Theor Appl Genet (2008) 117:621–628
DOI 10.1007/s00122-008-0806-3

ORIGINAL PAPER

Mapping of quantitative trait loci for a new source of resistance to bruchids in the wild species *Vigna nepalensis* Tateishi & Maxted (*Vigna* subgenus *Ceratotropis*)

Prakit Somta · Akito Kaga · Norihiko Tomooka · Takehisa Isemura · Duncan A. Vaughan · Peerasak Srinives

Received: 12 June 2007 / Accepted: 21 May 2008 / Published online: 19 June 2008

Abstract Azuki bean breeders have long been interested in producing azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] varieties with bruchid resistance. A new bruchid (*Callosobruchus* spp.) resistance source was found in *V. nepalensis* Tateishi & Maxted, a species that is cross compatible with azuki bean. Quantitative trait loci (QTLs) analysis for resistance to *C. chinensis* (L.) and *C. maculatus* (F.) was conducted using F_2 (*V. nepalensis* × *V. angularis*) and BC_1F_1 [(*V. nepalensis* × *V. angularis*) × *V. angularis*] populations derived from crosses between the bruchid resistant species *V. nepalensis* and bruchid susceptible species *V. angularis*. Resistance was measured using two traits, percentage of seeds damaged by bruchids and the time taken for adult bruchids to emerge from seeds. Based on the results from both populations seven QTLs were detected for bruchid resistance; five QTLs for resistance to *C. chinensis* and two QTLs for resistance to *C. maculatus*. The different locations found for some resistance QTL to the two bruchid species suggests different resistance mechanisms. QTLs on linkage group (LG) 1 and LG2 for bruchid resistance to *C. chinensis* co-localized with seed size QTLs suggesting that incremental increase in seed size accompanied susceptibility to *C. chinensis*. Based on linked markers the QTL on these two linkage groups appear to be the same as previously reported in other Asian *Vigna*. However, several other QTLs were newly detected including one on LG4 that appears unrelated to seed size. Transfer of these new sources of bruchid resistance from *V. nepalensis* to azuki bean will be aided by the progress being made in azuki genome mapping.

Communicated by H. T. Nguyen.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-008-0806-3) contains supplementary material, which is available to authorized users.

P. Somta · P. Srinives
Department of Agronomy,
Faculty of Agriculture at Kamphaeng Saen,
Kasetsart University, Nakhon Pathom 73140, Thailand

A. Kaga · N. Tomooka · T. Isemura · D. A. Vaughan (✉)
Genebank, National Institute of Agrobiological Sciences,
2-1-2 Kannondai, Tsukuba 305-8602, Japan
e-mail: duncan@affrc.go.jp

Introduction

Azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi], genus *Vigna* subgenus *Ceratotropis*, is an economically important legume crop in Asia. The bean is mainly produced and consumed in China, Japan and South Korea. In Japan and Korea it is the second most important legume crop after soybean (Lumpkin and McClary 1994). The bean is mainly used as an ingredient for cakes, ceremonial and religious foods.

Bruchid beetles (*Callosobruchus* spp. Coleoptera, Bruchidae), also known as seed weevils, are major post-harvest insects of grain legumes in sub-tropical and tropical zones. *C. chinensis* L. (azuki bean weevil) and *C. maculatus* F. (cowpea weevil) are the most important bruchids in Asia and cause considerable damage to *Vigna* seeds. Bruchid beetles first infest *Vigna* plants in the field where the adult bruchid lays eggs on pods, hatched larva bore into pods and feed on the seed (Southgate 1979). When the crop is harvested, stored bruchids continue feeding and eventually emerge as adults and cause secondary infestation, which at times results in total destruction of a seed lot within a period of 3–4 months (Banto and Sanchez 1972). The

damaged seeds are unsuitable for human consumption, commercial and agricultural uses.

Seed size is known to be one of the factors affecting resistance to bruchid species and bruchid species predation is considered to result in selection for smaller seeds in wild legumes (Janzen 1969; Winn 1988). Bruchid resistance in wild mungbean (*V. radiata* var. *sublobata*) is associated with smaller seed size (James et al. 1999). There are several possible explanations for the relationship between seed size and bruchid resistance including the fact that small seeded legumes have less food for bruchid larvae to feed on and seed size may pose a limit to their ability to grow (Janzen 1969).

Breeding for bruchid resistance has been a major objective of azuki bean improvement. To date no resistant variety of azuki bean has been developed because resistance sources have not been found in the cultivated gene pool. Wild relatives of crop plants are valuable sources of disease and insect resistance for crop improvement. Complete resistance against *C. chinensis* and *C. maculatus* was found in wild mungbean [*V. radiata* var. *sublobata* (Roxb.) Verdc.] and the resistance was conditioned by a single dominant gene (Kitamura et al. 1988; Fujii et al. 1989). The resistance gene was transferred to susceptible cultivated mungbean (Tomooka et al. 1992). A single dominant gene for resistance to bruchid species has also been reported in cultivated mungbean (Somta et al. 2007). Wild black gram (*V. mungo* var. *silvestris* Lukoki, Maréchal & Otoul) has also been reported to be immune to bruchid species (Fujii et al. 1989; Dongre et al. 1996). However, these resistance sources cannot be used to develop bruchid-resistant azuki bean because of cross-incompatibility. No source of bruchid resistance has been identified in wild azuki bean [*V. angularis* var. *nipponensis* (Ohwi) Ohwi & Ohashi]. Recently several species of the genus *Vigna* subgenus *Ceratotropis* are reported to possess bruchid resistance (Tomooka et al. 2000). Among these species, *V. nepalensis* Tateishi & Maxted, a wild relative of azuki bean, is a potentially useful source of bruchid resistance for azuki bean breeding because this species is cross-compatible with azuki bean (Vaughan et al. 2004). In addition, recently, genetic maps of azuki bean have been constructed using interspecific populations derived from azuki bean and *V. nepalensis* (Han et al. 2005; Isemura et al. 2007). These linkage maps can facilitate the study of the genetics of bruchid resistance in *V. nepalensis.*

In this paper, we report quantitative trait loci (QTLs) for resistance to *C. chinensis* and *C. maculatus* in *V. nepalensis.* The objectives of this study were to (1) identify QTLs controlling bruchid resistance in *V. nepalensis*, (2) to determine the relationships between bruchid resistance and seed size. We compare the bruchid resistance QTLs of *V. nepalensis* with previously reported resistance in other *Vigna* species.

Materials and methods

Plant materials

The mapping populations and DNA markers used in this study were the same as those of Han et al. (2005) and Isemura et al. (2007). The F_2 population consisted of 141 plants and BC_1F_1 population consisted of 187 plants. These populations were derived from the crosses between, *V. nepalensis* (accession JP107881), and Japanese cultivated azuki bean, *V. angularis* (accession JP81481 a landrace from Tokushima Prefecture, Japan). In brief, *V. nepalensis* was crossed onto azuki bean to produce F_1 hybrid plants. An F_1 plant was self-pollinated to produce the F_2 population and at the same time other F_1 plants were crossed as female parent with azuki bean to develop the backcross population (BC_1F_1). BC_1F_1 and F_2 were designated as populations "1" and "2" respectively. The genotype of the F_2 and BC_1F_1 populations has been characterized by restriction fragment length polymorphisms and simple sequence repeat markers to cover the whole genome with markers at 10–15 cM intervals (Han et al. 2005; Isemura et al. 2007).

Bioassay for bruchid resistance

The bruchid species *C. chinensis* and *C. maculatus* were used for the evaluation. The insects were reared on susceptible mungbean seeds and kept in an incubator at 30°C and 70% relative humidity. Five to ten replicates of 25–30 seeds from the parents, two replicates of 100 seeds from two F_1 plants (F_2 seeds), 25 seeds of each BC_1F_1 plant (BC_1F_2 seeds), and 30 seeds of each F_2 plant (F_3 seeds) were evaluated for *C. chinensis* and *C. maculatus* resistance. The seeds were placed in a 9 cm-diameter petri dish and then infested with newly emerged adult bruchids for 24 h. Five pairs (five males and five females) of bruchids were used for seeds from each parent and BC_1F_1 plant, and 15 pairs of bruchids for seeds from each F_1 plant and ten pairs of bruchids for seeds from each parent and F_2 plant. The infested seeds were maintained in incubators at 30°C and 70% relative humidity. When the beetles started emerging, the numbers of damaged seeds were recorded daily and then the insects and seeds from which they had emerged (counted as damaged seeds) were removed from the petri dish. Data collection was stopped at 50 days after insect introduction to avoid counting second-generation infestation. The average number of days from seed infestation to bruchid emergence from seeds (based on daily counts of damaged seeds) was used as days to adult bruchid emergence. The number of damaged seeds were the accumulated number of seeds removed from each petri dish expressed as a percentage.

QTL analysis

V. nepalensis expresses two mode of resistance to bruchids that are expressed as:

1. Low percentage of seeds damaged by bruchids;
2. Late emergence of adult bruchids (Tomooka et al. 2000).

Consequently, percentage of seed damage caused by bruchids and days to emergence of adult were subjected to QTL analysis. Arcsine transformation was applied to the percentage of seed damage and the resultant data were used for analysis. For delayed emergence of adults the average of number of days from insect introduction to adult emergence was used. QTL associated with resistance-related traits was analyzed using the software package MultiQTL ver. 2.5 according to the procedure described by Peng et al. (2003). In brief, the entire genome was scanned for each trait using general interval mapping with the following approach. First, a single QTL model was fitted for each trait-chromosome combination. Chromosome-wise statistical significance thresholds ($\alpha = 0.05$) for declaring putative QTL were obtained by 10,000 runs of a permutation test (Churchill and Doerge 1994). The QTL parameters (position, additive and dominance effect, and the percentage of variance explained) and standard deviation for the position were re-estimated based on 10,000 bootstrap sampling per LG (standard deviation are shown in supplementary data). When the LOD graph indicates the possibility of two QTLs, a two-linked QTL model (Korol et al. 1998) was fitted for each trait-chromosome combination. Putative two-linked QTL was declared at the same threshold described above. Further, the hypothesis of two-linked QTL in the chromosome (*H2*) was compared with single QTL (*H1*) at 1% level of significance using parametric bootstrap (Ronin et al. 1999). Multiple interval mapping (MIM, Kao et al. 1999) was then conducted to reduce the background variation by taking into account QTL effects from other chromosomes at 1% level of significance. The QTL effects were re-evaluated by fitting all positive QTLs and a global permutation test (10,000 runs) to get more precise estimates of significance. QTL nomenclature followed the style of Somta et al. (2006).

Results

Resistance in parents and F_1 plants

Infestation of seeds of *V. nepalensis*, *V. angularis* with *C. chinensis* and *C. maculatus* showed that *V. nepalensis* was highly resistant to both insect species, while *V. angularis* was completely susceptible (Fig. 1). The resistance was expressed by low percentage damaged seeds at the end of the experiment and delayed adult emergence (Fig. 1). The mean percentage of damaged seeds caused by *C. chinensis* and *C. maculatus* in *V. nepalensis* was $17.7 \pm 2.5\%$ and $6.6 \pm 2.7\%$, respectively. For *V. angularis* over 90% were damaged by both bruchid species. The mean number of days from infestation to average time to adult emergence in *V. nepalensis* for *C. chinensis* was 40.5 ± 0.8 days and for *C. maculatus* was 37.0 ± 0.8 days, whereas in *V. angularis* the figures were 22.8 ± 0.3 days and 25.4 ± 0.3 days, respectively. Seed damage in *V. angularis* reached 99.8% at 26 days after infestation by *C. chinensis* and 93.7% at 27 days by *C. maculatus*. In contrast, at these days, percentage of seed damage in *V. nepalensis* was 0% by *C. chinensis* and 0.1% by *C. maculatus* (Fig. 1).

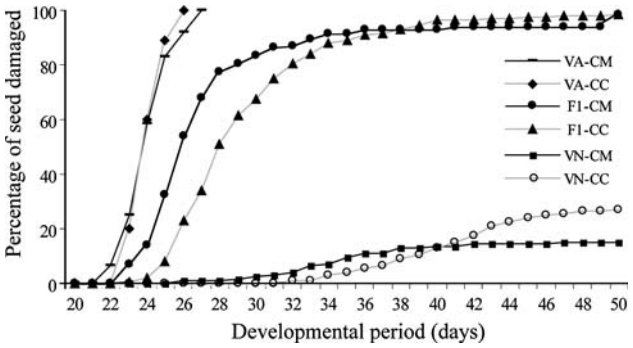

Fig. 1 Patterns of *C. chinensis* (*CC*) and *C. maculatus* (*CM*) infestation to *V. nepalensis* (*VN*), *V. angularis* (*VA*), and F_1 population (F_2 seeds) (*V. nepalensis* × *V. angularis*)

Results of infestation of seeds from F_1 (*V. nepalensis* × *V. angularis*) plants with *C. chinensis* and *C. maculatus* were similar to those for the susceptible parent, *V. angularis*. However, compared to the susceptible parent, they displayed delayed emergence of adult bruchids, but less pronounced than the resistant parent (Fig. 1).

Resistance in F_2 and BC_1F_1 populations

The resistance in the F_2 (*V. nepalensis* × *V. angularis*) population was evaluated based on individual plants using bulked F_3 seeds. Damaged seeds showed a skewed distribution towards the susceptible parent for both *C. chinensis* and *C. maculatus* (Fig. 2a). However, more resistant plants were observed in this population than the BC_1F_1 population (Fig. 2b). The mean percentage of seed damaged caused by *C. chinensis* was $80.4 \pm 2.1\%$ varying from 3.3 to 100% and for *C. maculatus* was $72.8 \pm 2.0\%$ ranging from 3.3 to 100%. Different F_2 plants had resistance to *C. chinensis* and *C. maculatus*. The F_2 population also showed segregation for average time to adult emergence. Frequency distribution for developmental period of both bruchids was skewed towards the susceptible parent (Fig. 3a). The average

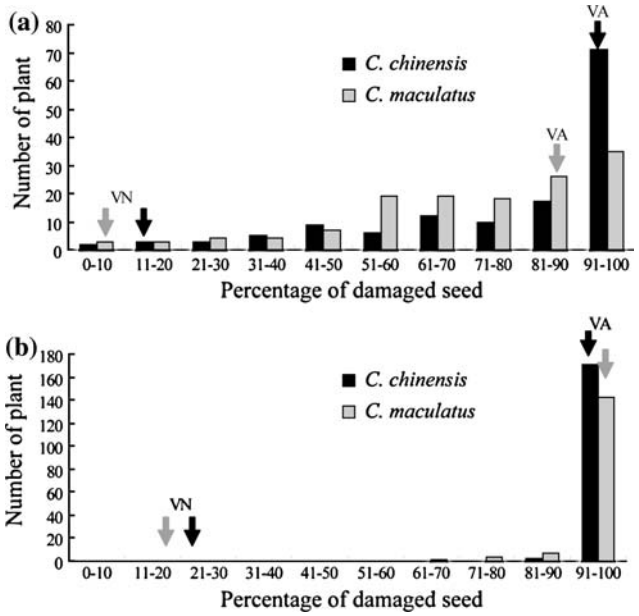

Fig. 2 Frequency distribution of percentage of damaged seed caused by *C. chinensis* and *C. maculatus* in F_2 (**a**) and BC_1F_1 (**b**) populations. *VN V. nepalensis* parent, *VA V. angularis* parent

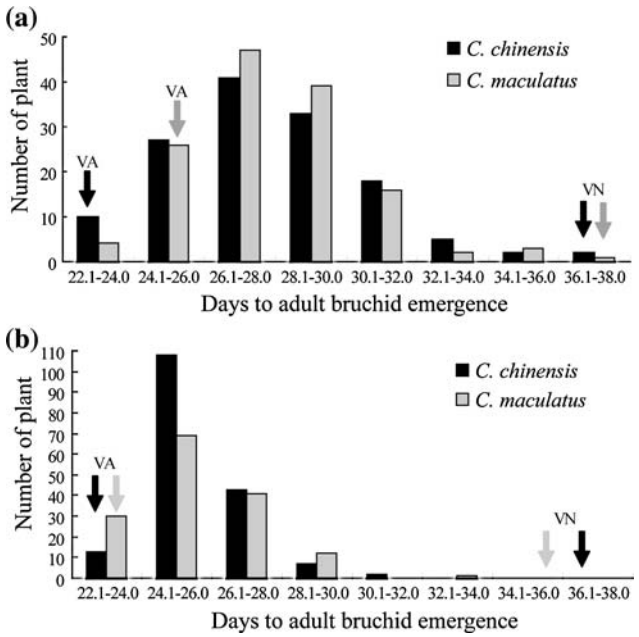

Fig. 3 Frequency distribution of average days to adult bruchid emergence of *C. chinensis* and *C. maculatus* in F_2 (**a**) and BC_1F_1 (**b**) populations. *VN V. nepalensis* parent, *VA V. angularis* parent

number of days from infestation to adult emergence for *C. chinensis* was 27.8 ± 0.2 days, and for *C. maculatus* was 27.9 ± 0.2 days.

In the BC_1F_1 [(*V. nepalensis* × *V. angularis*) × *V. angularis*] population, each plant was separately evaluated for resistance using bulked BC_1F_2 seeds. Most plants were as susceptible to *C. chinensis* and *C. maculatus* as the susceptible parent, *V. angularis* (Fig. 2b). The mean percentage of damaged seeds caused by *C. chinensis* was 99.4 ± 0.2% and by *C. maculatus* was 97.0 ± 0.4%. The mean for average days to adult emergence for *C. chinensis* was 25.6 ± 0.1 days, and for *C. maculatus* was 25.5 ± 0.1 days. The trait showed similar pattern between *C. chinensis* and *C. maculatus* (Fig. 3b).

Association of bruchid resistance with seed size

V. nepalensis has a seed weight of 1.7 ± 0.2 g and *V. angularis* has seed weight of 16.3 ± 0.1 g. In the F_2 population average seed weight was 5.0 ± 1.2 g and in the BC_1F_1 population was 9.1 ± 1.6 g. Significant positive correlations between percentage of seed damage and seed size were observed. In the F_2 population, the correlation coefficient between seed size and percentage of damaged seeds by *C. chinensis* was 0.62 ($P < 0.001$) and by *C. maculatus* was 0.53 ($P < 0.001$). For the BC_1F_1 population, analogous correlation for *C. chinensis* and *C. maculatus* was 0.21 ($P = 0.005$) and 0.31 ($P < 0.001$), respectively. The correlation coefficient between seed size and average days to adult emergence was significantly negative. In the F_2 population, the coefficient value in *C. chinensis* and *C. maculatus* was −0.48 ($P < 0.001$) and −0.52 ($P < 0.001$), respectively. The figure was −0.43 ($P = 0.002$) for *C. chinensis* and −0.25 ($P < 0.001$) for *C. maculatus*, in the BC_1F_1 population.

QTL for seed damage and days to adult emergence from seeds

Significant QTLs associated with bruchid resistance and days to adult bruchids emergence were detected in both populations and are summarized (Table 1; Fig. 4).

C. chinensis In both populations only a single QTL, *Brc*1.2.1, for damaged seeds by *C. chinensis* was identified on LG 2 (Table 1). The phenotypic variation explained (PVE) by this QTL in the F_2 population was higher (16.6%) than in BC_1F_1 population (4.2%). The *V. nepalensis* alleles reduced the percentage of damaged seeds. Four QTLs, *Brcde*1.1.1, *Brcde*1.2.1, *Brcde*1.4.1, and *Brcde*1.10.1, were identified related to number of days to emergence of adult *C. chinensis* in both populations (Table 1). *V. nepalensis* alleles prolonged the number of days to emergence of bruchids. Among them *Brcde*1.4.1 had a relatively large effect (PVE 17.6–21.7%) on bruchid emergence. *Brcde*1.2.1 was co-located with the seed damage QTL *Brc*1.2.1 (Fig. 4).

C. maculatus One QTL, *Brm*1.3.1, for damaged seeds by *C. maculatus* was identified on LG 3 in both populations (Table 1). PVE in the F_2 population was higher (18.2%) than in BC_1F_1 population (8.6%). The allele from *V. nepalensis* decreased the percentage of seed damage. One QTL, *Brmde*1.3.1, for days to emergence of adult *C. maculatus*

Table 1 QTLs detected in both the self pollinated (F_2) and backcrossed (BC_1F_1) populations

Linkage group	Trait[a]	QTL[b]	Population	Multiple interval mapping					
				LOD	*P*	PVE[c] (%)	Position (cM)	Additive effect	Dominant effect
1	BRCDE	*Brcde1.1.1*	BC	2.6	0.00748	4.4	85.4	0.55 day	
			F_2	3.8	0.00187	6.3	89.4	1.98 day	0.04 day
	SD100WT	*Sd100wt1.1.2*	BC	9.8	0.00093	11.5	92.9	−1.05 g	
			F_2	8.6	0.00093	10.2	86.7	−0.99 g	0.11 g
2	BRC	*Brc1.2.1*	BC	1.6	0.02991	4.2	41.6	−0.05%	
			F_2	4.5	0.00093	16.6	42.9	−7.12%	−0.29%
	BRCDE	*Brcde1.2.1*	BC	6.4	0.00093	12.0	38.4	0.91 day	
			F_2	7.4	0.00093	15.1	46.1	2.74 day	0.97 day
	SD100WT	*Sd100wt1.2.1*	BC	19.8	0.00093	24.3	32.4	−1.53 g	
			F_2	14.2	0.00093	24.2	44.5	−1.51 g	−0.22 g
3	BRM	*Brm1.3.1*	BC	3.0	0.00187	8.6	16.4	−0.36%	
			F_2	10.3	0.00093	18.2	22.4	−2.62%	0.10%
	BRMDE	*Brmde1.3.1*	BC	4.3	0.00093	10.7	14.5	1.08 day	
			F_2	10.6	0.00093	27.3	21.9	2.02 day	−0.43 day
4	BRCDE	*Brcde1.4.1*	BC	10.7	0.00093	21.7	9.5	1.22 day	
			F_2	7.6	0.00093	17.6	16.4	2.96 day	−1.04 day
8	SD100WT	*Sd100wt1.8.1*	BC	2.5	0.00187	2.2	38.6	−0.46 g	
			F_2	2.6	0.02243	2.3	24.3	−0.42 g	0.15 g
9	SD100WT	*Sd100wt2.9.1*	BC	5.5	0.00093	5.5	16.8	−0.73 g	
			F_2	9.7	0.00093	14.0	0.0	−0.70 g	−0.21 g
10	BRCDE	*Brcde1.10.1*	BC	2.9	0.00093	5.1	37.6	0.59 day	
			F_2	2.6	0.01869	5.6	46.0	1.60 day	0.68 day

[a] *BRCDE Callosobruchus chinensis* days to emergence, *BRC C. chinensis* damaged seeds, *BRMDE C. maculatus* days to emergence, *BRM C. maculatus* damaged seeds, *SD100WT* 100 seed weight

[b] QTL name declared when both populations and both interval and multiple interval mapping methods indicated the presence of a QTL

[c] Percentage of phenotypic variance explained by QTL

was identified at a similar location to *Brm*1.3.1 (Fig. 4). As with *C. chinensis*, *V. nepalensis* alleles at this QTL prolonged the number of days to emergence of bruchids.

In addition to these QTLs that were consistently found, a number of statistically significant genomic regions were detected in one or other population by interval mapping and/or multiple interval mapping (S1, S2). These were not named due to being found in only one population. Among them, a QTL in the middle of LG 2 in the F_2 population had a particularly high LOD score for damaged seeds by *C. maculatus* (S1).

Comparison of QTLs for bruchid resistance with QTLs for seed size

Genome locations of QTLs for resistance-related traits were compared to those of QTLs associated with seed size (Fig. 4). In both populations, four QTLs affecting seed size were found (Table 1; Fig. 4). In all cases, the *V. nepalensis* allele decreased seed size. When the QTL locations were compared, it was found that some QTLs related to bruchid resistance and seed size were co-localized (Fig. 4). In both the F_2 and BC_1F_1 populations, QTLs for days to adult bruchid emergence from seeds, *Brcde*1.1.1 and *Brcde*1.2.1, were co-located with seed size QTLs having large effect, *Sd100wt*1.1.2 and *Sd100wt*1.2.1, respectively. At these regions, *V. nepalensis* alleles decreased seed size and prolonged the number of days to emergence of bruchids. However, the QTL for bruchid resistance, *Brcde*1.4.1, located on LG 4 is presumed to be independent of seed size QTLs.

Discussion

There are no varieties of azuki bean with bruchid resistance because resistance sources have not been found in either cultivated or wild azuki bean gene pools. In the present study, inheritance of the bruchid resistance found in *Vigna nepalensis* was investigated. Damaged seeds and days to emergence of adult azuki bean weevil (*C. chinensis*), a

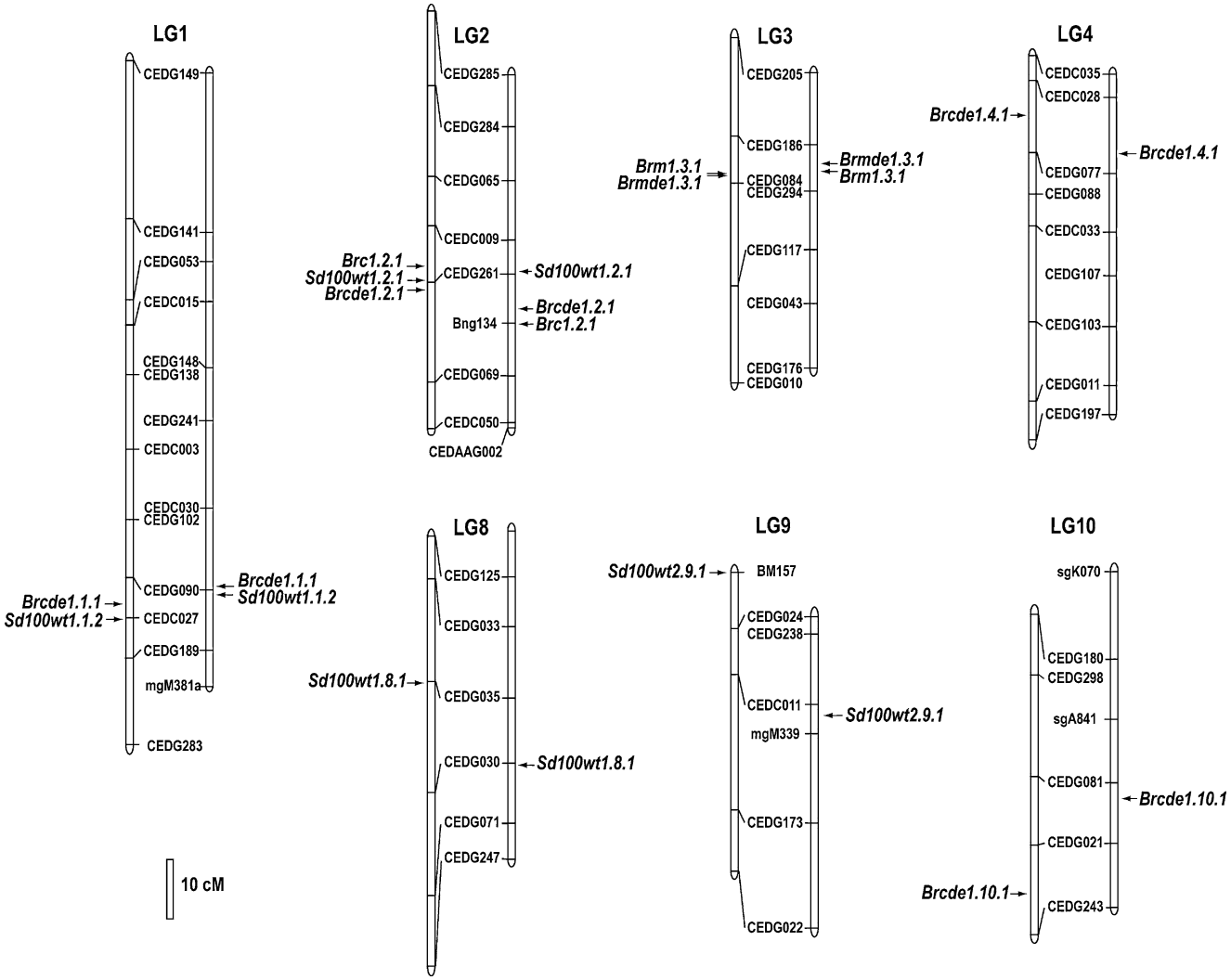

Fig. 4 QTL locations of *C. chinensis* resistance, *C. maculatus* resistance, and seed size on comparative linkage map between F_2 (*V. nepalensis* × *V. angularis*) and BC_1F_1 [(*V. nepalensis* × *V. angularis*) × *V. angularis*] maps. Linkage groups of F_2 and BC_1F_1 maps are aligned on the left and right sides, respectively. Lines connect common markers between linkage groups. *Solid arrow* represents QTL

major bruchid distributed in East Asia, and cowpea weevil (*C. maculatus*), a major bruchid distributed in Southeast Asia, were used to determine the level of resistance to bruchids. Seeds produced by F_1 hybrid plants between the resistant wild parent, *V. nepalensis*, and susceptible cultivated parent, *V. angularis*, were severely damaged by both bruchids compared to the resistant parent (Fig. 1). The progenies, comprising F_2 and BC_1F_1 populations revealed skewed distributions to the susceptible parent (Fig. 2). The F_2 showed a continuous distribution. The average time to emergence of adult bruchids from seeds in both populations showed normal distribution between parents but the mean value was skewed to the susceptible parent (Fig. 3). The segregation patterns indicate that the damaged seeds and days to adult emergence for both bruchid species are quantitative traits. The inheritance of resistance was confirmed by QTL analysis in the F_2 and BC_1F_1 populations.

Most QTLs related to bruchid resistance in *V. nepalensis* were found to be inherited additively which differs from some earlier reports of resistance genes in other *Vigna* species. A simple genetic model and straightforward division between resistant and susceptible classes based upon seed damage have been reported for bruchid resistance in other *Vigna* species; a single dominant gene controls *C. chinensis* and *C. maculatus* resistance in the wild mungbean accession TC1966 (Fujii et al. 1989), two dominant genes control *C. chinensis* and *C. maculatus* resistance in wild black gram (Dongre et al. 1996), two recessive genes involved in *C. maculatus* resistance in cowpea [*V. unguicalata* (L.) Walp.] accession TVu2027 (Adjadi et al. 1985). However, several researchers have proposed a more complex mode of bruchid resistance. The bruchid resistance in accession TVu2027 is reported to be controlled by one to two loci with major gene effects plus loci with minor gene effects

(Redden and McGuire 1983), resistance to *C. chinensis* and *C. maculatus* in cultivated mungbean are governed by a major dominant gene with modifiers (Somta et al. 2007), and resistance to *C. maculatus* in Australian wild mungbean is associated with seed size (James et al. 1999).

In the present study, seed size of the resistant parent, *V. nepalensis*, is 9.5-fold smaller than susceptible parent, *V. angularis*. Significant positive correlation between seed size and percentage of seeds damaged, and negative correlation between seed size and days to first adult emergence from seeds indicates that large seeds are a factor explaining bruchid susceptibility. The relationship between seed size and bruchid resistance were partially resolved into QTLs. It is not known whether the co-localization between QTLs of seed size and bruchid resistance is due to pleiotropy or linkage. If co-localization is due to linkage between bruchid resistance and seed size in *V. nepalensis* to transfer these QTL it will be necessary to minimize linkage drag.

Not all QTLs for bruchid resistance are co-localized with seed size QTLs. QTL for bruchid resistance on LG 4 appears to be independent of QTL for seed size and has not previously been reported. Since this QTL is independent of seed size it may be more readily be transferred to large seed azuki bean than other bruchid resistance QTLs.

F_2 plants with the same level of resistance as *V. nepalensis* (around 3% of damaged seed) had small seeds. The seed weight (5.0 g) of the F_1 plant is closer to *V. nepalensis* (1.7 g) than *V. angularis* (16.3 g). To determine if bruchid resistance and susceptibility occurs in seeds of the same size, three kinds of seeds from the same F_1 plant were tested; (1) F_1 plant crossed with *V. nepalensis* as the pollen donor, (2) F_1 plant crossed with *V. angularis* as the pollen donor, (3) self pollination of an F_1 plant. Although seed size were almost the same, the percentage of seeds damaged and days to first emergence of adult bruchids when *V. nepalensis* was used as a pollen donor were lower and longer than when *V. angularis* was used as a pollen donor or self-pollination (S3). These results indicate that a number of genes or a set of genes in a network derived from *V. nepalensis* in the seed is important for resistance expression and factors other than seed size are responsible for bruchid resistance.

Almost all seeds produced by most of BC_1F_1 plants, that have increased susceptible parent genetic background, were highly damaged by bruchids. To test the relationship between seed size and resistant factor concentration, artificial seeds as large as the susceptible parent seed made from various proportions of resistant and susceptible parent seed powder were evaluated (S4). As the proportion of cotyledon seed powder of the resistant parent in the artificial seed increased, seed damage decreased and days to first adult emergence increased for both bruchid species. The artificial seeds showed full resistance when they contained 75% resistant parent powder. This is a similar result to that previously reported for artificial seeds made with increasing proportions of bruchid resistant rice bean [*V. umbellata* (Thunb.) Ohwi & Ohashi] flour (US patent 6,770,630 B2 can be viewed from http://www.patft.uspto.gov/checked 14 February 2008). Rice bean flour in the proportion of 80% was effective against bruchids in artificial seeds but not at a 60% level. Therefore it seems that a certain level or concentration of resistance factor is required to effect resistance to bruchids in large seeds.

The co-localization of major QTLs for seed size were found for major QTLs for days to first adult emergence for *C. chinensis* on LG1 and LG2 (Fig. 4). Previous studies of bruchid resistance in the Asian *Vigna* species, wild mungbean (*V. radiata* var. *sublobata*) and rice bean (*V. umbellata*), have found QTLs for bruchid resistance at similar positions in relation to molecular markers as found here on LG 1 and 2 (Kaga 1996; Kaga and Ishimoto 1998; Somta et al. 2006). This suggests there is some conservation of resistance across these species.

This study has identified new sources of resistance to bruchids and their position on the azuki genome map. While some of these bruchid resistances QTLs are co-localized with seed weight QTL and others are not. The results should enable bruchid resistant azuki beans to be developed using *V. nepalensis*.

Acknowledgments This study was conducted when the first author (PS) was a recipient of a Royal Golden Jubilee Ph.D. Scholarship awarded from the Thailand Research Fund.

References

Adjadi O, Singh BB, Singh SR (1985) Inheritance of bruchid resistance in cowpea. Crop Sci 25:740–742

Banto SM, Sanchez FF (1972) The biology and chemical control of *Callosobruchus chinensis* (Linn) (Coleoptera: Bruchidae). Philipp Ent 2:167–182

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Dongre TK, Pawar SE, Thakare RG, Harwalker MR (1996) Identification of resistance sources to cowpea weevil in *Vigna* sp. and its inheritance of their resistance in black gram. J Stored Prod Res 32:201–204

Fujii K, Ishimoto M, Kitamura K (1989) Patterns of resistance to bean weevils (Bruchidae) in *Vigna radiata-mungo-sublobata* complex inform the breeding of new resistant varieties. Appl Entomol Zool 24:126–132

Isemura T, Kaga A, Konishi S, Ando T, Tomooka N, Han OK, Vaughan DA (2007) Genome dissection of traits related to domestication in azuki bean (*Vigna angularis*) and comparison with other warm season legumes. Ann Bot 100:1053–1071

James AT, Lawn RJ, Williams RW, Lambrides CJ (1999) Cross fertility of Australian accessions of wild mungbean (*Vigna radiata* spp. *sublobata*) with green gram (*V. radiata* spp. *radiata*) and black gram (*V. mungo*). Aust J Bot 47:601–610

Janzen DH (1969) Seed-eaters versus seed size, number, toxicity and dispersal. Evolution 23:1–27

Han OK, Kaga A, Isemura T, Wang XW, Tomooka N, Vaughan DA (2005) A genetic linkage map for azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]. Theor Appl Genet 111:1278–1287

Kaga A (1996) Construction and application of linkages map of azuki bean (*V. angularis*). Ph.D. Thesis, Kobe University

Kaga A, Ishimoto M (1998) Genetic localization of a bruchid resistance gene and its relationship to insecticidal cyclopeptide alkaloids, the vignatic acids, in mungbean (*Vigna radiata* L. Wilczek). Mol Gen Genet 258:378–384

Kao C-H, Zeng Z-B, Teasdale RD (1999) Multiple interval mapping for quantitative trait loci. Genetics 152:1203–1216

Kitamura K, Ishimoto M, Sawa M (1988) Inheritance of resistance to infestation with azuki bean weevil in *Vigna sublobata* and successful incorporation to *V. radiata*. Jpn J Breed 38:459–464

Korol AB, Ronin YI, Hayes P, Nevo E (1998) Multi-interval mapping of correlated trait complexes: simulation analysis and evidence from barley. Heredity 80:273–274

Lumpkin TA, McClary DC (1994) Azuki bean: botany, production and uses. CAB International, Wallingford

Peng J, Ronin Y, Fahima T, Roder MS, Li Y, Nevo E, Korol A (2003) Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. Proc Natl Acad Sci USA 100:2489–2494

Ronin Y, Korol A, Nevo E (1999) Single-and multiple-trait analysis of linked QTLs: some asymptotic analytical approximation. Genetics 151:387–396

Redden RJ, McGuire (1983) The genetic evaluation of bruchid resistance in seed of cowpea. Aust J Agric Res 34:707–715

Somta P, Kaga A, Tomooka N, Kashiwaba K, Isemura T, Chaitieng B, Srinives P, Vaughan DA (2006) Development of an interspecific *Vigna* linkage map between *Vigna umbellata* (Thunb.) Ohwi & Ohashi and *V. nakashimae* (Ohwi) Ohwi & Ohashi and its use in analysis of bruchid resistance and comparative genomics. Plant Breed 125:77–84

Somta P, Ammaranan C, Ooi PAC, Srinives P (2007) Inheritance of seed resistance to bruchids in cultivated mungbean (*Vigna radiata* L. Wilczek). Euphytica 155:47–55

Southgate BJ (1979) Biology of the Bruchidae. Annu Rev Entomol 24:449–473

Tomooka N, Lairungruang C, Nakeeraks P, Egawa Y, Thavarasook C (1992) Development of bruchid-resistant mungbean using wild mungbean germplasm in Thailand. Plant Breed 109:60–66

Tomooka N, Kashiwaba K, Vaughan DA, Ishimoto M, Egawa Y (2000) The effectiveness of evaluating wild species: searching for sources of resistance to bruchid beetles in the genus *Vigna* subgenus *Ceratotropis*. Euphytica 115:27–41

Vaughan DA, Tomooka N, Kaga A (2004) Azuki bean. In: Singh RJ, Jauhar PP (eds) Genetic resources, chromosome engineering, and crop improvement: grain legumes, CRC Press, Boca Raton, pp 341–352

Winn A (1988) Ecological and evolutionary consequence of seed size in *Prunella vulgaris*. Ecology 69:1537–1544