Theor Appl Genet (2016) 129:1673–1683
DOI 10.1007/s00122-016-2731-1

ORIGINAL ARTICLE

A gene encoding a polygalacturonase-inhibiting protein (PGIP) is a candidate gene for bruchid (Coleoptera: bruchidae) resistance in mungbean (*Vigna radiata*)

Sathaporn Chotechung[1] · Prakit Somta[1] · Jinbing Chen[2] · Tarika Yimram[1] · Xin Chen[2] · Peerasak Srinives[1]

Received: 17 February 2016 / Accepted: 13 May 2016 / Published online: 24 May 2016

Abstract
Key message **The *Br* locus confers bruchid resistance in mungbean; *VrPGIP2* (encoding a polygalacturonase inhibitor) is a strong candidate gene for this resistance. The *VrPGIP2* sequence differs between resistant and susceptible lines.**
Abstract Azuki bean weevil (*Callosobruchus chinensis*) and cowpea weevil (*Callosobruchus maculatus*) are serious insect pests of mungbean during storage. Bruchid resistance in mungbean is controlled by a single dominant locus, *Br*. Although the *Br* locus has been located on a genetic map, molecular basis and function of the gene remain unknown. In this study, high-resolution mapping using a $BC_{11}F_2$ population of 418 plants derived from a cross between 'Kamphaeng Saen 1' (KPS1; susceptible) and 'V2802' (resistant) using simple sequence repeat (SSR) markers delimited the *Br* locus to a genomic region of 38 Kb of chromosome 5 containing two annotated genes. EST-SSR marker DMB-SSR158 co-segregated perfectly with the *Br* locus. Bioinformatics analyses revealed that DMB-SSR158 corresponds to a gene encoding a polygalacturonase inhibitor (polygalacturonase-inhibiting protein PGIP) and was designated as *VrPGIP2*. Comparison of *VrPGIP2* coding sequences between four bruchid-resistant (V2802, V1128, V2817 and TC1966) and four bruchid-susceptible (KPS1, Sulu-1, CM and an unknown accession) mungbean lines revealed six single nucleotide polymorphisms (SNPs) between the resistant and susceptible groups. Three of the six SNPs resulted in amino acid changes; namely, alanine (A) to serine (S) at position 320, leucine (L) to proline (P) at position 332, and threonine (T) to P at position 335 of the *VrPGIP2* sequence in resistant lines, compared with that in susceptible lines. The A to S change at position 320 may affect the interaction between PGIP and polygalacuronase. These results indicate that *VrPGIP2* is very likely the gene at the *Br* locus responsible for bruchid resistance in mungbean.

Communicated by Matthew N Nelson.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-016-2731-1) contains supplementary material, which is available to authorized users.

✉ Prakit Somta
pksomta@gmail.com

[1] Department of Agronomy, Faculty of Agriculture at Kampaheng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand

[2] Institute of Vegetable Crops, Jiangsu Academy of Agricultural Science, 50 Zhongling Street, Nanjing 210014, China

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is among the most important grain legumes in Asia with an annual planting area of about 6 million hectares (Nair et al. 2012). It is a popular crop because of its short life cycle, drought tolerance, and ability to fix nitrogen in association with soil bacteria. It is mainly cultivated in rotation after rice or wheat (Somta and Srinives 2007). Mungbean seeds are important sources of dietary proteins and carbohydrates. The seeds are consumed directly or used to produce sprouts, noodles and starches.

The most important insect pest of stored mungbean seeds is bruchids or seed weevils (Coleoptera: bruchidae). Bruchids damage seeds in the field and after harvest. In storage, they can cause a total loss of the seed lot within 3–4 months (Srinives et al. 2007). Azuki bean weevil

(*Callosobruchus chinensis* L.) and cowpea weevil (*Callosobruchus maculatus* F.) are the main bruchid pests of mungbean. These bruchids also damage seeds of several other important *Vigna* crops such as cowpea (*Vigna unguiculata* L.), black gram (*Vigna mungo* (L.) Hepper), Bambara groundnut (*Vigna subterranea* (L.) Verdc.), and azuki bean (*Vigna angularis* (Willd.) Ohwi and Ohashi). The bruchid-damaged seeds are not suitable for consumption, or for agricultural or commercial uses. Therefore, breeding for resistance to bruchids is a major goal in mungbean breeding programs (Srinives et al. 2007).

Several sources of resistance to bruchids have been identified in mungbean. Fujii and Miyazaki (1987) showed that wild mungbean (*V. radiata* var. *sublobata*) accession TC1966 is completely resistant to *C. chinensis* and *C. maculatus*. Lambrides and Imrie (2000) found that wild mungbean accessions ACC23 and ACC41 are resistant to both bruchid species. Talekar and Lin (1992) reported that cultivated mungbean accessions V2709 and V2802 are highly resistant to *C. chinensis*. Previously, we found that V2709 and V2802 are also highly resistant to *C. maculatus*, while the cultivated accessions V1128 and V2817 are completely resistant to both bruchid species (Somta et al. 2008). The resistance in TC1966, V2709, V2802, V1128, and V2817 is due to biochemical compounds in the seeds (Talekar and Lin 1992; Somta et al. 2008).

Resistance to *C. chinensis* in the wild mungbean accession TC1966 is controlled by a single dominant gene locus, designated as *Br*, with modifying factor(s), and the degree of resistance depends on the seed genotype (Kitamura et al. 1988). Resistance to *C. chinensis* and *C. maculatus* in V2709 and V2802 is also controlled by a single dominant gene with modifiers (Somta et al. 2007). Since *V. radiata* var. *sublobata* is proposed to be the ancestor of cultivated mungbean (Maréchal et al. 1978), the bruchid resistance gene in V2709 and V2802 may have been inherited from the *V. radiata* var. *sublobata* progenitor. Gene mapping for *C. chinensis* resistance in TC1966 using restriction fragment length polymorphism (RFLP) markers revealed that the *Br* locus is located on linkage group (LG) VIII between the markers (probes) pA882 and pM151a (Young et al. 1992). Later, additional RFLP markers were added to the mungbean genetic map and LG VIII containing the *Br* gene was re-named LG9. The *Br* locus was labeled as the *Bruc* locus on this map (Menancio-Hautea et al. 1993). Kaga and Ishimoto (1998) narrowed down the *Br* locus to a region of 0.7 cM between the RFLP probes Bng110 and Bng143. Bng143 is tightly linked to *Br*, being 0.2 cM away from this locus. Using ACC41 as the resistance source, the RFLP marker mgM213 mapped onto LG9 was identified as being closely associated with *Br* (1.3 cM apart Miyagi et al. 2004; Mei et al. 2009). A few simple sequence repeat (SSR) and sequence-tagged site (STS) markers for the *Br* locus in ACC41 were developed from a bacterial artificial chromosome (BAC) clone containing marker mgM213 (Miyagi et al. 2004). One of those markers, STSbr2, was shown to be associated with *Br* in V2709 (Sun et al. 2008); Chen et al. (2007) developed seven cleaved amplified polymorphism (CAP) markers closely linked with *Br* in TC1966. Chotechung et al. (2011) reported that the mungbean SSR marker DMB-SSR158 (DMB158 in the original paper) perfectly associated with *Br* in V2802. Based on QTL mapping, Chen et al. (2013) showed that DMB-SSR158 is tightly linked with the major QTL for bruchid resistance in TC1966. The distance between DMB-SSR158 and this major QTL was <0.1 cM. These reports suggested that the same *Br* locus controls bruchid resistance in TC1966, ACC41, V2709 and V2802.

Although DNA markers closely linked to the *Br* locus have been reported, most of them are RFLP markers which are not suitable for marker-assisted selection (MAS) in a large population. In addition, the molecular basis of bruchid resistance is still unknown. Recently, a draft genome sequence of mungbean was reported (Kang et al. 2014). This sequence is useful for gene identification and development of DNA markers for trait(s) of interest in mungbean breeding. In this paper, we report high-resolution mapping of the *Br* locus in the cultivated mungbean accession V2802. The objectives of this study were to narrow down the genomic region of the *Br* locus and to identify candidate gene(s) for resistance.

Materials and methods

Plant materials and DNA extraction

Four bruchid-resistant mungbean accessions (V1128, V2802, V2817, and TC1966) and one bruchid-susceptible mungbean cultivar 'Kamphaeng Saen 1' (KPS1) were used in this study. A $BC_{11}F_2$ population developed from crossing KPS1 as the recipient parent and V2802 as the donor parent was used as the mapping population. Population development procedures are shown in Fig. 1. The population comprised 418 individuals from five $BC_{11}F_1$ plants. The $BC_{11}F_2$ plants were grown together with the parents in an experimental field of Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand, from February to April 2013. Seeds produced from each of the $BC_{11}F_2$ and parental plants were individually harvested and used to evaluate bruchid resistance.

Genomic DNA was extracted from young leaf tissue of all mungbean plants using a modified CTAB method (Lodhi et al. 1994).

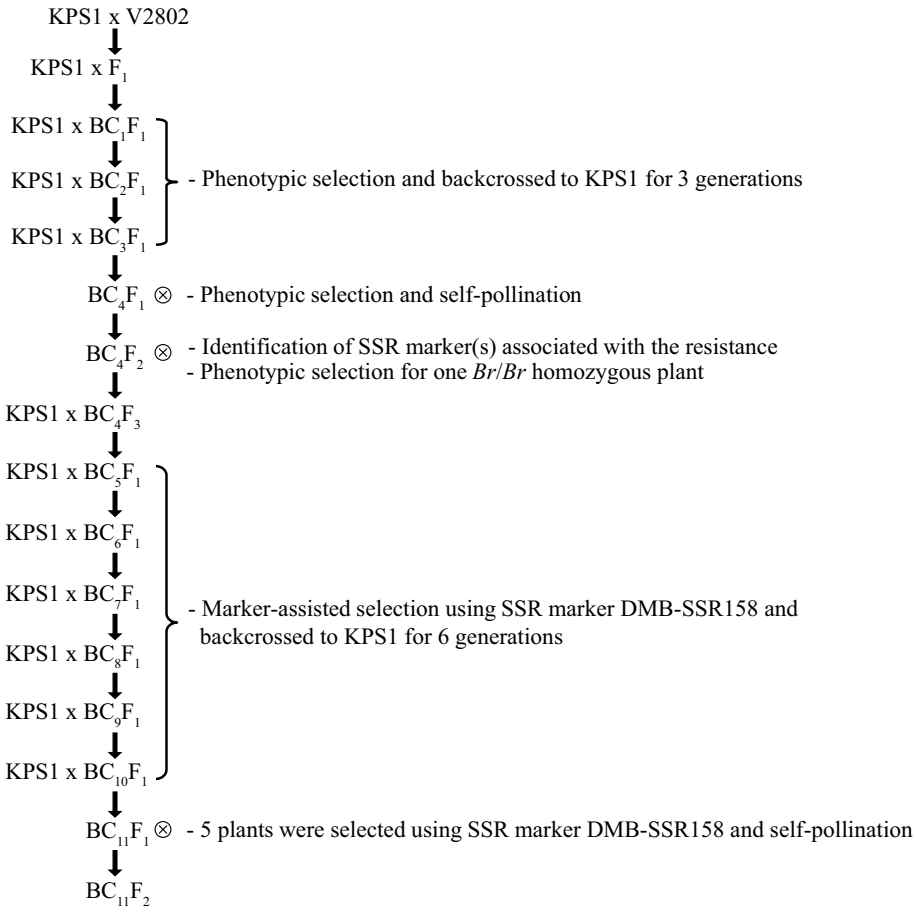

Fig. 1 Scheme showing procedures for the development of $BC_{11}F_2$ mungbean population used for QTL mapping of bruchid resistance

Evaluation of bruchid resistance

Mature seeds were evaluated by exposure to *C. chinensis* and *C. maculatus* reared on seeds of KPS1. The resistance evaluation was conducted as described previously (Somta et al. 2007) with modifications. Briefly, 30–40 seeds from each $BC_{11}F_2$ plant were placed in a plastic box. Then, 40 adult bruchids (20 pairs of 1–3 day old males and females) were added to the box and kept there for 7 days to lay eggs and then removed. At least three eggs were laid on each seed. Parental seeds were tested and replicated five times. The infested seeds were maintained at 28 °C and 70 % RH. The number of damaged seeds was counted at 60 days after introducing the insects and then used to calculate damage percentages.

Segregation analysis

To confirm the monogenic inheritance of bruchid resistance in V2802 (Somta et al. 2007; Chotechung et al. 2011), $BC_{11}F_2$ plants showing 0–80 % damaged seeds were characterized as resistant. These included the homozygous resistance genotype (highly resistant with 0–20 % damaged seeds) and the heterozygous resistance genotype (moderately resistant with 21–80 % damaged seeds), while $BC_{11}F_2$ plants showing 81–100 % damaged seeds were considered to be homozygous for the susceptible genotype (Somta et al. 2007). A Chi-squared (χ^2) test was used to determine the goodness of fit to a 3 (resistant):1 (susceptible) ratio. The test was conducted using software *R*-program 2.0.10 (R Development Core Team 2010).

The correlation between percentages of seeds damaged by *C. chinensis* and *C. maculatus* was calculated using *R*-program 2.0.10.

Bioinformatics analyses of *Br* locus location on mungbean genome

In a previous study, the *Br* locus was found to be located between the RFLP markers Bng110 and Bng143 (Kaga and Ishimoto 1998). Therefore, we used mungbean cDNA sequences of the probes Bng110 (GenBank accession AB020611) and Bng143 (GenBank accession AB020610) to conduct BLASTN searches against the mungbean reference sequence (Kang et al. 2014; http://plantgenomics.snu.ac.kr/sequenceserver). Once identified, the genome sequence between the probes Bng110 and Bng143 was downloaded and searched for SSRs using SSRIT software

(Temnykh et al. 2001). The primers were designed using Primer3 software (Untergasser et al. 2012).

We also conducted a BLASTN search for the location of the genic SSR marker DMB-SSR158 on the reference genome, because this marker was reported to completely co-segregate with the *Br* locus in V2802 (Chotechung et al. 2011). The cDNA sequence of this marker was used in the BLASTN search (GenBank accession AM696512; Somta et al. 2009).

DNA marker analysis

The SSR markers used to screen for polymorphisms between KPS1 and V2802 were those located on the same linkage group as the RFLP markers Bng110 and Bng143 [LG9 of mungbean reported by Kaga and Ishimoto (1998), Zhao et al. (2010) and LG2 of mungbean, azuki bean and cowpea reported by Isemura et al. (2012), Han et al. (2005) and Kongjaimun et al. (2012), respectively] together with the new SSR markers developed in this study (Supplementary Table S1). We also included one EST marker, RP, which was shown to be closely linked to *Br* in mungbean accession V2709 (Hong et al. 2015). The PCR amplifications, gel electrophoresis, and DNA band visualization were carried out as described previously (Somta et al. 2009). Polymorphic markers were used to analyze the DNA of the $BC_{11}F_2$ plants.

QTL mapping for bruchid resistance

A genetic linkage map was constructed using QTL IciMapping 4.0 software (Meng et al. 2015). Markers were grouped with log of odds (LOD) value of 3.0 and anchored based on their positions on the reference sequence. Distances between markers were calculated using Kosambi's mapping function. A QTL controlling bruchid resistance was located onto the linkage map by the inclusive composite interval mapping method (Li et al. 2007) using QTL IciMapping 4.0. The LOD threshold for declaring the QTL was determined by a 5000 permutation test (at $P = 0.001$).

Sequencing of candidate genes for the *Br* locus

Based on the results of QTL mapping *Vradi05g03940* and *Vradi05g03950* were considered as candidate genes for *Br* (see "Results"). Primer pairs were designed (Supplementary Table S2) to amplify all exonic regions of the genes in all mungbean accessions/cultivars used in this study. The PCR mixture (10 µl) contained 10 ng genomic DNA, 5 pmol each forward and reverse primers, 1 U Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), 1× Taq Buffer, 2 mM dNTPs, and 0.8 mM $MgCl_2$. The thermal cycling program (using a GeneAmp 9700 system) was as follows: 94 °C for 2 min, 40 cycles of 94 °C for 15 s, 57.5 °C for 30 s, 72 °C for 90 s, and 72 °C for 2 min. The amplified products were electrophoresed on a 1.0 % agarose gel, and then DNA bands with expected size were cut and purified with E.Z.N.A.® Gel Extraction Kit (Omega, Doraville, GA, USA) following the manufacturer's instructions. The purified DNA was sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) by Macrogen Inc. (Seoul, Korea).

Re-annotation of *Vradi05g03950*

The cDNA sequence of the marker DMB-SSR158 (GenBank accession AM696512) was used in BLASTN searches against the transcriptome shotgun assembly (TSA) of mungbean in GenBank and our in-house mungbean transcriptome sequence data generated from the susceptible mungbean accessions 'Sulu_1' and 'CM'. A transcript sequence from GenBank (GenBank accession GBXO01011683) and two transcript sequences from our laboratory were aligned with the *Vradi05g03950* sequence from the reference sequence (VC1973A), KPS1, V2802, V1128 and TC1966 using Clustal 2.1 to obtain a new annotated CDS and identify mutations among the mungbean accessions. The predicted protein sequences of *Vradi05g03950* from all these mungbean lines were also aligned.

Results

Bruchid resistance in the $BC_{11}F_2$ population

Seeds of V2802 were completely resistant to *C. chinensis* and *C. maculatus*, while all seeds of KPS1 were damaged by both bruchid species. In the $BC_{11}F_2$ population, the frequency distribution of the percentage of damaged seeds was non-continuous and bimodal, and skewed toward KPS1 (Fig. 2). The proportions of damaged seeds caused by *C. chinensis* and *C. maculatus* were similar, ranging from 0 to 100 % with a mean of 48.65 % for *C. chinensis* and 48.58 % for *C. maculatus*, with a correlation coefficient of 0.97 ($df = 402$, $P < 0.0001$). The high correlation coefficient confirmed the results reported by Somta et al. (2007) and Chotechung et al. (2011) and suggested that resistance to *C. chinensis* and to *C. maculatus* in V2802 is controlled by the same locus.

We classified resistant and susceptible plants in the $BC_{11}F_2$ population using 80 % damaged seeds as the cut-off level. This analysis revealed that resistance to *C. chinensis* and to *C. maculatus* did not fit a 3 (resistant):1 (susceptible) ratio ($\chi^2 = 25.27$, $P < 0.0001$ and $\chi^2 = 27.57$,

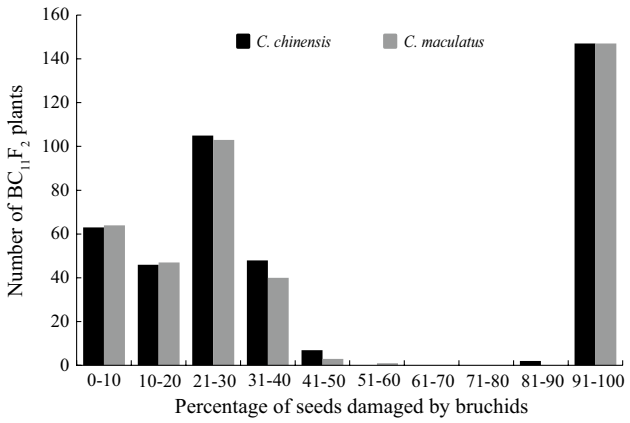

Fig. 2 Frequency distribution of the percentage of seeds damaged by *Callosobruchus chinensis* and *Callosobruchus maculatus* in the $BC_{11}F_2$ population

$P < 0.0001$, respectively). These findings contrasted with those reported by Somta et al. (2007) and Chotechung et al. (2011) that a single dominant gene with modifiers controls bruchid resistance in V2802.

Location of *Br* locus on mungbean reference genome

The mungbean chromosome region containing *Br* was identified by BLAST searches using the sequences of RFLP markers Bng143 and Bng110. The results showed that these markers corresponded to two annotated genes, *Vradi05g03740* (Vr05:5,049,905..5,054,628) and *Vradi05g04410* (Vr05:6,775,804..6,780,603), on chromosome 5 of mungbean, respectively (Fig. 3a). The markers Bng143 and Bng110 were found to be 1.72 Mbp apart on the mungbean reference genome. The search

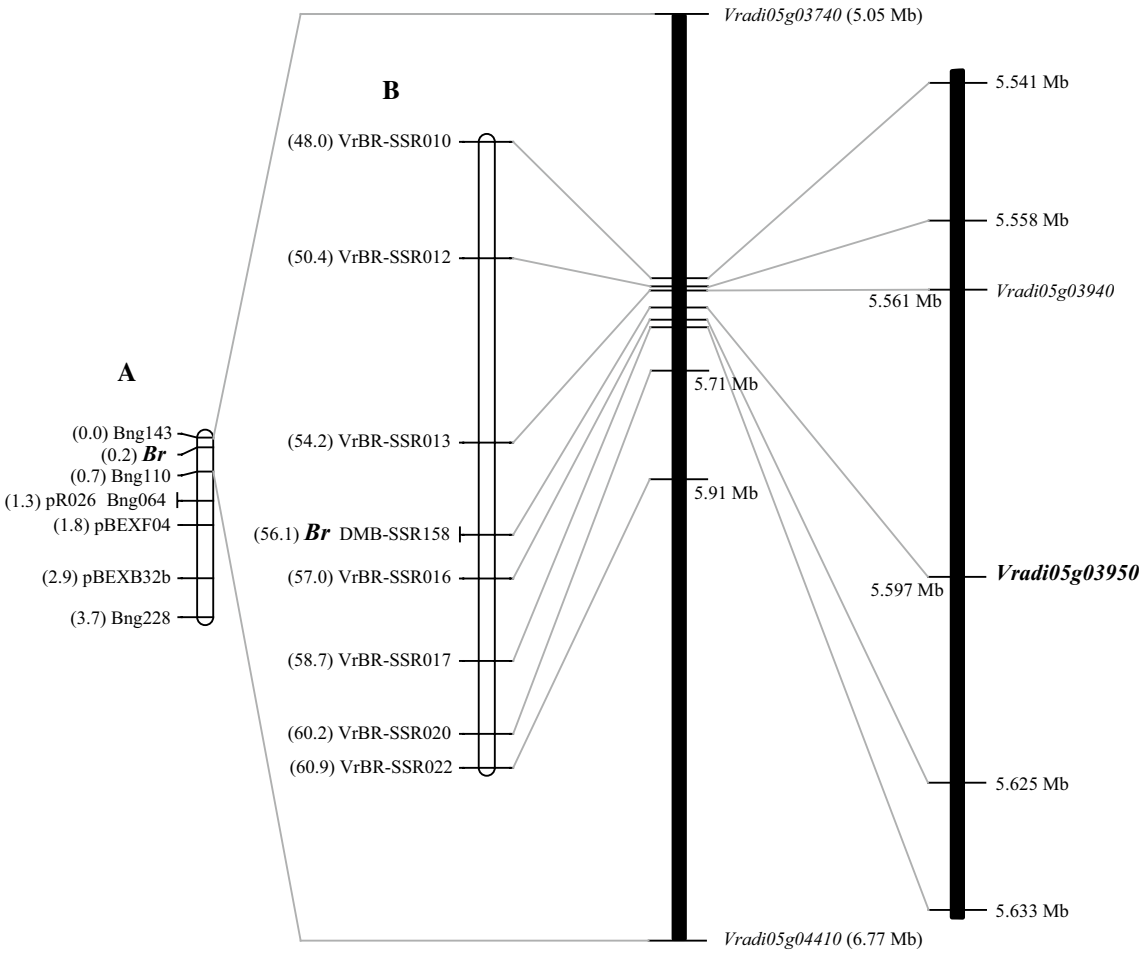

Fig. 3 Location of *Br* locus in wild mungbean accession TC1966 (as reported by Kaga and Ishimoto (1998)) on chromosome 5 of mungbean reference genome based on BLAST search of RFLP markers, Bng110 and Bng143 (**a**). Current map of the *Br* locus redrawn based on the map reported by the same authors. Location of the *Br* locus from cultivated mungbean accession V2802 detected in the $BC_{11}F_2$ population in this study, and its position and corresponding genes on chromosome 5 of the mungbean reference genome (**b**). *Numbers in parentheses* indicate the position of marker on the linkage map in centiMorgan units

Table 1 Location and effects of QTL controlling resistance to azuki bean weevil (*Callosobruchus chinensis*) and cowpea weevil (*Callosobruchus maculatus*) detected in $BC_{11}F_2$ mungbean population derived from the cross between KPS1 and V2802. The resistance was determined by percentage of seeds damaged by bruchids

Bruchid species	QTL name	Position (cM)	LOD score	Interval markers	PVE[a] (%)	Additive effect	Dominance effect
C. chinensis	*qBr*	56.0	245.61	VrBr-SSR013– DMB-SSR158	93.34	41.10	−38.13
C. maculatus	*qBr*	56.0	240.39	VrBr-SSR013– DMB-SSR158	93.84	40.89	−36.99

[a] Phenotypic variance explained by the QTL

also revealed that the RFLP marker mgM213 and SSR marker DMB-SSR158 corresponded to *Vradi05g06080* (Vr05:12,103,656..12,111,809) and *Vradi05g03950* (Vr05:5,590,573..5,598,731), respectively, on chromosome 5 of mungbean (Fig. 3). There were 76 annotated genes in the 1.72 Mb region.

We developed 40 SSR markers located between *Vradi05g03740* and *Vradi05g04410* (Supplementary Table S1). These new SSR markers, seven previously developed SSR markers, and one STS marker previously mapped to the same linkage group with the *Br* locus (48 markers in total) were used to detect polymorphisms between V2802 and KPS1. Of the 48 markers, 45 were able to amplify mungbean DNA and 10 were polymorphic between the two mungbean cultivars (Supplementary Fig. S1). Marker DMB-SSR158 detected two loci: one monomorphic and the other polymorphic. The polymorphic locus was inherited as a dominant marker (DNA band was present only in V2802).

High-resolution mapping of the *Br* locus

A genetic map for mungbean chromosome 5 was constructed using polymorphic DNA markers. The linkage group comprised ten markers and spanned a length of 60.9 cM. A QTL analysis identified one major QTL for resistance to each bruchid species (Table 1; Supplementary Fig. S2). These QTLs were located at the same position between markers VrBr-SSR013 and DMB-SSR158 and thus were named *qBr*. The *qBr* was only 0.1 cM away from DMB-SSR158 (Supplementary Fig. S2), and explained 93.34–93.84 % of the total variation in the proportion of damaged seeds caused by *C. chinensis* and *C. maculatus*, respectively.

When the genotypes were classified according to bruchid damage as resistant (*Br*/*Br* and *Br*/*br*) and susceptible (*br*/*br*), we found that *Br* co-segregated perfectly with the marker DMB-SSR158 (*Vradi05g03950*) (Supplementary Table S3). This result together with the QTL mapping result suggested that *Br* and *Vradi05g03950* are the same locus. Figure 3 shows the location of *Br* and the markers associated with it on chromosome 5. A BLASTP search against the NCBI database revealed that *Vradi05g03950* showed the strongest similarity to a polygalacturonase-inhibiting protein (PGIP) in *Medicago* (*Medicago truncatula* Gaertn.) (accession XP_003625218.1; e-value = $1e^{-118}$, score = 993, identity = 68.0 %).

Sequencing and re-annotation of candidate gene(s)

The coding DNA sequences (CDSs) of *Vradi05g03940* and *Vradi05g03950* in V2802 and KPS1 were determined. *Vradi05g03940* was sequenced because it is located next to *Vradi05g03950* and encodes a PGIP. In the mungbean reference sequence, the full CDS of these two genes consisted of 1302 and 1431 nucleotides, respectively.

A comparison of the CDSs showed that *Vradi05g03940* from VC1973A and KPS1 (both susceptible to bruchids) were the same, whereas the sequences of V2802, V1128, V2817, and TC1966 (all resistant to bruchids) were identical but different from that in susceptible mungbean. There were 12 single nucleotide polymorphisms (SNPs) between the two mungbean groups (Supplementary Fig. S3). The SNPs at positions 854 and 913 caused amino acid changes in the bruchid-resistant mungbean accessions, while the SNP at position 1087 resulted in a premature stop codon in these accessions. The predicted PGIP encoded by *Vradi05g03940* in the susceptible mungbeans consisted of 433 amino acids, while that in the resistant mungbeans consisted of only 362 amino acids (Supplementary Fig. S4). There were two changes in amino acids at positions 285 [asparagine (N) to serine (S)] and 305 [glutamic acid (E) to lysine (K)]. *Vradi05g03940* showed the strongest similarity to PGIP from *M. truncatula* (XP_003625218.1; identities = 69 %, E-value = 3×10^{-150}); therefore, we named it *VrPGIP1*.

We re-annotated the CDS of *Vradi05g03950* because a BLAST search for the position of DMB-SSR158 showed that the transcript sequence (GenBank accession AM696152) used to develop this marker aligned to both exonic and intronic regions of this gene. We conducted a BLAST search against mungbean TSA database together with our in-house transcriptome data and found that AM696512 was almost identical to transcripts of GBXO01011683, Sulu_1 and CM. We aligned these three transcript sequences and AM696152 to the reference

sequence of VC1973A and obtained a new annotation for *Vradi05g03950* (Supplementary Fig. S5). The new annotated gene was found to be intronless with 1011 nucleotides encoding a protein of 336 amino acids. A blast search of this protein against the GenBank database demonstrated that, similar to *VrPGIP1*, it showed the highest similarity to PGIP from *M. truncatula* (XP_003625218.1; identities = 69 %, E-value = 3×10^{-150}). Thus, we named this gene *VrPGIP2*.

We compared the nucleotide sequences of *VrPGIP2* from GBXO01011683, Sulu-1, CM, KPS1, V2802, V1128, V2817, and TC1966, and found that it was of the same length in all of the accessions. The *VrPGIP2* sequences in GBXO01011683, Sulu_1, CM, and KPS1 (all susceptible to bruchids) were identical. The *VrPGIP2* sequences in V2802, V1128, V2817, and TC1966 (all resistant to bruchids) were also identical. However, the *VrPGIP2* sequences differed between the resistant and the susceptible groups of cultivars (Supplementary Fig. S6). There were six SNPs (at nucleotide positions 958, 969, 972, 995, 1003, and 1008) between the two mungbean groups (Supplementary Fig. S6). The SNPs at positions 958, 995, and 1003 caused amino acid changes in *VrPGIP2* at positions 320 [from alanine (A) to S], 332 [from leucine (L) to proline (P)], and 335 [from threonine (T) to P] in the resistant mungbean lines, compared with the sequence in the susceptible lines (Fig. 4).

Discussion

Legume breeders and scientists have long been interested in identifying the bruchid resistance gene in mungbean. The *Br* locus confers resistance against five bruchid species including *C. chinensis*, *C. maculatus*, *C. phaseoli* (Gyllenhal), *Zabrotes subfasciatus* (Boheman), and *Acanthoscelides obtectus* (Say) (Fujii and Miyazaki 1987; Fujii et al. 1989; Lambrides and Imrie 2000), which are major storage pests of several legume crops around the world. Despite fine mapping of the *Br* locus 18 years ago (Kaga and Ishimoto 1998), identification of the gene(s) or candidate gene(s) responsible for resistance has been impossible due to the lack of genomic resources for mungbean. Recently, the whole-genome sequence of mungbean has been reported (Kang et al. 2014). Using this sequence together with QTL mapping and bioinformatics analyses, we were able to identify a candidate gene for *Br* in mungbean accession V2802.

Previous studies have shown that resistance to *C. chinensis* and *C. maculatus* in V2802 is controlled by a single dominant gene with some modifiers (Somta et al. 2007; Chotechung et al. 2011). In this study, the segregation of resistance to both insect species in $BC_{11}F_2$ was not consistent with monogenic inheritance, although the $BC_{11}F_2$ population used in this study was derived from the same BC_4F_2 population as that used in the study by Chotechung et al. (2011) (Fig. 1). These contrasting results may be because the $BC_{11}F_2$ population was a bulked population derived from five $BC_{11}F_1$ families, each consisting of 45–121 $BC_{11}F_2$ plants. The small number of plants in some families may have resulted in a deviation from the expected monogenic segregation.

Although segregation distortion of *Br* was evident in the $BC_{11}F_2$ population, QTL mapping detected a major QTL for resistance to both bruchids in V2802 in the genomic region previously identified to contain *Br*, as reported by Kaga and Ishimoto (1998) (Fig. 3). The QTLs for both bruchid species were localized to the same position and explained approximately 90 % of the variation in the proportion of damaged seeds. These results agreed with the high correlation between the two bruchids in terms of the proportion of damaged seeds found in this study and previously (Somta et al. 2007). The source of resistance used by Kaga and Ishimoto (1998) was the accession TC1966. Thus, the resistance gene in V2802 and that in TC1966 were predicted to be the same. The physical genome distance between markers VrBr-SSR13 and DMB-SSR158, which flank the bruchid-resistance QTL on the mungbean reference sequence, is only 38 kb, and contains two annotated genes, *Vradi05g03940* and *Vradi05g03950*, considered as candidates for *Br*. *Vradi05g03950* corresponding to DMB-SSR158 which co-segregated perfectly with bruchid resistance (Supplementary Table S3) and confirmed our previous result (Chotechung et al. 2011). Recently, Hong et al. (2015) reported that *C. chinensis* resistance in a mungbean cultivar that derived its resistance from V2709 is controlled by two tightly linked major QTLs, although our research group concluded that the resistance in this accession is controlled by a single gene (Somta et al. 2007; Chotechung et al. 2011). Nonetheless, based on blast searches of the primer sequences of the markers GBssr-MB87 and RP that were linked to each QTL as reported by Hong et al. (2015), we found that the position of GBssr-MB87 on chromosome 5 (5,602,082; identities = 94 %; E-value = 4.1) is very close to *Vradi05g03950*, while RP on chromosome 5 has multiple positions corresponding to the region around 5,210,000 to 5,520,000 (data not shown). Further research is required to explore the reasons for these contrasting results.

Both *Vradi05g03940* and *Vradi05g03950* encode a PGIP. Such enzyme inhibitor(s) play important roles in plant defense against phytophagous insects (Lawrence and Koundal 2002). For example, an α-amylase inhibitor in common bean seeds negatively affects the growth and development of *C. chinensis* and *C. maculatus* (Ishimoto and Kitamura 1989; Shade et al. 1994; Ishimoto

```
GBXO01011683    MRSLLMMLGLCVLVLSPFPVAVSERCHPQDKKVLLRFKKELNNPYFLASWDPKEDCCDWY   60
Sulu_1          MRSLLMMLGLCVLVLSPFPVAVSERCHPQDKKVLLRFKKELNNPYFLASWDPKEDCCDWY   60
CM              MRSLLMMLGLCVLVLSPFPVAVSERCHPQDKKVLLRFKKELNNPYFLASWDPKEDCCDWY   60
KPS1            MRSLLMMLGLCVLVLSPFPVAVSERCHPQDKKVLLRFKKELNNPYFLASWDPKEDCCDWY   60
V2802           MRSLLMMLGLCVLVLSPFPVAVSERCHPQDKKVLLRFKKELNNPYFLASWDPKEDCCDWY   60
V1128           MRSLLMMLGLCVLVLSPFPVAVSERCHPQDKKVLLRFKKELNNPYFLASWDPKEDCCDWY   60
V2802           MRSLLMMLGLCVLVLSPFPVAVSERCHPQDKKVLLRFKKELNNPYFLASWDPKEDCCDWY   60
TC1966          MRSLLMMLGLCVLVLSPFPVAVSERCHPQDKKVLLRFKKELNNPYFLASWDPKEDCCDWY   60
                ************************************************************

GBXO01011683    CVKCDDRNNRIYEVFLVSSFPDPNVTGQIPPSVGDLPYLEYLDFHKLPNLVGPIPTTITK   120
Sulu_1          CVKCDDRNNRIYEVFLVSSFPDPNVTGQIPPSVGDLPYLEYLDFHKLPNLVGPIPTTITK   120
CM              CVKCDDRNNRIYEVFLVSSFPDPNVTGQIPPSVGDLPYLEYLDFHKLPNLVGPIPTTITK   120
KPS1            CVKCDDRNNRIYEVFLVSSFPDPNVTGQIPPSVGDLPYLEYLDFHKLPNLVGPIPTTITK   120
V2802           CVKCDDRNNRIYEVFLVSSFPDPNVTGQIPPSVGDLPYLEYLDFHKLPNLVGPIPTTITK   120
V1128           CVKCDDRNNRIYEVFLVSSFPDPNVTGQIPPSVGDLPYLEYLDFHKLPNLVGPIPTTITK   120
V2802           CVKCDDRNNRIYEVFLVSSFPDPNVTGQIPPSVGDLPYLEYLDFHKLPNLVGPIPTTITK   120
TC1966          CVKCDDRNNRIYEVFLVSSFPDPNVTGQIPPSVGDLPYLEYLDFHKLPNLVGPIPTTITK   120
                ************************************************************

GBXO01011683    LTKLKSLSITYTGLSGSIPEFLGQIKTLEYIALSFNSLSGSIPSSLSQLPNLGSLQLDRN   180
Sulu_1          LTKLKSLSITYTGLSGSIPEFLGQIKTLEYIALSFNSLSGSIPSSLSQLPNLGSLQLDRN   180
CM              LTKLKSLSITYTGLSGSIPEFLGQIKTLEYIALSFNSLSGSIPSSLSQLPNLGSLQLDRN   180
KPS1            LTKLKSLSITYTGLSGSIPEFLGQIKTLEYIALSFNSLSGSIPSSLSQLPNLGSLQLDRN   180
V2802           LTKLKSLSITYTGLSGSIPEFLGQIKTLEYIALSFNSLSGSIPSSLSQLPNLGSLQLDRN   180
V1128           LTKLKSLSITYTGLSGSIPEFLGQIKTLEYIALSFNSLSGSIPSSLSQLPNLGSLQLDRN   180
V2817           LTKLKSLSITYTGLSGSIPEFLGQIKTLEYIALSFNSLSGSIPSSLSQLPNLGSLQLDRN   180
TC1966          LTKLKSLSITYTGLSGSIPEFLGQIKTLEYIALSFNSLSGSIPSSLSQLPNLGSLQLDRN   180
                ************************************************************

GBXO01011683    KLTGPIPASFGSFKKPGPDLKLSHNQLSGTLPPSLGNLDPDNIDLSRNNLVGDASFLFGS   240
Sulu_1          KLTGPIPASFGSFKKPGPDLKLSHNQLSGTLPPSLGNLDPDNIDLSRNNLVGDASFLFGS   240
CM              KLTGPIPASFGSFKKPGPDLKLSHNQLSGTLPPSLGNLDPDNIDLSRNNLVGDASFLFGS   240
KPS1            KLTGPIPASFGSFKKPGPDLKLSHNQLSGTLPPSLGNLDPDNIDLSRNNLVGDASFLFGS   240
V2802           KLTGPIPASFGSFKKPGPDLKLSHNQLSGTLPPSLGNLDPDNIDLSRNNLVGDASFLFGS   240
V1128           KLTGPIPASFGSFKKPGPDLKLSHNQLSGTLPPSLGNLDPDNIDLSRNNLVGDASFLFGS   240
V2817           KLTGPIPASFGSFKKPGPDLKLSHNQLSGTLPPSLGNLDPDNIDLSRNNLVGDASFLFGS   240
TC1966          KLTGPIPASFGSFKKPGPDLKLSHNQLSGTLPPSLGNLDPDNIDLSRNNLVGDASFLFGS   240
                ************************************************************

GBXO01011683    KKKTQVLDLSRNAFSFDLSPVTFPKKTLIWFDINHNKIYGSLPVALTKVENLQQLNVSYN   300
Sulu_1          KKKTQVLDLSRNAFSFDLSPVTFPKKTLIWFDINHNKIYGSLPVALTKVENLQQLNVSYN   300
CM              KKKTQVLDLSRNAFSFDLSPVTFPKKTLIWFDINHNKIYGSLPVALTKVENLQQLNVSYN   300
KPS1            KKKTQVLDLSRNAFSFDLSPVTFPKKTLIWFDINHNKIYGSLPVALTKVENLQQLNVSYN   300
V2802           KKKTQVLDLSRNAFSFDLSPVTFPKKTLIWFDINHNKIYGSLPVALTKVENLQQLNVSYN   300
V1128           KKKTQVLDLSRNAFSFDLSPVTFPKKTLIWFDINHNKIYGSLPVALTKVENLQQLNVSYN   300
V2817           KKKTQVLDLSRNAFSFDLSPVTFPKKTLIWFDINHNKIYGSLPVALTKVENLQQLNVSYN   300
TC1966          KKKTQVLDLSRNAFSFDLSPVTFPKKTLIWFDINHNKIYGSLPVALTKVENLQQLNVSYN   300
                ************************************************************

GBXO01011683    PQLKGQIPQGGELHRFDKYAFFHTKLCGSPLLPCTK   336
Sulu_1          PQLKGQIPQGGELHRFDKYAFFHTKLCGSPLLPCTK   336
CM              PQLKGQIPQGGELHRFDKYAFFHTKLCGSPLLPCTK   336
KPS1            PQLKGQIPQGGELHRFDKYAFFHTKLCGSPLLPCTK   336
V2802           PQLKGQIPQGGELHRFDKYSFFHTKLCGSPLPPCPK   336
V1128           PQLKGQIPQGGELHRFDKYSFFHTKLCGSPLPPCPK   336
V2817           PQLKGQIPQGGELHRFDKYSFFHTKLCGSPLPPCPK   336
TC1966          PQLKGQIPQGGELHRFDKYSFFHTKLCGSPLPPCPK   336
                ********************:*********** **.*
```

Fig. 4 Alignment of deduced amino acid sequences encoded by *VrPGIP2* in Sulu_1, CM, an unknown accession (GBXO01011683), KPS1, V2802, V2817, V1128, and TC1966. Sulu_1, CM, GBXO01011683 and KPS1 are susceptible to bruchids, while V2802, V2817, V1128, and TC19966 are resistant to bruchids. Polymorphic sites are shown in *bold*

et al. 2006). Polygalacturonase (PG) is a pectin-degrading enzyme that hydrolyzes α-1,4-glycosidic bonds between galacturonic acid units and acts preferentially on pectic acid. These proteins belong to the leucine-rich-repeat protein family. In plants, PGIPs are known to play roles in defense against disease and insects. Pectinesterases and PGs degrade pectin into oligosaccharides that can be absorbed in the insect gut. The main causes of plant damage by phytophagous insects are PGs from salivary glands (Girard and Jouanin 1999; Boyd et al. 2002). In *C. maculatus*, PGs are involved in digestion (Pedra et al. 2003; Pauchet et al. 2010; Nogueira et al. 2012; Santana 2013). Nogueira et al. (2012) noted that a high number of PG isoforms in larval *C. maculatus* found in their study and that of Pauchet et al. (2010) could be explained by the presence of PGIPs in cowpea seeds fed to the insects. They also noted that plants accumulate or induce enzyme inhibitors involved in polymer digestion, such as α-amylase inhibitors for starch and PGIPs for hemicellulose or pectin. Thus, PGs appear to play an important role in digestion in *C. maculatus*. In rice weevil [*Sitophilus oryzae* (Coleoptera: curculionidae)] feeding on cereal seeds, especially rice and wheat, pectinases including PG function as digestive enzymes to degrade pectin in the seeds as an energy source (Shen et al. 1996). In addition, degrading pectin helps other digestive enzymes to gain access to their substrates in insect food (Shen et al. 1996). The same is likely to be true for PGs in *Callosobruchus* weevils.

Although the *VrPGIP1* protein encoded by *Vradi05g03940* (*VrPGIP1*) differs between KPS1 and V2802, *VrPGIP1* is not likely to be the gene responsible for bruchid resistance in V2802 because the SSR marker VrBr-SSR13 within this gene did not completely co-segregate with resistance (Supplementary Table S3). Also, *Vradi05g03940* encodes an incomplete PGIP in V2802 and all other bruchid-resistant accessions (Supplementary Fig. S3). However, further research is necessary to determine whether the incomplete *VrPGIP1* plays any role in bruchid resistance. In this study, *Vradi05g03950* (*VrPGIP2*) was re-annotated and was found to encode a polymorphic *VrPGIP2* between KPS1 and V2802. The marker DMB-SSR158, which was developed from this gene's sequence, co-segregated perfectly with resistance, suggesting that *VrPGIP2* is the gene responsible for bruchid resistance at the *Br* locus. However, Liu et al. (2016) recently showed that DMB-SSR158 was not completely associated with *Br* in recombinant inbred lines of NM92 × TC1966. The contrasting results between our study and that of Liu et al. (2016) are worthy of further investigation. Among the three polymorphic amino acids in *VrPGIP2* between V2802 and KPS1 (between resistant and susceptible mungbeans) encoded by *VrPGIP2*, the change from S to A at position 320 is likely to play an important role in bruchid resistance. Leckie et al. (1999) showed that an S to A mutation and vice versa at this position (equivalent to position 326 of the common bean PGIP1 and PGIP2) affects the PGIP–PG interaction. Nonetheless, the perfect co-segregation between DMB-SSR158 (*VrPGIP2*) and *Br* and the *VrPGIP2* polymorphisms between KPS1 and V2802 found in this study indicate that *VrPGIP2* is a very strong candidate as the gene responsible for resistance at the *Br* locus. Gene cloning and complementation analyses should be conducted to confirm the role of *VrPGIP2* in bruchid resistance.

Previous studies have shown that PGIPs play roles in the feeding behavior of Heteropteran insects. For example, PGIP3 and PGIP4 from common bean inhibit PGs of mirid bugs (Frati et al. 2006). *VrPGIP2* may also confer resistance to other piercing–sucking insects by preventing the digestion of plant tissue by insect PGs. This could explain why *Br* confers resistance to not only bruchids, but also to pod-sucking bug [*Riptortus clavatus* (Thunb.)] (Heteroptera: alydidae) (Kaga and Ishimoto 1998).

The SSR marker system has become standard for MAS using standard laboratory facilities because SSRs are easy to detect (PCR-based), multi-allelic, highly polymorphic, and highly reproducible. Previously, only a few SSR markers linked to bruchid resistance have been reported for mungbean, including DMB-SSR158 (Chotechung et al. 2011; Chen et al. 2013), GBssr-MB87 (Chotechung et al. 2011; Hong et al. 2015), and SSRbr1 (Miyagi et al. 2004). In this study, we developed several SSR markers located physically very close to the *Br* locus, and the marker DMB-SSR158 located within the candidate gene sequence (Supplementary Table S1and Fig. 3). These SSR markers will be very useful for MAS for bruchid resistance and will save 3–4 months in each selection cycle as compared to phenotypic selection.

Author contribution statement S.C. carried out major parts of the experiments and analyzed data. S.C., P. Somta, T.Y., and P. Srinives prepared plant materials. S.C and T.Y. evaluated bruchid resistance, J.C. and X.C. developed markers and sequenced DNA. P. Somta initiated and coordinated the study, and analyzed the results. P. Somta and P. Srinives wrote the manuscript. All authors approved the manuscript.

Acknowledgments This research was funded by the Center for Advanced Studies for Agriculture and Food (CASAF), Kasetsart University under the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand to P. Somta. We also acknowledge initial support from Thailand's National Science and Technology Development Agency to P. Srinives.

Compliance with ethical standards

Ethical standards The experiments carried out in this study comply with the current laws of Thailand.

Conflict of interest The authors declare that they have no conflict of interest.

References

Benchimol LL, de Campos T, Carbonell SAM, Colombo CA, Chioratto AF, Formighieri EF, Gouvêa LRL, de Souza AP (2007) Structure of genetic diversity among common bean (*Phaseolus vulgaris* L.) varieties of Mesoamerican and Andean origins using new developed microsatellite markers. Genet Resour Crop Evol 54:1747–1762

Boyd DW Jr, Cohen AC, Alverson DR (2002) Digestive enzymes and stylet morphology of *Deraeocoris nebulosus* (Hemiptera: miridae), a predacious plant bug. Ann Entomol Soc Am 95:395–401

Chen HM, Liu CA, Kuo GC, Chien CM, Sun HC, Huang CC, Lin YC, Ku HM (2007) Development of a molecular marker for a bruchid (*Callosobruchus chinensis* L.) resistance gene in mungbean. Euphytica 157:113–122

Chen HM, Ku HS, Schafleitner R, Bains TS, Kuo GC, Liu CA, Nair RM (2013) The major quantitative trait locus for mungbean yellow mosaic Indian virus resistance is tightly linked in repulsion phase to the major bruchid resistance locus in a cross between mungbean [*Vigna radiata* (L.) Wilczek] and its wild relative *Vigna radiata* ssp. *sublobata*. Euphytica 192:205–216

Chotechung S, Somta P, Chankaew S, Srinives P, Somta P (2011) Identification of DNA markers associated with bruchid resistance in mungbean. Khon Khan Agri J 39(S3):221–226 **(in Thai with English abstract)**

Development Core Team R (2010) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Frati F, Galletti R, De Lorenzo G, Salerno G, Conti E (2006) Activity of endo- polygalacturonases in mirid bugs (Heteroptera: miridae) and their inhibition by plant cell wall proteins (PGIPs). Eur J Entomol 103:515–522

Fujii K, Miyazaki S (1987) Infestation resistance of wild legumes (*Vigna sublobata*) to azuki bean weevil, *Callosobruchus chinensis* (L.) (Coleoptera: bruchidae) and its relationship with cytogenetic classification. Appl Ent Zool 22:229–230

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

Gaitán-Solís E, Duque MC, Edwards KJ, Tohme J (2002) Microsatellite repeats in common bean (*Phaseolus vulgaris*): isolation, characterization and cross-species amplification in *Phaseolus* ssp. Crop Sci 42:2128–2136

Girard C, Jouanin L (1999) Molecular cloning of cDNAs encoding a range of digestive enzymes from a phytophagous beetle, *Phaedon cochleariae*. Insect Biochem Mol Biol 29:1129–1142

Grisi MCM, Blair MW, Gepts P, Brondani C, Pereira PAA, Brondani RPV (2007) Genetic mapping of a new set of microsatellite markers in a reference common bean (*Phaseolus vulgaris*) population BAT93 × Jalo EEP558. Genet Mol Res 6:691–706

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

Hong MG, Kim KH, Ku JH, Jeong JK, Seo MJ, Park CH et al (2015) Inheritance and quantitative trait loci analysis of resistance genes to bruchid and bean bug in mungbean (*Vigna radiata* L. Wilczek). Plant Breed Biotechnol 3:39–46

Isemura T, Kaga A, Tabata S, Somta P, Srinives P, Shimizu T et al (2012) Construction of a genetic linkage map and genetic analysis of domestication related traits in mungbean (*Vigna radiata*). PLoS ONE 7:e41304

Ishimoto M, Kitamura K (1989) Growth inhibitory effects of an α-amylase inhibitor from kidney bean, *Phaseolus vulgaris* (L.) on three species of bruchids (Coleoptera: bruchidae). Appl Ent Zool 24:281–286

Ishimoto M, Sato T, Chrispeels MJ, Kitamura K (2006) Bruchid resistance of transgenic azuki bean expressing seed α-amylase inhibitor of common bean. Entomol Exp Appl 79:309–315

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

Kang YJ, Kim S, Kim MY, Lestari P, Kim KH, Ha BK et al (2014) Genome sequence of mungbean and insights into evolution within *Vigna* species. Nat Commun 5:5443. doi:10.1038/ncomms6443

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

Kongjaimun A, Kaga A, Tomooka N, Somta P, Shimizu T, Shu Y et al (2012) An SSR-based linkage map of yardlong bean (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata Sesquipedalis* group) and QTL analysis of pod length. Genome 55:81–92

Lambrides CJ, Imrie BC (2000) Susceptibility of mungbean varieties to the bruchid species *Callosobruchus maculatus* (F.), *C phaseoli* (Gyll.), *C chinensis* (L.), and *Acanthoscelides obtectus* (Say) (Coleoptera: chrysomelidae). Aust J Agric Res 51:85–89

Lawrence PK, Koundal KR (2002) Plant protease inhibitors in control of phytophagous insects. Electron J Biotechnol 5(1):3. doi:10.2225/vol5-issue1-fulltext-3Electronic

Leckie F, Mattei B, Capodicasa C, Hemmings A, Nuss L, Aracri B et al (1999) The specificity of polygalacturonase-inhibiting protein (PGIP): a single amino acid substitution in the solvent-exposed beta-strand/beta-turn region of the leucine-rich repeats (LRRs) confers a new recognition capability. EMBO J 418:2352–2363

Li H, Ye G, Wang J (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361–374

Liu MS, Kuo TCY, Ko CY, Wu DC, Li KY, Lin WJ et al (2016) Genomic and transcriptomic comparison of nucleotide variations for insights into bruchid resistance of mungbean (*Vigna radiata* [L.] R. Wilczek). BMC Plant Biol 16:46

Lodhi MA, Ye GN, Weeden NF, Reisch BI (1994) A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep 12:6–13

Maréchal R, Mascherpa JM, Stainier F (1978) Etude taxonomiqe d'un group d'espécies des geners *Phaseolus* et *Vigna* (Papilionaceae) sur la basedenees morphologiques, traitees, pour 1' analyse informatique. Boisseira 28:1–278

Mei L, Cheng XZ, Wang SH, Wang LX, Liu CY, Sun L et al (2009) Relationship between bruchid resistance and seed mass in mungbean based on QTL analysis. Genome 52:589–596

Menancio-Hautea D, Kumar L, Danesh D, Young ND (1993) A genome map for mungbean (*Vigna radiata* L. Wilczek) based on DNA genetic markers ($2N = 2X = 22$). In: O'Brien SJ (ed) Genetic maps: locus maps of complex genomes, Cold Spring Harbor Laboratory Press, New York, pp 6.259–6.261

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J 3:269–283

Miyagi M, Humphry M, Ma ZY, Lambrides CJ, Bateson M, Liu CJ (2004) Construction of bacterial artificial chromosome libraries and their application in developing PCR-based markers closely linked to a major locus conditioning bruchid resistance in mungbean (*Vigna radiata* L. Wilczek). Theor Appl Genet 110:151–156

Nair RM, Schafleiner R, Kenyon L, Srinivasan R, Easdown W, Ebert AW, Hanson P (2012) Genetic improvement of mungbean. SABRAO J Breed Genet 45:177–190

Nogueira FCS, Silva CP, Alexandre D, Samuels RI, Soares EL, Aragão FJL et al (2012) Global proteome changes in larvae of *Callosobruchus maculatu*s (Coleoptera: chrysomelidae: bruchinae) following ingestion of a cysteine proteinase inhibitor. Proteomics 12:2704–2715

Pauchet Y, Wilkinson P, Chauhan R, Ffrench-Constant RH (2010) Diversity of beetle genes encoding novel plant cell wall degrading enzymes. PLoS One 5:e15635

Pedra JHF, Brandt A, Westerman R, Lobo N, Li HM, Romero-Severson J, Murdock LL, Pittendrigh BR (2003) Transcriptome analysis of the cowpea weevil bruchid: identification of putative proteinases and alpha-amylases associated with food breakdown. Insect Mol Biol 12:405–412

Santana MC (2013) Identificação de poligalacturonases expressas no sistema digestório de *Callosobruchus maculatus* (Coleoptera: chrysomelidae: bruchinae). Ph.D. Thesis, Universidade Federal de Santa Catarina, Brazil

Shade RE, Schroeder HE, Pueyo JJ, Tabe LL, Murdock TJV, Higgins MJ, Chrispeels MJ (1994) Transgenic pea seeds expressing the α-amylase inhibitor of the common bean are resistant to bruchid beetles. Bio/Technol 12:793–796

Shen Z, Reese JC, Reeck GR (1996) Purification and characterization of polygalacturonase from the rice weevil, *Sitophilus oryzae* (Colecoptera: curculionidae). Insect Biochem Mol Biol 26:427–433

Somta P, Srinives P (2007) Genome research in mungbean [*Vigna radiata* (L.) Wilczek] and blackgram [*V. mungo* (L.) Hepper]. Sci Asia 33(S1):69–74

Somta P, Ammaranan C, Ooi PAC, Srinives P (2007) Inheritance of seed resistance to bruchids in cultivated mungbean (*Vigna radiata* (L) Wilzcek). Euphytica 155:49–55

Somta C, Somta P, Tomooka N, Ooi PAC, Vaughan DA, Srinives P (2008) Characterization of new sources of mungbean (*Vigna radiata* (L) Wilczek) resistance to bruchids, *Callosobruchus* spp (Coleoptera: bruchidae). J Stored Prod Res 44:316–321

Somta P, Seehalak W, Srinives P (2009) Development, characterization and cross-species amplifcation of mungbean (*Vigna radiata*) genic microsatellite markers. Conserv Genet 10:1939–1943

Srinives P, Somta P, Somta C (2007) Genetics and breeding of resistance to bruchids (*Callosobruchus* spp) in *Vigna* crops: a review. NU Int J Sci 4:1–17

Sun L, Cheng XZ, Wang SH, Wang LX, Liu CY, Mei L, Xu N (2008) Heredity analysis and gene mapping of bruchid resistance of a mungbean cultivar V2709. Agric Sci China 7:672–677

Talekar NS, Lin CL (1992) Characterization of *Callosobruchus chinensis* (Coleoptera: bruchidae) resistance in mungbean. J Econ Entomol 85:1150–1153

Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Res 11:1441–1452

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3—new capabilities and interfaces. Nucleic Acids Res 40(15):e115

Young N, Kumar L, Menancio-Hautea D, Danesh D, Talekar NS, Shanmugasundaram S, Kim DH (1992) RFLP mapping of a major bruchid resistance gene in mungbean (*Vigna radiata*, L Wilczek). Theor Appl Genet 84:839–844

Yu K, Park SJ, Poysa V, Gepts P (2000) Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). J Hered 91:429–434

Zhao D, Cheng XZ, Wang LX, Wang SH, Ma YL (2010) Integration of mungbean (*Vigna radiata*) genetic linkage map. Acta Agron Sin 36:932–939