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Construction of an integrated map and location of a bruchid resistance gene in mung bean

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ABSTRACT

Bruchid beetle (*Callosobruchus chinensis*) poses a serious threat to the production and storage of mung bean (*Vigna radiata*). Mapping bruchid resistance (*Br*) will provide an important basis for cloning the responsible gene(s) and elucidating its functional mechanism, and will also facilitate marker-assisted selection in mung bean breeding. Here, we report the construction of the genetic linkage groups of mung bean and mapping of the *Br1* locus using an RIL population derived from a cross between Berken, a bruchid-susceptible line, and ACC41, a bruchid-resistant line. A total of 560 markers were mapped onto 11 linkage groups, with 38.0% of the markers showing distorted segregation. The lengths of the linkage groups ranged from 45.2 to 117.0 cM with a total coverage of 732.9 cM and an average interval of 1.3 cM between loci. *Br1* was located on LG9 between BM202 (0.7 cM) and Vr2-627 (1.7 cM). Based on 270 shared SSR markers, most of the linkage groups were assigned to specific chromosomes. These results should further accelerate the genetic study of this crop.

1. Introduction

Mung bean (*Vigna radiata*) is widely grown throughout Asia, where it is a major source of protein [1]. Mung bean has been a traditional food in China for thousands of years and is the main agricultural crop in dry and semi-dry regions of the northwest of the country. Mung bean is frequently used as an intercrop and in crop rotation, because of its short growth period and nitrogen fixation. One of the most serious pests affecting the production and marketing of mung bean is the bruchid beetle (*Callosobruchus chinensis*). It attacks mung bean both in the field and in storage, resulting in heavy or even complete loss [2,3]. Searching for sources of resistance to bruchid beetles and breeding resistant varieties is the best and most effective way to protect mung bean production [1].

To date, several mung bean genotypes have been identified as resistant to bruchid beetle [4–7]. It has been reported that bruchid resistance (*Br*) is controlled by a major locus in two highly resistant wild genotypes, TC1966 and ACC41 [5]. RFLP markers have been used to map the resistance gene in both of these genotypes [8–11], but this marker system is not practical for marker-assisted selection (MAS) because of its complicated protocol. By contrast, SSR markers can be conveniently used in MAS. However, owing mainly to the limited number available in mung bean, SSR markers have not been widely used in mapping loci conferring bruchid resistance.

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Following the development and validation of a large number of SSR markers in mung bean [12], we constructed linkage maps and located the *Br1* locus using a RIL population derived from Berken and ACC41 [13]. Based on the analysis of sequences flanking mapped SSR markers [12], we also assigned linkage groups to specific chromosomes. We believe that these results will further accelerate genetic research on mung bean and related species.

2. Materials and methods

2.1. Plant materials and DNA preparation

A mapping population consisting of 201 F_{10} recombinant inbred lines (RILs) derived from a cross between Berken (cultivated type, 100% susceptible to bruchid) and ACC41 (wild type, 100% resistant to bruchid) was used in this study [14]. Fresh leaves from 5 to 6 individuals of each line and the parents were used for genomic DNA extraction using the CTAB method [15]. After assessment of quality and quantity, prepared genomic DNA was stored at −20 °C.

2.2. Measurement of bruchid resistance

Bruchid resistance was assayed in each of the RILs in a 2012 experiment, based on a method released by the National Scientific and Technological Project [16]. Owing to limited numbers of seeds, only 190 RILs were assayed again in 2013. Briefly, 30 healthy seeds, replicated three times for each line, together with Zhonglyu No. 5 (a susceptible cultivar) as a control, were placed separately into plastic dishes (diameter, 3.5 cm × 0.5 cm) without lids. All dishes were then placed in a large plastic box (diameter, 66 cm × 44 cm × 18 cm) with a cup of water to maintain humidity. Approximately 400–500 adult bruchid beetles were released into the box to ensure that each line had more than 20 adult insects to lay eggs on the seed surface. The box was covered with two layers of black cloth to maintain darkness, and placed in a room with an ambient temperature of 27 ± 2 °C. The water level was monitored regularly throughout the infestation period. Forty days later, the dishes were taken out and the damaged seeds of each line were examined and recorded. The percentage of seed damage was calculated by the formula: $SDR = \frac{\Sigma NSD}{N} \times 100\%$, where *SDR* denotes the damage percentage, *NSD* the number of damaged seeds, and *N* the total number of inoculated seeds. The percentage of damaged seeds for each RIL was used to classify each line as either resistant or susceptible as previously described [10,17].

2.3. Molecular marker analysis

Molecular markers used in this study were from several sources including SSR, EST-SSR, and STS derived from mung bean [12,18], adzuki bean [19], common bean (http://isa.ciat.cgiar.org/molphas/micros.jsp), and cowpea [20] as well as a set of RFLP markers [9,14]. PCR analysis was performed in 20-μL reactions containing 1× PCR buffer, 100 μmol L^{-1} of each dNTP, 0.4 μmol L^{-1} of each primer, 20 ng genomic DNA, and 1 U of *Taq* DNA polymerase. PCR amplification was performed using an EDC-810 thermal cycler (Dongsheng Co.) with 35 cycles of 94 °C for 30 s, 47 °C for 30 s, and 72 °C for 30 s followed by a final 5-min extension. The product was fractionated by 8% SDS-polyacrylamide gel electrophoresis (PAGE) with 0.5× TBE as buffer at 220 V. The running time was adjusted according to the expected size of products and usually ranged from 1.0 to 1.5 h. To visualize the fragments, gels were stained in 0.2% $AgNO_3$ and then developed in a solution of 1.5% NaOH plus 0.5% formaldehyde.

2.4. Linkage group construction and gene mapping

Based on profiles of all polymorphic markers in each of the RILs assessed, a linkage map was constructed using JoinMap 4.0 [21] with a minimum LOD score of 3 for grouping and a recombination frequency of 0.25 by the Kosambi mapping function [22]. Double crossovers between adjacent loci were confirmed visually. Chi-square tests were used to evaluate the segregation distortion of mapped markers. Based on the locations of SSR markers in the linkage groups and their physical positions in the mung bean genome [12,23], the linkage groups were assigned to specific chromosomes of mung bean.

3. Results

3.1. Bruchid resistance assays

Seeds of the control genotype were completely damaged in both of the two experiments conducted. Damage percentages of individual RILs ranged from 0 to 100%, with an average of 46.5% in one of the experiments and 47.7% in the other. The phenotypic values of bruchid resistance were highly correlated ($r = 0.98$) between the two experiments. Chi-square tests showed that the segregation of resistance and susceptibility in the RIL population in the 2012 and 2013 experiments fitted a 1:1 ratio.

3.2. Map construction and gene location

The 547 markers used in this study produced a total of 560 polymorphic loci. Among the RFLP markers, seven generated more than one polymorphic locus each. Among the markers used, 364 (or 66.9%) were newly developed SSRs from the mung bean genome. The 560 loci were mapped on 11 linkage groups covering a total of 732.9 cM (Fig. 1). The lengths of linkage groups ranged from 45.2 to 117.0 cM with an average of 66.6 cM. The average interval between two loci was 1.3 cM. The number of loci on each linkage group varied from 33 to 87 with an average of 50.9. Based on the resistance assay data, the bruchid resistance gene *Br1* was mapped between BM202 (a SSR marker from common bean) and Vr2-627 (a SSR marker from mung bean), covering a region of 2.4 cM on LG9 (Fig. 1).

3.3. Segregation of markers in the mapping population

There were 70 and 143 loci that showed segregation distortion at levels of $P < 0.05$ and $P < 0.01$, respectively, accounting for 12.5% and 25.5% of all markers. Among these markers showing distorted segregation, 93.9% favored alleles from the female parent Berken. The number of distorted markers