SSR MAP CONSTRUCTION AND QUANTITATIVE TRAIT LOCI (QTL) IDENTIFICATION OF MAJOR AGRONOMIC TRAITS IN MUNGBEAN (Vigna radiata (L.) Wilczek)

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SUMMARY

Mungbean (Vigna radiata) (2n = 2x = 22) is an important annual legume in Asia. It is widely grown in South and Southeast Asia, as well as China. The aim of this research was to use SSR markers to construct a linkage map and identify chromosome regions controlling some agronomic traits in mungbean. The mapping population comprised 186 F₂ plants derived from a cross between an annual cultivated mungbean line ‘KUML29-1-3’ (Vigna radiata var. radiata) and an Australian wild perennial mungbean accession ‘W021’ (Vigna radiata var. sublobata). A total of 150 SSR primers were composed into 11 linkage groups, each containing at least 5 markers. The map spans 1,174.2 cM with the average distance between the adjacent markers of 7.8 cM. Comparing the mungbean map with azuki bean (Vigna angularis) and blackgram (Vigna mungo) linkage maps revealed extensive genome conservation between the three species. Twenty QTLs controlling major agronomic characters including days to first flower (FLD), days to first pod maturity (PDDM), days to harvest (PDDH), 100 seed weight (SD100WT), number of seeds per pod (SDNPPD) and pod length (PDL) were located on the linkage map. Most of the QTLs were located on linkage groups 7 and 5.

Keywords: mungbean, Vigna radiata, comparative genome mapping, agronomic traits, simple sequence repeat, quantitative trait loci

Manuscript received: December 14, 2011; Decision on manuscript: January 14, 2012; Manuscript accepted in revised form: January 28, 2012.

Communicating Editor: Bertrand Collard
INTRODUCTION

Mungbean (Vigna radiata (L.) Wilczek: 2n = 2x = 22) is one of the most important annual legumes. It is native to India and becomes an economic crop in many countries in Asia, Africa and South America. Mungbean seed is consumed as a protein source for human and animals. Mungbean plants can be made into hay and green manure. It is usually cultivated in cropping systems. The production of mungbean grain in the world is around 3.5 to 4 M tons per year (Weinberger, 2003). Products from mungbean seed are rich in vitamins, minerals and easily digested proteins. However, the average yield of mungbean is still low due to susceptibility to pests and diseases, its indeterminate growth habit and photoperiod sensitivity (Fernandez and Shanmugasundaram, 1988).

All mungbean cultivars are annual crop with two broad growth stages, vegetative (V) and reproductive (R). Pookpakdi et al. (1992) proposed a system that further classifies both stages based on soybean growth stages published by Fehr et al. (1971). V stages are determined by counting the number of developed nodes on the main stem, beginning with the unifoliolate nodes as the first nodes (stage \( V_1 \)) and the final node is the node which has fully developed trifoliolate leaf (stage \( V_n \)) when the leaf at the node above is unrolled sufficiently. R stages are determined from \( R_1 \) (beginning bloom), \( R_2 \) (beginning pod), \( R_3 \) (beginning seed), \( R_4 \) (full seed), \( R_5 \) (beginning maturity), \( R_6 \) (first harvest), and \( R_7 \) (second harvest).

Difference in number of dates specifying to each growth stage can affect seed yield. Khattak et al. (1995) found that number of days to flowering is negatively correlated with number of pods per plant and total seed weight with especially strong direct effect on total seed weight.

To perform a breeding process effectively, inheritance of dates specifying different growth stages should be investigated in order to manipulate developmental stages of mungbean through selection. In addition, molecular markers associated with the traits should be determined in order to save time used in selection cycles through marker-assisted selection. Mungbean has a very small genome with the size of 579 Mbp/1C (Arumanganthan and Earle, 1991). Thus one would expect short chromosomes in each linkage group. The first linkage map of mungbean was constructed from RFLP markers by Menancio-Hautea et al. (1992). Then Young et al. (1992 and 1993) located a major bruchid insect resistance and powdery mildew disease resistance genes onto this map. However, the RFLP marker map has not been further used due to limitation that it requires a large amount of good quality DNA for analysis. The technique is time-consuming and expensive, making it less suitable for large-score screening programs in plant breeding. Moreover, the RFLP markers are not distributed throughout the genome. Although, Lambrides et al. (2000) added RAPD markers into the map, there was no report of its further use. The main reason is that the RAPD markers are dominant markers and
thus cannot distinguish between homozygous and heterozygous genotypes. Yet, RAPD technique is not always repeatable. This made a specific PCR-based marker, especially Simple Sequence Repeat or SSR marker the marker of choice.

SSR is the variable in short tandem repeat of DNA bases, giving co-dominant markers which can distinguish between homozygote and heterozygote. Nowadays, there are research reports using SSR makers for mapping the mungbean genome and locating QTLs. Kasetratanan et al. (2010) located QTLs conferring resistance to powdery mildew disease on a SSR partial linkage map of mungbean. Chankaew et al. (2011) reported a QTL mapping for Cercospora leaf spot (CLS) resistance in mungbean. Recently, Zhao et al. (2010) reported construction of a mungbean genetic linkage map by combining 76 RFLP markers from Humphry et al. (2002) and 103 new loci consisting of 97 SSR, 4 RAPD and 2 STS markers. The number of PCR-based markers in their map was too low to make use of the map.

The objectives of this study were: (1) to estimate heritability of yield components and dates specifying growth stages in mungbean; (2) to construct a SSR linkage map of mungbean; and (3) to map QTLs controlling the traits.

**MATERIALS AND METHODS**

**Mapping population and DNA extraction**

The population used in this study was an F2 population of 186 plants developed from an inter-subspecies cross between cultivated mungbean line ‘KUML29-1-3’ (V. radiata var. radiata) (hereafter called KUML) and a wild mungbean accession ‘W021’ (V. radiata var. sublobata). KUML29-1-3 was developed from the Project on Genetics and Breeding of Field Legumes for Thailand, Kasetsart University, Kamphaeng Saen Campus. The line has high and stable seed yield. W021 was obtained from the National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan. It is a small-seeded wild perennial mungbean with long vegetative and reproductive growth stages.

Young leaves of 7 days old from parental lines and F1 plants were extracted for DNA using a CTAB method (Lodhi et al., 1994). The DNA concentration was estimated by comparing with λ DNA standard on agarose gel electrophoresis. The DNA concentration was adjusted to 1ng/μl for PCR amplification.

**Phenotyping and data analysis**

Twenty plants each of KUML, W021, F1 (KUML x W021) and F2 (W021 x KUML), and all 186 F2 plants were individually grown in 12-inch pots each filled with 5 kg of soil. All plants were placed in a net house during February to October 2010 at Kasetsart University, Kamphaeng Saen, Thailand (14° 01’ N, 99° 59’ E, 7.5m ASL). Data were recorded from individual plants on days to first flower (FLD), days to first pod maturity (PDDM), days to harvest (PDDH), pod width (PDW) in mm,
pod length (PDL) in cm, number of seeds per pod (SDNPPD), total number of pods per plant (PDTN), 100 seed weight (SD100WT) in g, and total seed weight (SDTWT) in g. The data were analyzed by an analysis of variance (ANOVA) of a completely randomized statistical design (CRD). Difference between trait means is declared by Duncan’s Multiple Range Test (DMRT) at $P \leq 0.05$. Variance of each trait was calculated in KUML, W021, F1, F1 and F2 populations and used to estimate broad-sense heritability ($h^2$) based on the equation $h^2 = \frac{\sigma^2_g}{\sigma^2_p}$. Where $\sigma^2_g$ is the genotypic variance component and $\sigma^2_p$ is the phenotypic variance component. In this experiment, $\sigma^2_g$ was estimated from $V_{F_2} - (V_{P_1} + V_{P_2} + V_{F_1} + V_{F_1})/4$; where $V_{F_2}$, $V_{P_1}$, $V_{P_2}$, $V_{F_1}$ and $V_{F_1}$ are the variation between plants within the specified genotypes, and $\sigma^2_p$ was estimated from $V_{F_2}$ (Fehr, 1987). Phenotypic correlation coefficients between traits were calculated from 186 F2 plants using software R-program v. 2.8.1 (http://www.r-project.org/).

SSR analysis
Nine hundred and forty-five SSR markers were screened to detect polymorphism between the parents. Of these, 628 markers were developed from mungbean (Kumar et al., 2002a and 2002b; Gwag et al., 2006; Somta et al., 2008 and 2009; Seelahak et al., 2009 and Tangphatsornruang et al., 2009), 191 were from azuki bean (Wang et al., 2004), 119 were from common bean (Blair et al., 2003 and Buso et al., 2006) and 7 were from cowpea (Li et al., 2001). PCR reaction and amplification were the same as described by Somta et al. (2008). The DNA product was electrophoresed on 4.5% polyacrylamine gel with 0.5x TE buffer for 1-2 h. The DNA bands were visualized by silver staining.

Linkage map construction and QTL analysis
A linkage map was developed by JoinMap 3.0 program (van Ooijen, 2009). A minimum LOD score of 3.0 was used as a threshold value for grouping the markers. Genetic distance between markers was calculated using Kosambi map function (Kosambi, 1944). Linkage groups were named after azuki bean linkage groups (Han et al., 2005). QTL analysis for each character was performed using composite interval mapping (CIM) by WinQTL Cartographer 2.5 program (Wang et al., 2007). A permutation test (Churchill and Doerge, 1994) was run for 2,500 times at the significance level of $P = 0.01$ to determine a LOD score threshold for declaring a significant QTL.

RESULTS
Phenotypic data and broad-sense heritability
Mean and standard deviation of the parents and F2 population were presented in Table 1. All traits were different among the parents but not different between F1 and F1, revealing no maternal effect conditioning these traits. KUML showed determination, while W021 showed indetermination in growth habit. Days to first flower (FLD) of KUML was only 31 while that of W021 was 65. The same relationship was also found in days 74.
to first pod maturity (PDDM) (47 vs 82), and days to harvest (PDDH) (76 vs 140). Pod length (PDL) of KUML was longer than W021 (8.2 vs 4 cm), and pod width (PDW) was also wider (4.7 vs 3.1 mm). Total number of pods per plant (PDTN) of KUML was lower than W021 (25 vs 109), while number of seeds per pod were not different. The F2 population can be classified into different classes according to days to first flower, days to first pod maturity, days to harvest, 100 seed weight, number of seeds per pod, total number of pods per plant, pod length, pod width and total seed weight (Figure 1). All traits, except 100 seed weight showed transgressive segregation. Days to first flower, pod maturity and harvest of the F2 population ranged respectively from 29-76, 44-96 and 79-178 days, demonstrated skewing toward KUML. The progenies showed positive segregation when compared with W021 (Figure 1a, 1b and 1c). Yield components such as number of seeds per pod, total seed weight and pod length showed transgressive segregation, while 100 seed weight fell between the parents. The F2 plants had 100 seed weight of 1.0 g to 3.0 g whereas W021 and KUML had 0.6 and 4.2 g per 100 seeds, respectively (Fig. 1d). When compared with KUML, total seed weight and pod width showed positive transgressive segregation but number of seeds per pod showed negative segregation. The progenies also showed positive transgressive segregation in total number of pods per plant when compared with W021.

Broad-sense heritabilities (h²) as calculated from the F2 data of each trait were presented in Table 1. The broad-sense heritability of flowering dates, viz. FLD, PDDM and PDDH were 88.6%, 91.2% and 86.8%, respectively, which were considered highly heritable. The heritabilities of yield components were high in PDL (92.4%), PDW (97.5%), SDNPPD (91.2%) and SD100WT (90%), and medium-high in PDTN (77.0%), SDTWT (65.1%).

Correlation between agronomic characters

The phenotypic correlation coefficients among 9 quantitative traits are given in Table 2. FLD showed positive correlation with PDDM (r = 0.966**) and PDDH (r = 0.693**). These traits tended to correlate negatively with yield components and total seed weight. 100 seed weight had positive correlation with SDTWT (0.535**), PDL (0.574**) and PDW (0.376**). Correlation between yield components were high in number of seeds per pod with pod length (0.781**), and 100 seed weight with pod length (0.574**). This result indicated that yield depended on seed size and pod size (pod length and pod width), number of seeds per pod, and 100 seed weight. In this experiment, pod length, pod width and 100 seed weight were positively correlated to total seed weight, while days to flower was negatively correlated with seed weight. Thus an optimum number of days to flower should be considered as a selection criterion together with yield components.
Kajonphol et al. (2012)

(a) Days to first flower

(b) Days to first pod maturity

(c) Days to harvest

(d) 100 seed weight

(e) Number of seeds per pod

(f) Total seed weight

(g) Pod length

(h) Pod width
Linkage map construction
Nine hundred and forty-five SSR markers were screened between the two parents and 152 markers (16.08%) were found polymorphic. One hundred and fifty markers could be assigned into 11 linkage groups of mungbean chromosomes plus a small linkage group (CEDG144 and CEDG149) with the total coverage of 1,174.2 cM, giving the average chromosome length of 97.9 cM. The average distance between SSR loci on the map is 7.8 cM (Figure 2). Each chromosome was tagged with five or more markers. Of these 150 markers, 75 are mungbean loci, 61 are azuki bean loci, 13 are common bean loci and one is cowpea locus.

QTL analysis
Twenty putative QTLs of agronomic traits were detected by CIM with WinQTL Cartographer 2.5 (Figure 2; Table 3). Four, 3, 3, 6, 2 and 2 QTLs were detected for FLD, PDDM, PDDH, SD100WT, SDNPBD and PDL, respectively (Table 3). The amount of phenotypic variation in each trait explained by its respective QTLs ranges from 6.3 to 28.6%. The number of QTLs per trait range between 2 and 6 loci. QTLs for different traits were co-located on the map (Figure 2). These include Fld2, Pddm2 and Pddh2 on LG2 and Fld4.1, Pddm4.1 and Pddh4.1 on LG4, to name a few.

Comparative linkage map between mungbean, azuki bean and black gram
The mungbean linkage map was compared with azuki bean linkage map (Han et al., 2005), and black gram linkage map (Chaitieng et al., 2006). Sixty-two and 21 SSR markers were common between azuki bean and mungbean, and between black gram and mungbean, respectively. Most of the common markers were mapped on the same linkage groups and orders, with a few exceptions (Fig. 3). Marker order in our study was 42 out of 62 loci (68%) colinear with azuki bean, and 19 out of 21 loci (90%) colinear with black gram. Reverse regions were identified between mungbean map and azuki bean map on LG1, 2 and 5. One inversion region between mungbean map and black gram map was tagged by BW212 and CEDG056 on LG9.
Kajonphol et al. (2012)

Table 1. Mean and standard deviation of major agronomic traits observed from parents and progenies from the cross between an annual cultivated mungbean line ‘KUML29-1-3’ and a wild perennial mungbean accession ‘W021’, with their variances and corresponding heritabilities.

<table>
<thead>
<tr>
<th>Generations/parameters</th>
<th>FLD</th>
<th>PDDM</th>
<th>PDDH</th>
<th>PDL (cm)</th>
<th>PDW (mm)</th>
<th>SDNPPD</th>
<th>PDTN</th>
<th>SD100 WT (g)</th>
<th>SD- TWT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁ (29-1-3)</td>
<td>31.2c</td>
<td>47.4e</td>
<td>76.4b</td>
<td>8.2a</td>
<td>4.7a</td>
<td>12.0a</td>
<td>25.0c</td>
<td>4.2a</td>
<td>9.6a</td>
</tr>
<tr>
<td>± 1.6</td>
<td>± 1.1</td>
<td>± 0.5</td>
<td>± 0.1</td>
<td>± 0.2</td>
<td>± 0.1</td>
<td>± 0.3</td>
<td>± 0.4</td>
<td>± 0.1</td>
<td>± 0.1</td>
</tr>
<tr>
<td>P₂ (W021)</td>
<td>65.5a</td>
<td>81.8a</td>
<td>139.6a</td>
<td>4.0b</td>
<td>3.1c</td>
<td>9.7ab</td>
<td>109.5ab</td>
<td>0.6c</td>
<td>1.4b</td>
</tr>
<tr>
<td>±6.5</td>
<td>±6.5</td>
<td>±19.2</td>
<td>±0.2</td>
<td>±0.1</td>
<td>±0.9</td>
<td>14.8</td>
<td>±0.04</td>
<td>±0.2</td>
<td></td>
</tr>
<tr>
<td>F₁</td>
<td>42.5b</td>
<td>57.0bc</td>
<td>124.0a</td>
<td>5.3ab</td>
<td>4.0ab</td>
<td>8.8b</td>
<td>149.6a</td>
<td>1.9b</td>
<td>12.0a</td>
</tr>
<tr>
<td>±2.2</td>
<td>±1.5</td>
<td>±6.0</td>
<td>±0.3</td>
<td>±0.1</td>
<td>±3.0</td>
<td>±21.2</td>
<td>±0.1</td>
<td>±4.1</td>
<td></td>
</tr>
<tr>
<td>F₂</td>
<td>47.8b</td>
<td>62.7b</td>
<td>123.5a</td>
<td>5.3ab</td>
<td>4.0b</td>
<td>8.4b</td>
<td>151.4a</td>
<td>2.0b</td>
<td>12.1a</td>
</tr>
<tr>
<td>±11.0</td>
<td>±12.0</td>
<td>±28.6</td>
<td>±2.7</td>
<td>±0.7</td>
<td>±2.6</td>
<td>±41.2</td>
<td>±0.4</td>
<td>±6.6</td>
<td></td>
</tr>
<tr>
<td>LSD₀5</td>
<td>11.1</td>
<td>12.1</td>
<td>28.1</td>
<td>3.0</td>
<td>0.7</td>
<td>2.9</td>
<td>52.9</td>
<td>0.4</td>
<td>8.1</td>
</tr>
<tr>
<td>V₁</td>
<td>120.3</td>
<td>143.6</td>
<td>818.2</td>
<td>1.1</td>
<td>0.4</td>
<td>6.8</td>
<td>1695.9</td>
<td>0.2</td>
<td>43.8</td>
</tr>
<tr>
<td>V₁²</td>
<td>13.7</td>
<td>12.6</td>
<td>108.3</td>
<td>0.1</td>
<td>0.01</td>
<td>0.6</td>
<td>390.1</td>
<td>0.02</td>
<td>15.3</td>
</tr>
<tr>
<td>h²(%)</td>
<td>88.6</td>
<td>91.2</td>
<td>86.8</td>
<td>92.4</td>
<td>97.5</td>
<td>91.2</td>
<td>77.0</td>
<td>90.0</td>
<td>65.1</td>
</tr>
</tbody>
</table>

Means of each trait followed by the same letter are not different as compared by DMRT at P ≤ 0.05.

FLD = days to first flower, PDDM = days to first pod maturity, PDDH = days to harvest, PDL = pod length (cm), PDW = pod width (mm), SDNPPD = number of seeds per pod, PDTN = total number of pods per plant, SD100 WT = 100 seed weight (g) and SD TWT = total seed weight (g).

Table 2. Correlation between number of days in each growth stage and yield components of the F₂ plants.

<table>
<thead>
<tr>
<th></th>
<th>PDDM</th>
<th>PDDH</th>
<th>PDW</th>
<th>PDL</th>
<th>SDNPPD</th>
<th>PDTN</th>
<th>SD100 WT</th>
<th>SD TWT</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLD</td>
<td>0.966**</td>
<td>0.693**</td>
<td>-0.152*</td>
<td>-0.286**</td>
<td>-0.244**</td>
<td>-0.278**</td>
<td>-0.373**</td>
<td>-0.293**</td>
</tr>
<tr>
<td>PDDM</td>
<td>0.700**</td>
<td>-0.162*</td>
<td>-0.349**</td>
<td>-0.314**</td>
<td>-0.319**</td>
<td>-0.395**</td>
<td>-0.347**</td>
<td></td>
</tr>
<tr>
<td>PDDH</td>
<td>-0.146*</td>
<td>-0.303**</td>
<td>-0.272**</td>
<td>-0.021**</td>
<td>-0.312**</td>
<td>-0.118**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDW</td>
<td>0.328**</td>
<td>0.190**</td>
<td>0.134*</td>
<td>0.376**</td>
<td>0.212**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDL</td>
<td>0.781**</td>
<td>0.232**</td>
<td>0.574**</td>
<td>0.522**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDNPPD</td>
<td>0.235**</td>
<td>0.226**</td>
<td>0.472**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDTN</td>
<td>0.295**</td>
<td>0.822**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD100W</td>
<td>0.535**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0.535**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns.* ** non significant and significant at 0.01 level of probability (df=184), respectively.

FLD = days to first flower, PDDM = days to first pod maturity, PDDH = days to harvest, PDL = pod length (cm), PDW = pod width (mm), SDNPPD = number of seeds per pod, PDTN = total number of pods per plant, SD100 WT = 100 seed weight (g), SD TWT = total seed weight (g).
Figure 2. SSR linkage map of mungbean constructed from the F2 population. Cumulative distances in centiMorgans (Kosambi's) and marker names are shown on the left and right sides of the linkage group, respectively. QTL intervals detected at LOD ≥ 2.0 are presented as boxes on the left of the linkage groups.
Figure 3. A comparative linkage map between mungbean from this study Vs azuki bean (left) (Han et al. 2005) and black gram (right) (Chaitieng et al. 2006), based on azuki common markers.
## Table 3. QTLs conditioning six traits detected in the F2 population of a cross between cultivated mungbean line ‘KUML29-1-3’ and accession ‘W021’.

<table>
<thead>
<tr>
<th>Traits</th>
<th>QTL names</th>
<th>Linkage groups</th>
<th>Markers in F2 population</th>
<th>Position (cM)</th>
<th>LOD score</th>
<th>QTL effect</th>
<th>R² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to first flower (FLD)</td>
<td>Fld2</td>
<td>2</td>
<td>VR0364</td>
<td>72.70</td>
<td>13.3</td>
<td>-6.2</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>Fld4.1</td>
<td>4</td>
<td>CEDG241-VR-SSR019</td>
<td>9.85</td>
<td>6.5</td>
<td>-3.98</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>Fld4.2</td>
<td>4</td>
<td>DMB-SSR199-CEDG107</td>
<td>69.27</td>
<td>20.1</td>
<td>-7.91</td>
<td>4.37</td>
</tr>
<tr>
<td></td>
<td>Fld11</td>
<td>11</td>
<td>VR0216-CEDG168</td>
<td>14.01</td>
<td>5.3</td>
<td>-4.73</td>
<td>0.59</td>
</tr>
<tr>
<td>Days to first pod maturity (PDDM)</td>
<td>Pddm2</td>
<td>2</td>
<td>VR0364</td>
<td>72.70</td>
<td>10.2</td>
<td>-6.04</td>
<td>2.76</td>
</tr>
<tr>
<td></td>
<td>Pddm4.1</td>
<td>4</td>
<td>CEDG241-VR-SSR019</td>
<td>9.85</td>
<td>7.1</td>
<td>-4.8</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Pddm4.2</td>
<td>4</td>
<td>DMB-SSR199-CEDG107</td>
<td>69.27</td>
<td>18.5</td>
<td>-8.46</td>
<td>4.67</td>
</tr>
<tr>
<td>Days to harvest (PDDH)</td>
<td>Pdhd2</td>
<td>2</td>
<td>VR0364</td>
<td>72.70</td>
<td>8.4</td>
<td>-12.95</td>
<td>8.93</td>
</tr>
<tr>
<td></td>
<td>Pdhd4.1</td>
<td>4</td>
<td>CEDG241-VR-SSR019</td>
<td>9.85</td>
<td>6.5</td>
<td>-10.19</td>
<td>10.58</td>
</tr>
<tr>
<td></td>
<td>Pdhd4.2</td>
<td>4</td>
<td>VR0313</td>
<td>17.3</td>
<td>5.9</td>
<td>-9.61</td>
<td>11.60</td>
</tr>
<tr>
<td>100 seed weight (g) (SD100WT)</td>
<td>Sd100wt2.1</td>
<td>2</td>
<td>VR078-CEDG065</td>
<td>4.01</td>
<td>6.6</td>
<td>0.19</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>Sd100wt2.2</td>
<td>2</td>
<td>VR17-VR0200</td>
<td>19.13</td>
<td>7.7</td>
<td>0.19</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>Sd100wt4</td>
<td>4</td>
<td>VR0366-VR035</td>
<td>35.08</td>
<td>4.9</td>
<td>0.18</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Sd100wt8</td>
<td>8</td>
<td>VR-SSR031-VR0225</td>
<td>52.22</td>
<td>5.0</td>
<td>0.15</td>
<td>0.05</td>
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<tr>
<td></td>
<td>Sd100wt9</td>
<td>9</td>
<td>CEDG259-CEDG166</td>
<td>19.21</td>
<td>4.5</td>
<td>0.15</td>
<td>-0.03</td>
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<tr>
<td></td>
<td>Sd100wt11</td>
<td>11</td>
<td>MB-SSR104-VR-SSR011</td>
<td>53.08</td>
<td>5.2</td>
<td>0.16</td>
<td>-0.03</td>
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<td>Number of seeds per pod (SDNPPD)</td>
<td>Sdnppd1.1</td>
<td>1</td>
<td>VR-SSR015-VR-SSR018</td>
<td>34.69</td>
<td>5.7</td>
<td>1.69</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>Sdnppd1.2</td>
<td>1</td>
<td>VR0194-VR0198</td>
<td>46.18</td>
<td>5.6</td>
<td>1.7</td>
<td>0.80</td>
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<tr>
<td>Pod length (cm) (PDL)</td>
<td>Pdl7</td>
<td>7</td>
<td>CEDG111-VR0126</td>
<td>66.86</td>
<td>5.2</td>
<td>0.41</td>
<td>-0.12</td>
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<tr>
<td></td>
<td>Pdl8</td>
<td>8</td>
<td>VR-SSR005-VR-SSR031</td>
<td>50.43</td>
<td>4.9</td>
<td>0.36</td>
<td>0.23</td>
</tr>
</tbody>
</table>


Kajonphol et al. (2012)

**DISCUSSION**

In this study, broad-sense heritabilities of days to first flower (FLD), days to first pod maturity (PDDM) and days to harvest (PDDH) were high (88.6, 91.2 and 86.8%, respectively). Sripadhet et al. (2007) studied inheritance of agronomic traits and their interrelationship in RIL mungbean lines obtained from the cross between wild mungbean 'ACC 41' and the cultivated 'Berken'. They found that flowering date skewed towards ACC 41, but the narrow-sense heritability was high at 88.0%. They also reported an abnormal distribution in FLD, PDDM and PDDH data. Similar results were also reported by Siddique et al. (2006) that there were high heritabilities in days to first flower and days to harvest. Rohman et al. (2003) reported that days to first flower, days to harvest, 100 seed weight and plant height had high heritability, while total number of pods per plant and number of seeds per pod were low in heritability.

Correlation analysis in this study revealed that days to flower showed positive correlation with days to first pod maturity and days to harvest. Days to flowering and days to maturity showed negative correlation with yield components such as 100 seed weight. According to Khattak et al. (1995), days to flowering was positively correlated with days to maturity, but negatively correlated with total number of pods per plant and total seed weight, while days to maturity was negatively correlated with total seed weight. In contrast, Rohman et al. (2003) found that days to flowering showed negative correlation with days to maturity but showed positive correlation with 100 seed weight and total seed weight. Rajan et al. (2000) worked in mungbean and found similar results to ours that total seed weight had positive genotypic correlation with pods per plant, seeds per pod and one hundred seed weight. Thus the genetic of total seed weight can be improved by indirectly selecting characters showing positive correlation (PDTN, SD100WT, PDL and SDNPPD), as well as negative correlation (FLD and PDDM).

Our research can assign 150 SSR markers into 11 linkage groups, corresponding to the haploid number of mungbean chromosomes. In the previous research, Menancio-Hauetea et al. (1992) used 171 RFLP markers to construct a map grouping into 14 linkage groups that span a total of 1,570 cM with an average distance of 9 cM. Humphry et al. (2002), clustered 255 RFLP probes into 13 linkage groups, with a total length of the map spanned 737.9 cM at an average distance between markers of 3.0 cM and a maximum distance between linked markers of 15.4 cM. While our SSR map has covered 1,174.2 cM, with the average distance between adjacent loci of 7.8 cM. Han et al. (2005) analyzed azuki bean genetic linkage map from a backcross population of (V. nepalensis × V. angularis) × V. angularis. They used 486 markers comprising 205 SSR, 187 AFLP and 94 RFLP to saturate the map. Their map covers altogether 11 linkage groups as our results, but spanned 832.1 cM with an average marker distance of 1.85 cM. Our result showed longer
genome coverage than both maps, with the longer average marker distance. Recently, Zhao et al. (2010) constructed a mungbean integrated map including 97 SSRs, 76 RFLPs, 4 RAPDs and 2 STSs. Their prime objective was to locate the bruchid-resistance Br1 gene. Among the SSR markers located on the map, 91 were from azuki bean, blackgram, common bean and cowpea. The linkage map spans 1,831.8 cM with the average marker distance of 10.2 cM. However, their marker names were presented as codes and thus were not available to the public. Considering the low polymorphism found in mungbean germplasm, our work is the most successful in developing an SSR linkage map resolving 11 mungbean linkage groups.

Azuki bean SSR markers were used for constructing a black gram linkage map (Chaitieng et al. 2006), and mungbean linkage map in this study. Our map construction capitalized the co-linearity between genomes of the Asian Vigna, viz. mungbean, black gram and azuki bean. Although many azuki bean markers can be assigned into mungbean genome, the number of common markers were more conserved between mungbean and black gram than that between mungbean and azuki bean. This result supported the propose of Tomooka et al. (2002) that mungbean and black gram are in the same section Ceratotropis, while azuki bean is in section Angulares. Chaitieng et al. (2006) later reported highly co-linearity (88%) between black gram and azuki bean, revealing the use of cross-species genetic markers among Vigna species.

Our results showed internal insertion/deletion on LG1, 2 and 5 between mungbean and azuki bean maps, and on LG9 between mungbean and black gram maps. These results indicated that genomes in the subgenus Ceratotropis have accumulated a small number of insertions/deletions. The chromosome aberrations detected between mungbean, black gram and azuki bean linkage maps may play important roles in evolution among these species.

ACKNOWLEDGEMENTS

This research was supported by the Commission on Higher Education under the Strategic Scholarships for Frontier Research Networks for Thai Doctoral Degree Program, Ministry of Education, Thailand and the National Science and Technology Development Agency, Thailand. We also thank the Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Thailand for lab facilities.

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