5	0.0000		−2.0		28.8	0.8	1, 2, 3, m	1, 2, 3, m
I	*BP20-1*	AB002807–Satt614	13.0	4.0–21.6	3.5	0.0000				4.3	0.1	1	
					XG								
H	*BP12-1*	GNE229–**Sat_218**	45.9	44.9–47.0	4.4	0.0000				40.8		2	2
					SX								
D1a	*BP1-1*	*Pap*–**Sat_346**	21.6	17.0–24.6	−7.8	0.0000				27.0		3	3

Boldface indicates markers that are closely linked in the corresponding linkage group of the consensus genetic map (Song et al. 2004). *BP2-1* and *BP2-2* are recognized as different QTL because they locate in different marker regions with a distance more than 10 cM between their nearest support interval boundaries on D1b of the consensus genetic map, even though their positions on respective maps are similar (see Fig. 2)

LG linkage group, *marker region* the flanking markers of the QTL, *position* the distance between QTL and the first marker of the relevant linkage group, *support interval* the confidence interval of the QTL position calculated with an experiment-wise type I error under alpha = 0.05, here QTL Network uses *F* test to test the QTL effects rather than LOD score with the critical *F* value calculated from 1,000 permutation tests, *A* the estimated additive effect, *AE1*, *AE2*, and *AE3* additive by yearly interaction effects for 2004, 2005, and 2006, respectively, $h^2_{(a)}$ phenotypic variation explained by the additive QTL, $h^2_{(ae)}$ phenotypic variation explained by the additive × year interaction effects

[a] The numbers 1, 2, 3, and m indicate that the QTL was detected in 2004, 2005, 2006 and over 3 years, respectively. An underlined number or m indicates that the QTL was detected in the linkage group, but the position is out of the confidence interval obtained from QTL Network V2.0

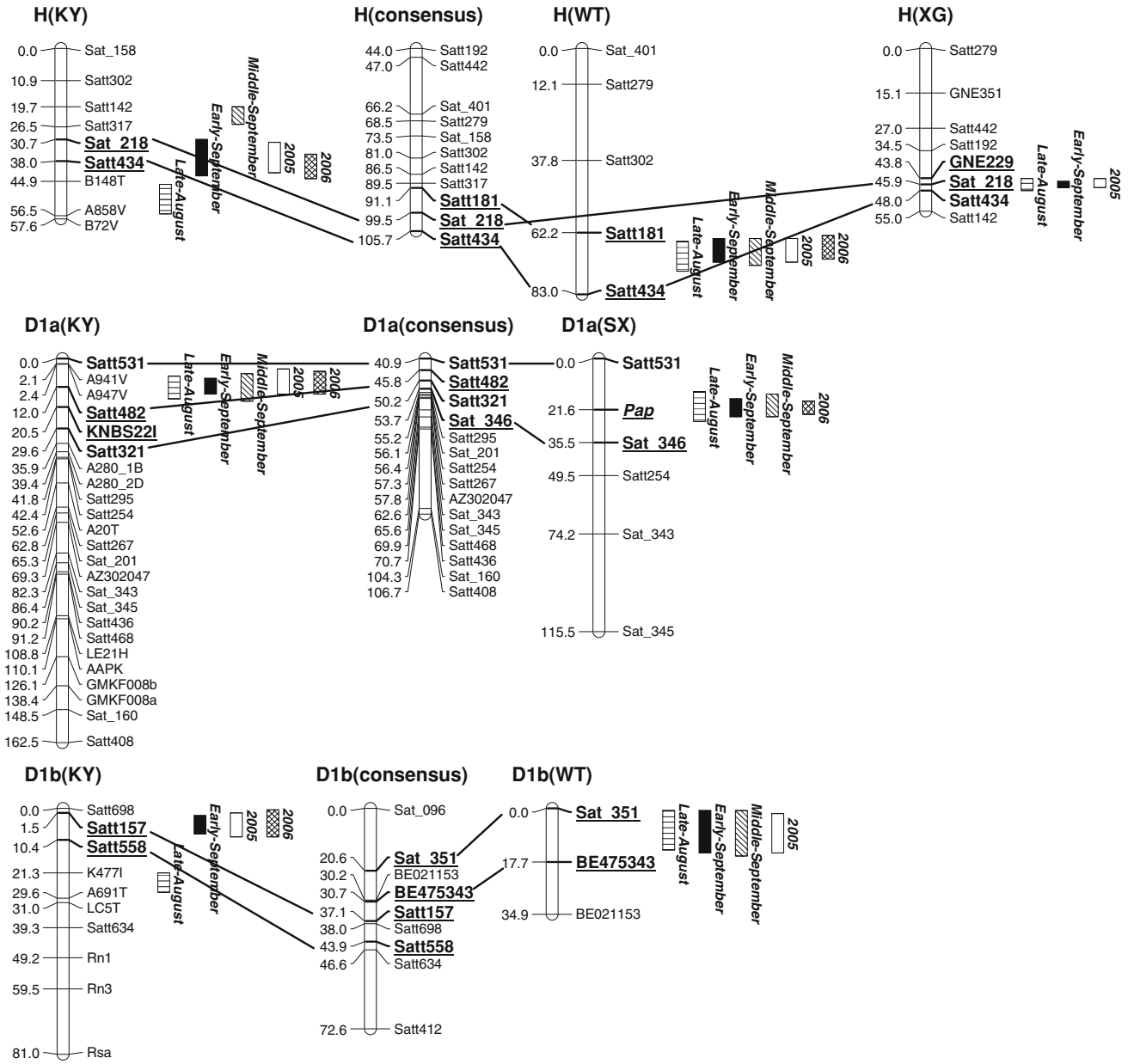


Fig. 2 Locations of QTL conferring resistance to BP in soybean detected with the MCIM procedure. The *upper part* indicates the locations of *BP12-1* across the KY, WT, and XG populations compared with Song et al.'s (2004) consensus genetic linkage map; the *middle part* indicates the locations of *BP1-1* across the KY and SX populations compared with Song et al.'s (2004) consensus genetic linkage map; the *lower part* indicates that *BP2-1* of WT and *BP2-2* of KY are located at distant places on D1b of the consensus genetic map even though their positions on respective maps are similar. QTL are marked with *bars*. The *bar length* represents the support interval of a QTL. The *bars with horizontal lines* represent the QTL detected from the data collected in late August in 2004–2006. The *solid black bars* represent the QTL detected from the data collected in early September in 2004–2006. The *bars with slash lines* represent the QTL detected from the data collected in the middle of September in 2004–2006. The *blank bars* represent the QTL detected for all three dates in 2005. The *bars with cross lines* represent the QTL detected for all three dates in 2006. In *parentheses* beside a linkage group, the KY, WT, SX, XG, and consensus indicate the source population of the respective linkage group. The flanking markers of a QTL are in *boldface* and *underlined*, with its support interval (bar length) at the *right side* of each LG. The locations of the *boldface* markers are closely linked on the corresponding linkage group of Song et al.'s (2004) consensus genetic linkage map

groups), among which two had additive effects, six had both additive and epistatic effects, and nine had only epistatic effects. Eight epistatic QTL pairs were observed, among which three pairs involved two QTL with additive effects, one involved one QTL with additive effect, and four involved no QTL with additive effects (Tables 4, 5). A QTL may have an additive effect, an epistatic effect, or both, and an epistasis QTL pair may be composed of zero,

Table 4 Epistatic QTL pairs conferring resistance to BP in the KY and WT populations

Pair	QTL	LG	Marker region	Position	Support interval	AA	*P* value	$h^2_{(aa)}$ (%)	$h^2_{(aae)}$ (%)
				KY					
BP-e1	*BP1-1*	D1a	Satt482–KNBS22I	12.0	8.4–15.0	1.8	0.0002	1.3	0.0
	BP12-1	H	Sat_218–Satt434	34.7	30.7–43.0				
BP-e2	*BP5-1*	A1	Satt648–K418_2V	86.8	82.5–91.8	2.0	0.0000	1.9	0.3
	BP20-2	I	Satt440–A644V	101.7	95.0–107.7				
BP-e3	*BP4-1*	C1	LE39T–Satt607	100.6	86.1–110.6	2.7	0.0000	1.8	0.0
	BP18-1	G	LD6T–K11_2T	235.7	231.7–235.7				
				WT					
BP-e4	*BP2-1*	D1b	Sat_351–BE475343	4.7	0.7–14.7	3.5	0.0000	2.0	0.2
	BP20-1	I	AB002807–Satt614	13.0	4.0–21.6				
BP-e5	*BP2-1*	D1b	Sat_351–BE475343	4.7	0.7–14.7	3.8	0.0000	1.3	0.1
	BP10-2	O	BE801128–Satt331	101.0	90.0–110.5				
BP-e6	*BP4-2*	C1	Sat_311–Satt338	53.7	38.7–53.7	3.4	0.0000	2.1	0.2
	BP17-1	D2	Satt372–Satt135	2.0	0.0–15.0				
BP-e7	*BP6-1*	C2	Sat_246–Satt643	11.6	9.0–16.6	4.1	0.0000	3.2	0.3
	BP10-3	O	Satt331–Sat_190	122.5	114.5–126.5				
BP-e8	*BP13-1*	F	Sat_298–Satt160	107.3	96.3–117.3	−6.3	0.0000	4.0	0.3
	BP10-1	O	Satt479–BE801128	44.8	34.7–57.8				

Pair epistatic QTL pair, *LG* linkage group, *marker region* the flanking markers of QTL, *position* the distance between a QTL and the first marker of the relevant chromosome, *support interval* the confidence interval of the QTL position calculated with an experiment-wise type I error under alpha = 0.05 as indicated in a note of Table 3, *AA* the estimated additive by additive epistatic effect, $h^2_{(aa)}$ the phenotypic variation explained by additive × additive interaction, $h^2_{(aae)}$ the phenotypic variation explained by *aa* × environment interaction

The epistatic effect of parental two-locus genotypes is positive, while that of non-parental two-locus genotypes (recombinants) is negative. The underlined QTL denotes a QTL with an additive effect

one, or two QTL with additive effects; therefore, the additive QTL and the epistatic QTL are uniform by nature.

The additive QTL for BP resistance in the four populations detected with MICM of QTL Network V2.0 are shown in Table 3 and Fig. 2. There were eight QTL in KY with five showing additive effects and three epistatic pairs, and there were ten QTL in WT with four showing additive effects and five epistatic pairs; however, only one additive QTL was detected in both XG and SX (Tables 3, 4, 5). Figure 2 illustrates that the locations of QTL *BP12-1* and *BP1-1*, as well as *BP2-1* and *BP2-2*, were relatively consistent among/between years and dates, indicating that the detection of major QTL was relatively stable.

The KY population

Five QTL with additive effects were mapped on Chr 1, 2, 4, 6 and 12 (formerly LGs D1a, D1b, C1, C2, and H, respectively). The QTL *BP12-1* mapped between Sat_218 and Satt434 on Chr 12 (LG H) and showed the largest contribution to PV (13 %, Table 3). The QTL *BP1-1* mapped to a 6.6-cM interval between Satt482 and KNBS22I on Chr 1 (LG D1a) and accounted for 10 % of PV. Another additive QTL (*BP6-2*) that accounted for 10 % of PV was detected between A748V and A397I on Chr 6 (LG C2). In addition, two other QTL, *BP2-2* on Chr 2 (LG D1b) and *BP4-1* on Chr 4 (LG C1), contributed 8 and 2 % of PV, respectively. The above results correlated well with those obtained from CIM of Windows QTL Cartographer V2.5 and ICIM of IciMapping V2.2 (Table 3). Four resistance alleles at the five loci came from NN1138-2, while another resistance allele on Chr 4 was from Kefeng No. 1. No additive × environment (AE) interaction was observed for *BP6-2*, and the interactions observed for other four QTL were small in this population (Table 3).

Three epistatic QTL pairs in KY were detected, and all six loci were distributed on different linkage groups; in total, these accounted for 5 % of PV (Tables 2, 4). Among the three epistatic pairs, both components of the pair *BP-e1* [*BP12-1* on Chr 12 (LG H) and *BP1-1* on Chr 1 (LG-D1a)] had additive effects, while the two components of the pair *BP-e2* did not have additive effects, and *BP-e3* had one QTL (*BP4-1*) with an additive effect (Tables 4, 5). For the three detected additive × additive interactions (*BP-e1–BP-*

Table 5 QTL detected in populations with additive or epistatic effects

Chromosome code	Linkage group	QTL	KY		WT		XG	SX
			Additive effect	Epistatic effect	Additive effect	Epistatic effect	Additive effect	Additive effect
1	D1a	*BP1-1*	a	a × a (*BP12-1*)				a
2	D1b	*BP2-1*			a	a × a (*BP20-1*)		
						a × a (*BP10-2*)		
		BP2-2	a					
4	C1	*BP4-1*	a	a × a (*BP18-1*)				
		BP4-2				a × a (*BP17-1*)		
5	A1	*BP5-1*		a × a (*BP20-2*)				
6	C2	*BP6-1*				a × a (*BP10-3*)		
		BP6-2	a					
10	O	*BP10-1*				a × a (*BP13-1*)		
		BP10-2			a	a × a (*BP2-1*)		
		BP10-3				a × a (*BP6-1*)		
12	H	*BP12-1*	a	a × a (*BP1-1*)	a		a	
13	F	*BP13-1*				a × a (*BP4-1*)		
17	D2	*BP17-1*				a × a (*BP4-2*)		
18	G	*BP18-1*		a × a (*BP4-1*)				
20	I	*BP20-1*			a	a × a (*BP2-1*)		
		BP20-2		a × a (*BP5-1*)				

a QTL with additive effect, *a* × *a* this QTL interacted with the QTL in parentheses

e3), the parental types of the two interacting loci were susceptible, while the recombinant types were resistant. No additive × additive × environment (AAE) interaction was observed for *BP-e1* and *BP-e3*, and the interaction observed for *BP-e2* was small in this population.

The WT population

Four QTL with additive effects were mapped to Chr 2, 10, 12, and 20 (LG D1b, O, H, and I, respectively), and they contributed 6, 3, 29, and 4 % to the PV, respectively, with *BP12-1* on Chr 12 being the predominant QTL for resistance to BP (Table 3; Fig. 2). *BP12-1* in WT was also confirmed by Windows QTL Cartographer V2.5 and QTL IciMapping V2.2 and was continuously detected throughout the 3-year study period (Table 3). All resistance alleles at the four loci came from the parent TSBPHDJ. No AE interaction was observed for *BP2-1*, and the interactions observed for other three QTL were small in this population (Table 3).

Epistasis analysis indicated that there were five significant additive × additive interaction QTL pairs for resistance to BP in WT involving nine loci in seven chromosomes; these accounted for 13 % of the PV (Tables 2, 4). One pair of epistatic QTL (*BP-e4*) was composed of two additive QTL, *BP2-1* on Chr 2 (LG D1b) and *BP20-1* on Chr 20 (LG I). Another epistatic QTL pair (*BP-e5*) was also composed of two additive QTL, *BP2-1,* and *BP10-2* (Tables 4, 5). Here, *BP2-1* was involved in both pairs and had epistatic effects with two different QTL. The other three epistatic QTL pairs (*BP-e6–BP-e8*) did not display additive effects. Among the four pairs of detected additive × additive interactions (*BP-e4–BP-e7*), the parental types of the two interacting loci were susceptible, while the recombinant types were resistant. A fifth pair of loci (*BP-e8*) was detected as an exception in which the opposite scenario was observed. For the five pairs, a small negligible variation in AAE was observed in this population.

The XG population

Only *BP12-1* on Chr 12 (LG H) was associated with BP resistance in this population (Table 3; Fig. 2). The QTL was also detected by Windows QTL Cartographer V2.5 and QTL IciMapping V2.2, and the resistance allele was from Gantai-2-2.

The SX population

Only *BP1-1* on Chr 1 (LG D1a) was associated with BP resistance in this population (Table 3; Fig. 2). The QTL was also detected by Windows QTL Cartographer V2.5 and QTL IciMapping V2.2, and the resistance allele was from Su 88-M21.

The major QTL in each population and their allelic effects

According to the above results, *BP12-1* and *BP1-1* are the major QTL in the populations studied here; *BP12-1* accounted for 14, 29, and 41 % of the PV and 15, 31, and 50 % of the total genetic variation in the KY, WT, and XG populations, respectively, and *BP1-1* accounted for 12 and 27 % of PV and 13 and 32 % of genetic variation in the KY and SX, respectively (with both additive and epistatic effects included). Small interaction effects between genotype and environment were detected for the two QTL in KY and WT, but all were negligible.

To verify the allelic differences, the allelic effects of RLP for *BP12-1* and *BP1-1* were roughly estimated by grouping the RILs into two allele type groups at the nearest marker locus. In this way, both additive and epistatic effects were included in the allelic effect. The *t* tests (based on an error mean square from ANOVA) showed that significant differences existed between the two alleles of the two respective loci in the populations. Figure 3 shows that the resistance allele caused consistently less RLP than the susceptible allele across observation dates, years, and populations.

The allelic differences for RLP might be related to allelic differences for yield. The allelic effect on yield was estimated and tested in the same way as that for RLP. Table 6 illustrates the yield reduction due to the susceptible allele. It was highly significant for the two loci across the 3 years (for *BP12-1*, we observed 17.7–19.0, 22.8–40.9, and 19.3 % yield reductions in the KY, WT, and XG populations, respectively; for *BP1-1,* we observed 16.7–21.3 and 18.2 % yield reductions in the KY and SX populations, respectively). An exception to this was *BP12-1* in the KY population in 2004, in which the yield reduction was not significant (6.8 %). It is very likely that the yield reduction results were due to susceptible alleles because the yield data from the row plots in 2004 and the hill plots in 2005 and 2006 (all utilizing a randomized block design) showed the same trend, and the significant differences were recognized across almost all of the tested populations and years for the two loci regardless of replication number or type of plot used (row or hill). This trend was true even for *BP12-1* in the KY population in 2004, in which the yield reduction due to the susceptible allele was not significant (up to 6.8 %).

Additionally, because *BP12-1* and *BP1-1* interacted as an epistatic QTL pair (*BP-e1*) in the KY population, their epistatic effect can be roughly estimated based on the effect of the interaction between the RIL marker type groups. The RILs were classified into four genotypic groups according to the allele types of tightly linked markers (Sat_218 for *BP12-1* and Satt482 for *BP1-1*) (Figs. 2, 4). A general linear model analysis using the marker genotype as the predictor variable and RLP data as the response variable was performed for the two-way ANOVA, which revealed that there were significant interactions between *BP12-1* and *BP1-1* in all 3 years. The results verified that there was a positive interaction between the two loci that was consistent throughout the years and coincided with the results shown in Table 4. A positive epistatic effect of additive × additive (AA) interactions indicates that the two epistatic loci with homozygous alleles from the susceptible parent could increase RLP or reduce BP resistance. Meanwhile, a negative AA indicates that the two epistatic loci with one allele from a resistant parent and another from a susceptible parent could increase RLP.

Differences in genetic constitution among the four populations

Tables 2 and 5 summarize the results of the genetic dissection of resistance to BP, which was mentioned in the above text. The genetic constitutions of the four populations were different from each other. For the detected QTL, the major QTL *BP12-1* was found in the KY, WT, and XG populations, and *BP1-1* was found in the KY and SX populations. The other QTL performed as additive or epistatic QTL in the KY and WT populations, respectively. Six loci, *BP1-1*, *BP2-1*, *BP4-1*, *BP10-2*, *BP12-1,* and *BP20-1* had both additive and epistatic effects, especially *BP2-1*, which had epistatic effects with two QTL. The KY, WT, and XG populations shared the same QTL *BP12-1*, but the number of QTL detected in the KY and WT populations was more than that in the XG population. Similarly, the KY and SX shared the same QTL *BP1-1*, but the number of QTL detected in the KY was more than that in the SX population. Thus, the KY population has five additive QTL detected with both the major QTL, *BP12-1* and *BP1-1*, which is more than four additive QTL detected with the major QTL *BP12-1* only in WT, and in turn more than only one major QTL detected in XG and SX, respectively, therefore, has the most important genetic components that could confer a phenotype of BP resistance.

The genetic contribution of major QTL is only a part of the total genetic variation. Another large genetic contribution came from a collection of unmapped minor QTL, which accounted for approximately 41–68 % of the total genetic variation.

The above results highlight 17 QTL with large or small additive and epistatic effects and a collection of unmapped minor QTL that are involved in resistance to BP in the four populations. The obtained information on the genetic constitution of the populations is not necessarily a complete scenario because the population sizes, marker numbers, and years tested are different among the populations and may not be large enough for analysis. Thus, more QTL may be detected if the mapping conditions could be improved; this would ensure that the genetic contribution from the collective unmapped minor QTL would be reduced.

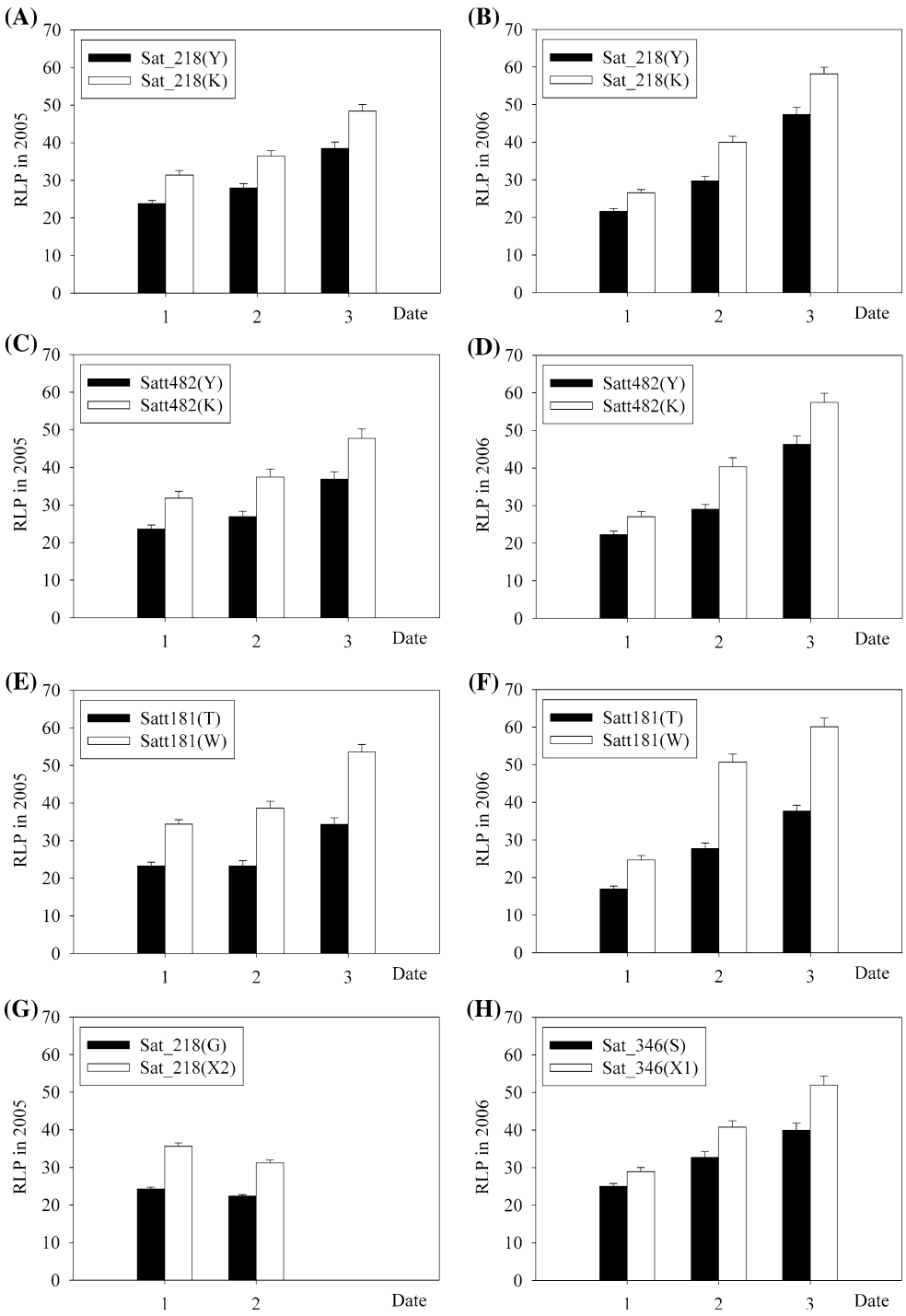


Fig. 3 Allelic effects of *BP12-1* and *BP1-1* at different dates in different populations estimated from their nearest marker genotypes. **a**, **b** Allelic effects of *BP12-1* on different dates in the KY population estimated from Sat_218 in 2005 and 2006, respectively; **c**, **d** allelic effects of *BP1-1* on different dates in the KY population estimated from Satt482 in 2005 and 2006, respectively; **e**, **f** allelic effects of *BP12-1* on different dates in the WT population estimated from Satt181 in 2005 and 2006, respectively; **g** allelic effects of *BP12-1* on different dates in the XG population estimated from Sat_218 in 2005; **h** allelic effects of *BP1-1* on different dates in the SX population estimated from Sat_346 in 2006. *1* late August, *2* early September, *3* middle September. In the legend, the *letter in parentheses* denotes the source parent of the allele, where *K* Kefeng No. 1, *Y* NN1138-2, *W* Wan82-178, *T* TSBPHDJ, *X2* Xianjin 2, *G* Gantai-2-2, *S* Su 88-M21 and *X1* XYXHD

Discussion

Relative reliance on the mapping results

Both data precision and mapping strategy may affect the mapping results. In the present study, two major factors might influence the phenotypic precision. They are the measurement precision of the resistance indicator RLP under natural infestation, and changes of plot and experiment design among years.

Data shown in Table 1 provide an evaluation of the relative precision of the experiment. Here both the resistant and susceptible parents can be considered as checks. The descriptive statistics in Table 1, including parent means, differences between parents, RIL frequency distributions, means of RIL populations, CVs, GCVs and h^2s are

Table 6 Average yield effect of alleles of *BP12-1* and *BP1-1* estimated with their nearest marker genotypes

Locus and LG	Population (marker)	Year	Plot (reps)	*R* (kg ha^{-1})	*S* (kg ha^{-1})	Difference (%)	*t* value
BP12-1 H	KY (Sat_218)	2004	Row (3)	656.3	611.7	6.8	1.5
		2005	Hill (6)	1,042.9	844.6	19.0	4.6**
		2006	Hill (3)	583.9	480.4	17.7	3.3**
	WT (Satt181)	2004	Row (2)	708.5	547.0	22.8	3.7**
		2005	Hill (6)	1,080.4	707.1	34.5	6.7**
		2006	Hill (3)	550.0	325.0	40.9	6.7**
	XG (Sat_218)	2005	Hill (6)	1,200.0	967.9	19.3	6.3**
BP1-1 D1a	KY (Satt482)	2004	Row (3)	691.3	574.7	16.9	3.3**
		2005	Hill (6)	1,050.0	875.0	16.7	3.1**
		2006	Hill (3)	603.6	475.0	21.3	3.3**
	SX (Sat_346)	2006	Hill (3)	835.7	683.9	18.2	3.6**

Difference (%) = $(R - S)/R \times 100$ (%)

Reps replications, *R* RILs with marker alleles from the resistant parent, *S* RILs with marker alleles from the susceptible parent

** Significance at the 0.01 probability level

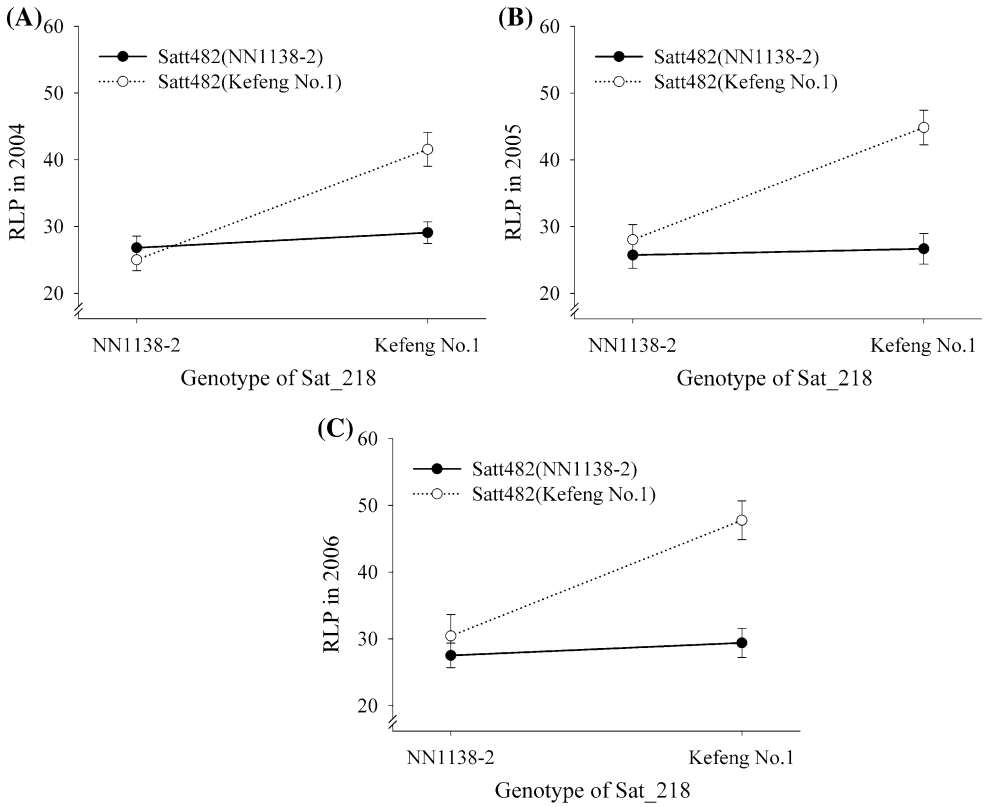


Fig. 4 Positive interaction of RLP between the two markers nearest to *BP12-1* and *BP1-1* of the epistasis QTL pair *BP-e1* in the KY population. **a** 2004, **b** 2005, **c** 2006. Sat_218 and Satt482 are the nearest markers for *BP12-1* and *BP1-1*, respectively

relatively consistent among years in KY and WT, respectively. Using CV estimated from error mean square as an indicator of random error on per plot basis, it ranges from 22.6 to 39.3 % for KY and 20.4 to 34.7 % for WT among individual years, while those from a joint ANOVA are 34.9 and 30.1 % for KY and WT, respectively, which ranks a small to medium size random error. Using heritability as a measure of relative importance of genetic variation versus random error variation, the heritability values for joint data are all around 90 % in KY and WT, and those for single year are more than 81 % except 1 year in KY (75.5 %). This indicates that the random error variation is relatively small in comparison with that of genetic variation. All the above facts indicate the relative reliability of natural infestation and therefore the relative precision and consistency of the data and the estimated parameters among years. Here the heritability estimates in the present study are also higher than those reported in the literature. For

example, Rufener et al. (1989) obtained the heritability value of 33–48 % for antibiosis resistance to Mexican bean beetle (*E. varivestis*) using F_1, F_2, and F_3 generations (five F_3 plants per F_2 plant) of crosses between 'Williams' (susceptible) and L76-0049, L78-608, and L76-0328 (resistant); Komatsu et al. (2004) obtained the heritability value of 71.3 % for common cutworm (*S. litura*) resistance using 143 F_2 plants, two plants from each parent and two F_1 plants. It is obvious that the experiment error on per plant basis must be larger than that on per plot (multiple plants) basis, and in turn larger than that on per multiple plot basis and that on per multiple plot multiple environment basis as in the present study and in Yesudas et al. (2010) where the heritability estimates of resistance to Japanese beetle (*P. japonica*) in soybean were also as high as 82–95 % using RIL population on per experiment basis.

As for the influence from the changes of plot design in the joint ANOVA over 3 years for KY and WT, the data were analyzed under random statistical model. The *F* tests showed all effects were significant except year × line interaction in KY. Statistically, the plot design effect is confounded with the year effect and their joint variation (as the year term) can be subtracted from the total variation in joint ANOVA. The possible influence of plot design changes is to cause plot design × line interaction which confounds with the real year × line interaction making the year × line term in joint ANOVA inflated and therefore causing the test for RIL variation to be less sensitive. This is because the year × line component is included in the error term used for the significance test. Since the estimated year × line mean square (MS, plot design effect confounded in) was relatively small and the CVs estimated from joint ANOVA (experiment error) were relatively small also, the plot design changes, in fact, did not cause much additional variation, and therefore did not influence the significance test for line variance. Furthermore, the genotypic variance of RILs was estimated from the line MS subtracted with those of year × line and experiment error, i.e. $\sigma_g^2 = \sigma_p^2 - \sigma_{gy}^2/n - \sigma_e^2/nr$, where $\sigma_p^2 = \mathrm{MS(RIL)}/nr$, since $\mathrm{MS(RIL)} = \sigma_e^2 + r\ \sigma_{gy}^2 + nr\ \sigma_g^2$. Thus, after the year × line variation (including plot design × line interaction) being removed, the genotypic variance of RILs was used to calculate the heritability values (Tables 1, 2). As indicated above, the high heritability values imply the relative accuracy of the genetic analysis.

Regarding the genotypic data (genetic maps) of the tested populations, they are not saturated, but are relatively consistent with the consensus map. The 17 mapped QTL, including the eight epistatic QTL pairs on 11 LGs in the four populations are a conservative result, relative only to the established maps and may have more loci detected if the genetic maps are more saturated. Among the identified QTL, some major QTL detected from the joint data over 3 years were also detected in individual years, even under different mapping procedures. In particular, *BP12-1* and *BP1-1* were found in a similar region within their confidence intervals in two or three populations (Table 3). Thus, we treated the detected QTL with different emphasis, with the QTL *BP12-1* and *BP1-1* confirmed and analyzed further for their average effects, while the others were mentioned, but left for further confirmation in a future study.

In summary, the outcomes of both experimental error analysis (ANOVA) and QTL mapping are reasonable and relatively stable, but with certain fluctuation and not being fully explored, it implies the relative reliance on natural infestation, as well as the relative reliance on the results obtained.

Strategic considerations for QTL mapping

As indicated above, mapping strategy may be another factor affecting the mapping results. From a simulation study, Su et al. (2010) reported that different mapping procedures might give different mapping results, even for the same set of data. This effect could be due to the different genetic models and algorithms used and the fact that mapping statements provide only a probability statement rather than an absolute conclusion. Therefore, whole-genome scanning with multiple mapping procedures was recommended for mapping QTL. The results from the present study support this point. Using MICM in the QTL Network V2.0 program (Yang et al. 2008), the various genetic effects of QTL (A and AA) as well as environmental effects and interactions between QTL and the environment (AE and AAE) were evaluated and then the detected additive QTL were checked with CIM in Windows QTL Cartographer V2.5 and ICIM in QTL IciMapping V2.2. We were successful in detecting QTL with both additive and epistatic effects, epistatic QTL pairs and interactions between QTL and the environment, which are often neglected in some complex trait studies. In fact, interactions among loci or between genes and environmental factors make a substantial contribution to complex trait variation (Carlborg and Haley 2004). The consideration of interactions between loci allowed us to identify several novel QTL and trait-specific relationships between loci within and across chromosomes (Große-Brinkhaus et al. 2010). Some QTL contribute both additive and epistasis effects, and their contribution to total genotypic and phenotypic variances may depend on the genetic background where they locate in.

The present results also confirm that multiple populations are needed for wide range QTL detection and genotypic distinction among breeding materials. Here, 17 QTL with large or small additive and epistasis effects were found

to be involved in resistance to BP in the four populations, indicating that this complex trait is controlled by a number of QTL with quite different genetic effects and that there are likely to be other QTL organized in diverse genetic constitutions that have not yet been detected in soybean germplasm. Furthermore, from the present study, it should also be emphasized that information on the total genetic variation of the population (or heritability) estimated from an ANOVA procedure is very important. This is because the information about the collection of unmapped minor QTL can be found and estimated from the difference between the total genetic variation and the overall contribution from each mapped QTL, as has been indicated in the above text. With this approach, a full genetic model with all types of genetic effects (MICM here) should be used for mapping all types of QTL; otherwise, the contribution from the collection of unmapped minor QTL might be overestimated. Thus, for a thorough QTL analysis, a full model scan checked with other relevant mapping procedures that are based on multiple mapping populations, multiple environments, and an appropriate estimation of total genetic variation should be utilized as an optimal strategy.

Genetic structure of mechanisms of resistance to BP

Antibiosis, antixenosis, and tolerance are the three principal mechanisms of plant resistance to insects (Painter 1951). Antibiosis describes resistance in which the normal relationship of the insect with a host plant causes physiological or developmental detriment to the insect, whereas antixenosis, or nonpreference, describes resistance in which the insect is either repelled from or not attracted to its normal host plant. Their effects may overlap, i.e. an antibiotic chemical may also repel (Rector et al. 1999, 2000). In studying the QTL related to soybean resistance to corn earworm, Rector et al. (2000) considered antibiotic and antixenotic traits as being distinct because among the nine QTL detected in the four genotypes tested, only one (on LG M) was both antibiotic and antixenotic. Tao et al. (2003) also identified two different mechanisms (antibiotic and antixenotic) for sorghum midge *Stenodiplosis sorghicola* (Coquillett) resistance through QTL mapping. Long et al. (2004) found both antibiotic and antixenotic mechanisms for resistance to BP in soybeans. In the present study, RLP was used as the resistance (or susceptibility) indicator to detect the resistant (or susceptible) QTL. Because the moths and larvae of BP move freely in the field and the larvae roll the leaflets when causing damage, RLP is a symptom associated with the plants rather than the insects themselves; therefore, it is considered mainly an indicator of antixenosis, and the obtained results basically indicate the QTL related to antixenosis. Accordingly, further efforts toward mapping QTL for resistance to BP should be considered to distinguish the similarity versus the difference of genetic systems for various mechanisms, especially for antibiosis. However, because the artificial feeding of BP has not been successful, it is difficult to have sufficient BP larvae to meet the requirements of a BP resistance bioassay. For this reason, the present study was limited to identifying QTL under natural infestation conditions and using RLP as an indicator, which is basically associated with antixenosis of soybean to BP. In future studies seeking to evaluate the genetic structure of QTL or mapping the QTL for various mechanisms of BP resistance, a necessary step will be the development of an artificial BP feeding technology.

Comparisons of major QTL related to resistance between BP and other insects in soybeans

Thus far, a number of major QTL or genes conferring resistance to corn earworm (*H. zea*), common cutworm (*S. litura*), pod borer (*L. glycinivorella*), globular stink bug (*M. cribraria*), soybean aphid (*A. glycines*), Japanese beetle (*P. japonica*), and whitefly (*B. tabaci*) have been reported in soybean. The QTL for resistance to *H. zea* were detected in linkage groups M, H, G, and F (Rector et al. 1998, 1999, 2000; Narvel et al. 2001); for *S. litura*, resistance QTL were found in LG M, D1b, and O (Komatsu et al. 2005; Fu et al. 2007); for *L. glycinivorella*, resistance QTL were found in LG H and G (Zhao et al. 2008); for *M. cribraria*, resistance QTL were found in LG D1a, H, C2, and D1b (Xing et al. 2008b); for *A. glycines*, resistance QTL were found in LG F and M (Li et al. 2007b; Mian et al. 2008; Hill et al. 2009; Zhang et al. 2009); for *P. japonica*, resistance QTL were found in LG A2 and D2 (Yesudas et al. 2010); and for *B. tabaci*, resistance QTL were found in LG H, G, and L (Perez-Sackett et al. 2011). It appears that LG M, H, D1a, and F are major LGs for insect resistance. In LG M, resistance QTL were identified for *H. zea* (Rector et al. 1998, 1999, 2000; Narvel et al. 2001), *S. litura* (Komatsu et al. 2005) and *A. glycines* (Li et al. 2007b; Zhang et al. 2009). The major QTL for *H. zea* resistance in linkage group M from PI 229358 have been fine-mapped (Zhu et al. 2006); similarly, QTL for *S. litura* resistance from the cultivar "Himeshirazu" (Komatsu et al. 2008) and gene for *A. glycines* resistance from the cultivar "Dowling" (Kim et al. 2010) have also been fine-mapped. In LG H, another group of QTL was identified, including *BP12-1* for BP resistance from the present study, which is in the same region as a QTL conferring resistance to *M. cribraria* (Xing et al. 2008b) and *B. tabaci* (Perez-Sackett et al. 2011), but in a different region than a QTL conferring resistance to *H. zea* (Rector et al. 1998, 1999; Narvel et al. 2001) and *L. glycinivorella* (Zhao et al. 2008). In LG D1a, the QTL for BP resistance and *M. cribraria*

resistance (Xing et al. 2008b) were located in a similar region, although this needs to be confirmed with further fine mapping. In LG F, a resistance QTL/gene for *H. zea* (Rector et al. 1999, 2000) and *A. glycines* (Mian et al. 2008; Hill et al. 2009; Zhang et al. 2009) was identified. In summary, additional research on QTL for insect resistance will be necessary to characterize the genetic structure of these traits since the previous studies for various insect species were very limited with regard to mapping populations, tested environments, and mapping procedures.

Implications for breeding for BP resistance

Because the results of the present study indicated that the heritability value of RLP was approximately 81–92 % and that all types of genotype × environment interactions were relatively small, it can be expected that BP resistance may be effectively improved with phenotypic selection. Among the 17 QTL found in our study, *BP12-1* and *BP1-1* exist in three and two different populations, respectively, which validates their existence. Additionally, they are recognized as major QTL with both additive and epistatic effects accounting for 15–50 % and 13–32 % of the genetic variation, respectively. In this case, they could be used in breeding for BP resistance with the help of marker-assisted selection (Satt181, Sat_218 and Satt434 for *BP12-1* and Satt482 and Sat_346 for *BP1-1*).

However, it should be noted that a large part of genetic variation (41–68 %), in addition to additive and epistatic QTL, is attributed to a collection of unmapped minor QTL. This phenomenon indicates that in breeding programs, marker-assisted selection could not be used for accumulation of these small-effect QTL, although they could play an essential role in the improvement of BP resistance. In this situation, conventional breeding procedures, such as composite crossing and recurrent selection, might be required to utilize this aspect of genetic variation.

Cui et al. (1997a) reported that BP and common cutworm were the most abundant insects in the Nanjing area from a field and lamp trapping inspection during 1983–1984 and 1990–1994. In field observations, it was found that the responses of soybean cultivars to BP and common cutworm were different. The accessions that were highly resistant to common cutworm, such as 'Lamar', PI 171451 and PI 229358, are highly susceptible to BP. In addition, the parents that are resistant to BP, such as NN1138-2, TSBPHDJ and Su 88-M21, are susceptible to common cutworm. According to the above results, the major QTL for BP resistance have been mapped to LGs H and D1a, while the major QTL for common cutworm resistance had previously been mapped to LG M (Komatsu et al. 2005). Therefore, it is possible that recombination could occur between the different resistances QTL.

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References

Boerma HR, Walker DR (2005) Discovery and utilization of QTL for insect resistance in soybean. Genetica 123:181–189

Boethel DJ (2004) Integrated management of soybean insects. In: Boerma HR, Specht JE (eds) Soybeans: improvement, production, and uses, 3rd edn. Madison, Wisconsin, pp 853–881

Carlborg Ö, Haley CS (2004) Epistasis: too often neglected in complex trait studies. Nat Rev Genet 5:618–625

Choi IY, Hyten DL, Matukumalli LK, Song QJ, Chaky JM, Quigley CV, Chase K, Lark KG, Reiter RS, Yoon MS, Hwang EY, Yi SI, Young ND, Shoemaker RC, van Tassell CP, Specht JE, Cregan PB (2007) A soybean transcript map: gene distribution, haplotype and single-nucleotide polymorphism analysis. Genetics 176:685–696

Choi KH, Hong YK, Chang YJ, Moon JS, Kim CS, Choi DC, Kim TH (2008) Development under constant temperatures and seasonal prevalence in soybean field of the bean pyralid, *Omiodes indicatus* (Lepidoptera: Crambidae). Korean J Appl Entomol 47:353–358

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, Specht JE (1999) An integrated genetic linkage map of the soybean genome. Crop Sci 39:1464–1490

Cui ZL, Gai JY, Ji DF, Ren ZJ (1997a) A study on leaf-feeding insect species on soybeans in Nanjing area. Soybean Sci 16:12–20

Cui ZL, Gai JY, Ji DF, Ren ZJ (1997b) Evaluation of soybean germplasm for resistance to leaf-feeding insects. Soybean Sci 16:93–102

Fu SX, Wang H, Wu JJ, Liu H, Gai JY, Yu DY (2007) Mapping insect resistance QTL of soybean with RIL population. Hereditas 29:1139–1143

Große-Brinkhaus C, Jonas E, Buschbell H, Phatsara C, Tesfaye D, Jüngst H, Looft C, Schellander K, Tholen E (2010) Epistatic QTL pairs associated with meat quality and carcass composition traits in a porcine Duroc × Pietrain population. Genet Sel Evol 42:39

Hanson HL, Robinson HF, Comstock RE (1956) Biometrical studies of yield in segregating populations of Korean Lespodeza. Agron J 48:268–272

Hill CB, Kim KS, Crull L, Diers BW, Hartman GL (2009) Inheritance of resistance to the soybean aphid in soybean PI 200538. Crop Sci 49:1193–1200

Kim KS, Bellendir S, Hudson K, Hill C, Hartman G, Hyten D, Hudson M, Diers B (2010) Fine mapping the soybean aphid resistance gene *Rag1* in soybean. Theor Appl Genet 120: 1063–1071

Komatsu K, Okuda S, Takahashi M, Matsunaga R (2004) Antibiotic effect of insect-resistant soybean on common cutworm (*Spodoptera litura*) and its inheritance. Breed Sci 54:27–32

Komatsu K, Okuda S, Takahashi M, Matsunaga R, Nakazawa Y (2005) QTL mapping of antibiosis resistance to common cutworm (*Spodoptera litura* Fabricius) in soybean. Crop Sci 45:2044–2048

Komatsu K, Takahashi M, Nakazawa Y (2008) Antibiosis resistance of QTL introgressive soybean lines to common cutworm (*Spodoptera litura* Fabricius). Crop Sci 48:527–532

Korir PC, Qi B, Wang YF, Zhao TJ, Yu DY, Chen SY, Gai JY (2011) A study on relative importance of additive, epistasis and unmapped QTL for aluminium tolerance at seedling stage in soybean. Plant Breed 130:551–562

Lander ES, Green P (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Li HH, Ye GY, Wang JK (2007a) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361–374

Li Y, Hill CB, Carlson SR, Diers BW, Hartman GL (2007b) Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol Breed 19:25–34

Long LP, Yang SZ, Chen HZ, Qin JL, Li CY, Sun ZD (2004) Effects of different genotypes of soybean varieties on the experimental population of bean pyralid (*Lamprosema indicata* Fabricius). Chin J Oil Crop Sci 26:67–70

Mian MAR, Kang ST, Beil SE, Hammond RB (2008) Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. Theor Appl Genet 117:955–962

Narvel JM, Walker DR, Rector BG, All JN, Parrott WA, Boerma HR (2001) A retrospective DNA marker assessment of the development of insect resistant soybean. Crop Sci 41:1931–1939

Painter RH (1951) Insect resistance in crop plants. Macmillan & Co., New York

Perez-Sackett PT, Cianzio SR, Kara PC, Aviles M, Palmer RG (2011) QTL mapping of whitefly resistance in soybean. J Crop Improvement 25:134–150

Rector BG, All JN, Parrott WA, Boerma HR (1998) Identification of molecular markers linked to quantitative trait loci for soybean resistance to corn earworm. Theor Appl Genet 96:786–790

Rector BG, All JN, Parrott WA, Boerma HR (1999) Quantitative trait loci for antixenosis resistance to corn earworm in soybean. Crop Sci 39:531–538

Rector BG, All JN, Parrott WA, Boerma HR (2000) Quantitative trait loci for antibiosis resistance to corn earworm in soybean. Crop Sci 40:233–238

Rufener GK II, St. Martin SK, Cooper RL, Hammond RB (1989) Genetics of antibiosis resistance to Mexican bean beetle in soybean. Crop Sci 29:618–622

Simon M, Loudet O, Durand S, Berard A, Brunel D, Sennesal FX, Durand-Tardif M, Pelletier G, Camilleri C (2008) Quantitative trait loci mapping in five new large recombinant inbred line populations of *Arabidopsis thaliana* genotyped with consensus single-nucleotide polymorphism markers. Genetics 178:2253–2264

Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

Stout M, Davis J (2009) Keys to the increased use of host plant resistance in integrated pest management. In: Peshin R, Dhawan AK (eds) Integrated pest management: innovation-development process. Springer, The Netherlands, pp 163–181

Su CF, Zhao TJ, Gai JY (2010) Simulation comparisons of effectiveness among QTL mapping procedures of different statistical genetic models. Acta Agron Sin 36:1100–1107

Sun ZD, Gai JY (2000) Studies on the inheritance of resistance to cotton worm *Prodenia litura* (Fabricius). Acta Agron Sin 26:341–346

Sun ZD, Yang SZ, Chen HZ, Wei DW (2001) A study on leaf-feeding insect species on soybeans in Nanning. Guangxi Agric Sci 2:104–106

Sun ZD, Yang SZ, Chen HZ, Li CY, Long LP (2005) Identification of soybean resistance to BP (*Lamprosema indicata* Fabricius) and oviposition preference of BP on soybean varieties. Chin J Oil Crop Sci 27:69–71

Tao YZ, Hardy A, Drenth J, Henzell RG, Franzmann BA, Jordan DR, Butler DG, McIntyre CL (2003) Identifications of two different mechanisms for sorghum midge resistance through QTL mapping. Theor Appl Genet 107:116–122

Terry LI, Chase K, Jarvik T, Orf J (2000) Soybean quantitative trait loci for resistance to insects. Crop Sci 40:375–382

Van Duyn JW, Turnipseed SG, Maxwell JD (1971) Resistance in soybeans to the Mexican bean beetle I: source of resistance. Crop Sci 11:572–573

Van Ooijen JW, Voorrips RE (2001) JoinMap 3.0 software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTL. J Hered 93:77–78

Wang YF (2009) Genomic characterization of simple sequence repeats and establishment, integration and application of high density genetic linkage map in soybean. PhD dissertation, Nanjing Agricultural University, Nanjing, People's Republic of China

Wang CS, Rutledge JJ, Gianola D (1994) Bayesian analysis of mixed linear models via Gibbs sampling with an application to litter size in Iberian pigs. Genet Sel Evol 26:91–115

Wang SC, Basten CJ, Zeng ZB (2006) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Wilson RF (2008) Soybean: market driven research needs. In: Stacey G (ed) Genetics and genomics of soybean. Springer, New York, pp 3–15

Wu MX, Xu KT (2002) Preliminary studies on the pests of green soybean in Fuzhou suburbs. Wuyi Sci J 18:27–32

Xing GN, Zhao TJ, Gai JY (2008a) Inheritance of resistance to *Lamprosema indicata* (Fabricius) in soybean. Acta Agron Sin 34:8–16

Xing GN, Zhou B, Zhao TJ, Yu DY, Xing H, Chen SY, Gai JY (2008b) Mapping QTL of resistance to *Megacota cribraria* (Fabricius) in soybean. Acta Agron Sin 34:361–368

Yang J, Zhu J, Williams RW (2007) Mapping the genetic architecture of complex traits in experimental populations. Bioinformatics 23:1527–1536

Yang J, Hu CC, Hu Han, Yu RD, Xia Z, Ye XZ, Zhu J (2008) QTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics 24:721–723

Yencho GC, Cohen MB, Byrne PF (2000) Applications of tagging and mapping insect resistance loci in plants. Annu Rev of Entomol 45:393–422

Yesudas CR, Sharma H, Lightfoot DA (2010) Identification of QTL in soybean underlying resistance to herbivory by Japanese beetles (*Popillia japonica*, Newman). Theor Appl Genet 121:353–362

Zhang WK, Wang YJ, Luo GZ, Zhang JS, He CY, Wu XL, Gai JY, Chen SY (2004) QTL mapping of ten agronomic traits on the soybean (*Glycine max* (*L.*) Merr.) genetic map and their association with EST markers. Theor Appl Genet 108:1131–1139

Zhang HM, Zhou B, Zhao TJ, Xing H, Chen SY, Gai JY (2008) QTL mapping of tofu and soymilk output in RIL population NJRISX of soybean. Acta Agron Sin 34:67–75

Zhang GR, Gu CH, Wang DC (2009) Molecular mapping of soybean aphid resistance genes in PI 567541B. Theor Appl Genet 118:473–482

Zhao GY, Wang J, Han YP, Teng WL, Sun GL, Li WB (2008) Identification of QTL underlying the resistance of soybean to pod borer, *Leguminivora glycinivorell*a (Mats.) obraztsov, and correlations with plant, pod and seed traits. Euphytica 164:275–282

Zhu S, Walker DR, Boerma HR, All JN, Parrott WA (2006) Fine mapping of a major insect resistance QTL in soybean and its interaction with minor resistance QTL. Crop Sci 46:1094–1099