Kasetsart J. (Nat. Sci.) 48 : 197 - 204 (2014)

Quantitative Trait Loci Associated with Seed Weight in Mungbean (*Vigna radiata* (L.) Wilczek)

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ABSTRACT

Seed weight is an important yield component of pulse crops. In mungbean (*Vigna radiata* (L.) Wilczek), varieties with higher seed weight (larger seed size) are generally preferred for cultivation and consumption. Large seed size is thus a major target trait in mungbean breeding. This study considered quantitative trait loci (QTL) mapping in mungbean using a population of 142 $F_{2:3}$ lines derived from a cross between BARImung 1 (low seed weight) and BARImung 6 (medium seed weight). The population was grown under field conditions in two locations in Bangladesh in 2012 and analyzed with 72 polymorphic simple sequence repeat markers. A single marker analysis suggested at least four loci controlling seed weight. Composite interval mapping consistently identified four QTL— *qSWT1, qSWT6, qSWT8* and *qSWT9*— on linkage groups 1, 6, 8 and 9 in both locations. These QTL accounted for 5.80 to 19.96% and 8.31 to 33.72% of the seed weight variation depending on the location. *qSWT1, qSWT8* and *qSWT9* were common to QTL for seed weight detected previously in mungbean, while *qSWT6* appears to be a new locus. *qSWT1, qSWT6, qSWT8* and *qSWT9* are conserved in other *Vigna* crops.

Keywords: microsatellite markers, seed size, genetic mapping

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is an important food legume crop widely grown in Southern and Southeast Asia because it is relatively drought tolerant and has a short life cycle of 60–75 d (Alam *et al*., 2010). Major mungbean growing countries include Australia, Bangladesh, China, India, Myanmar, Pakistan, Thailand and Vietnam. India is the largest producer with about 3 million ha.yr^{-1} (Nair *et al*., 2012). In Southern Asian countries, mungbean is principally grown after rice and wheat, or intercropped with other crops such as sugar cane (Nair *et al*., 2012). Seeds of mungbean contain a high amount of protein (25%) with high quality amino acids and are an inexpensive source of dietary proteins for people in this region.

Seed weight is an important yield component and positively correlated with yield. However, most cultivars released in South Asian countries have small seeds and low yields. The average productivity of the crop is less than 400 kg.ha^{-1} which is lower than that of most other pulse crops (Nair *et al.*, 2012). Hence, breeders wish to develop varieties carrying larger seeds

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Received date : 24/12/13 Accepted date : 24/02/14

together with other desirable traits. Seed weight is a quantitatively inherited polygenic trait with average heritability values of 86.3% in mungbean (Isemura *et al*. 2012). The transfer of quantitative traits such as seed weight can be achieved with high confidence using molecular markers. The availability of molecular maps facilitates marker-assisted selection, map-based cloning and mapping of the quantitative trait loci (QTL) of agronomically important traits in crop plants (Paterson *et al.*, 1995).

QTL that control the seed weight of mungbean have been identified by Fatokun *et al*. (1992); Humphrey *et al*. (2005); Mei *et al*. (2009); Isemura *et al*. (2012); Kajonphol *et al*. (2012); Sompong *et al*. (2012); Chen *et al*. (2013). For QTL robustness, it is important to evaluate the phenotypic data from multiple environments. In addition, it is also important that different DNA markers linked to the trait identified in one population may not associate with the same trait in the other populations. Although there are up to six reports on QTL mapping for seed weight in mungbean, all except one of them identified QTL in more than one environment. The present study considered the identification of QTL for seed weight in mungbean using an $F_{2:3}$ population grown in two environments in Bangladesh.

MATERIALS AND METHODS

Mapping population and DNA extraction

An F_2 population comprising 142 F_2 individuals was used for locating the QTL that control the seed weight in mungbean. This population was the same population used previously for QTL mapping for yellow mosaic disease resistance reported by Alam (2014). Briefly, an F_1 plant was developed from a cross between the mungbean cultivars 'BARImung 6' (BM6) as the male and 'BARImung 1' (BM1) as the female. Both these cultivars were released by the Bangladesh Agricultural Research Institute (BARI), Bangladesh (Alam *et al*., 2010). BM6 is an improved cultivar having medium seed size (approximately 5.5 g per 100 seeds), whereas BM1 is a landrace cultivar having small seed size (approximately 3 g per 100 seeds). The parents and the F_2 plants were grown at the field laboratory of the Agronomy Department, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand from February to April in 2012. The total genomic DNA of the parents and individual F_2 plants were extracted from fresh young leaf tissue using the CTAB method (Lodhi *et al.*, 1994).

Seed weight evaluation

The $F_{2:3}$ progenies and their parents were grown for seed weight evaluation at BARI during May to July 2012 in Gazipur and Madaripur, Bangladesh. In each location, the experiment was conducted using a randomized complete block design with two replications. Each entry was sown in a two-row plot 2 m long with a between row distance of 40 cm and spacing between adjacent plants of 10 cm. Fertilizers were applied at 40 $kg.ha^{-1}$ of urea, 90 $kg.ha^{-1}$ triple super phosphate, 60 $kg.ha^{-1}$ muriate of potash and 40 $kg.ha^{-1}$ gypsum according to a Bangladeshi guide on fertilizer application prior to soil preparation (BARC, 2005). Weeding and harvesting were done manually. A sample of 100 healthy seeds of each entry in each replicate were weighed using a digital balance. The heritability of the seed weight was estimated by using variance components from the analysis of variance as suggested by Fehr (1987).

Linkage and quantitative trait loci analyses

A simple sequence repeat (SSR)-based linkage map developed from the F_2 population as reported by Alam (2014) was used to locate QTL for seed weight. In brief, the map was constructed using 61 polymorphic SSR markers screened from 1,165 mungbean, azuki bean (*V. angularis* (Ohwi) Ohwi & Ohashi), cowpea (*V. unguiculata* (L.) Walp.) and common bean (*Phaseolus vulgaris* L.). The map was composed of 11 linkage groups

(LGs) with a total genetic distance of 393 cM and an average density of 6.42 cM per marker.

Line mean data from individual experiments and average data from the two experiments were subjected to QTL analysis. A preliminary analysis was done by single regression analysis using the *R* program version 2.10.0 (R Development Core Team, 2013). Only markers showing a significant level at $P \leq 0.001$ were considered as linked markers. Then, composite interval mapping (CIM) according to Zeng (1994) was performed to locate the positions of the QTL controlling seed weight using WinQTL Cartographer 2.5 (Wang *et al*., 2012). A permutation test (Churchill and Doerge, 1994) was run 3,000 times to determine a log of odds (LOD) score threshold at the significance level of $P = 0.05$ for declaring a significant QTL. One-LOD support was used to indicate the position of the QTL. The QTL identified in different locations with overlapping 1-LOD supports were considered as the same QTL.

RESULTS

The frequency distribution of seed weight showed a continuous and normal distribution for both Gazipur and Madaripur (Figure 1), suggesting polygenic inheritance of the trait. At Gazipur, the 100-seed weight of $F_{2:3}$ plants ranged from 2.8 to 5.5 g with a mean of 4.05 g, whereas at Madripur, the 100-seed weight varied between 2.9 and 5.8 g with a mean of 4.16 g. None of the $F_{2:3}$ progenies set seeds as large as BM6 at Gazipur.

There was a significant correlation for seed weight between Gazipur and Madaripur ($r = 0.71$; $P = 0.0001$). Heritability of seed weight calculated for Gazipur, Madaripur and combined data was high and comparable being 80.17, 83.64 and 85.36%, respectively. This indicated that seed weight in mungbean is largely controlled by genetic factors.

Markers associated with seed weight were preliminarily determined by single marker analysis at $P = 0.001$ (Table 1). At Gazipur, five markers from LGs 1, 6 and 9 were associated with seed

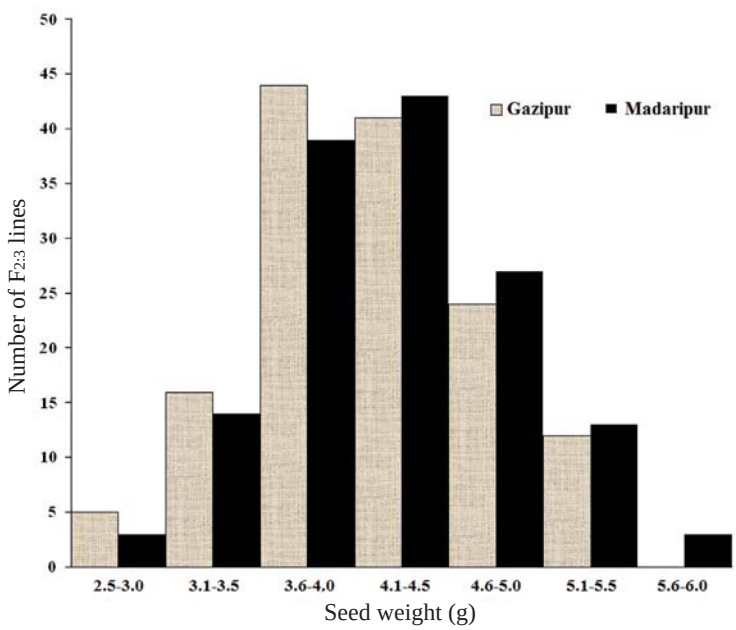

Figure 1 Frequency distribution of 100-seed weight in an F_2 mungbean population from BM1 × BM6.

weight, while at Madaripur, eight markers from LGs 1, 6, 8 and 9 were associated with the trait (Table 1). All the markers that were significant at Gazipur were also significant at Madaripur except for the marker on LG 8. When the combined data from both locations were used, six markers from LGs 1, 6 and 9 showed an association with seed weight. These results suggested that at least four QTL control seed weight in the F_2 population.

Composite interval mapping (CIM) was employed to locate the QTL on a linkage map. Significant thresholds for the QTL were determined by a permutation test. CIM identified four QTL for seed weight on different linkage groups (Table 2 and Figure 2). The QTL were each on LG1, LG6, LG8 and LG9 and were designated as *qSWT1.1*, *qSWT6.1*, *qSWT8.1* and *qSWT9.1*, respectively. All of these QTL except *qSWT8.1* were detected using Gazipur, Madaripur and combined data. *qSWT8.1* was identified only in the Madaripur and combined data. From the combined data of the two locations, *qSWT9.1* had the largest effect on seed weight and explained as much as 39.4% of the variation. Of all QTL detected, alleles from BM6 increased seed weight.

DISCUSSION

Seed size is one of the major target traits in breeding programs of pulse crops. Seed weight in pulse crops is a quantitative trait with high heritability (Fery, 1980). Heritability for seed weight in mungbean is reported to be greater than 80% (Imrie *et al*., 1985; Fatokun *et al*., 1992; James *et al*., 1999; Humphrey *et al*., 2005; Sriphadet *et al*., 2007; Isemura *et al*., 2012). The heritabilities estimated for mungbean seed weight in the current study fell between 80 and 84%, which were comparable to those in the previous reports and indicated that breeding for large mungbean seed size is possible. Seed weight is a quantitative trait and heritability estimates for this trait are important in selecting suitable genotypes for efficient yield improvement. High heritability implies that there are a few major genes together with a number of minor genes or modifiers controlling this trait. High heritability also indicates that the environment has less influence on these major genes, but not the modifiers. The transfer of agronomically important traits to the cultivars by conventional breeding methods is quite laborious and time consuming and almost impractical when the trait is governed by polygenes. The use of molecular marker technology can help to accelerate the mungbean improvement process through a year-round selection using the marker-assisted selection technique. When more markers surrounding both

Table 1 Simple sequence repeat markers associated with 100-seed weight in an F_2 mungbean population from BM1 × BM6 detected by single regression analysis.

Marker (LG)	Location					
	Gazipur		Madaripur		Combined	
	Probability	R^2 (%)	Probability	R^2 (%)	Probability	R^2 (%)
MB-SSR179 (1)	<0.0001	9.72	<0.0001	10.06	<0.0001	11.57
CEDG048 (1)	<0.0001	5.74	0.0006	8.16	0.0005	8.31
cp01225 (6)	0.0003	2.53	<0.0001	3.10	<0.0001	3.19
CEDG286 (8)	Ns	-	0.0001	10.11	Ns	-
cp00228 (8)	Ns	-	<0.0001	11.26	Ns	-
DMB-SSR043 (9)	<0.0001	14.99	<0.0001	12.57	<0.0001	16.05
CEDG025 (9)	<0.0001	17.35	<0.0001	15.34	<0.0001	19.07
VR354 (9)	Ns	20.85	<0.0001	17.99	<0.0001	22.66

Probability is significant at P = 0.0001; Ns = Not significant.

major and minor genes are captured, seed size can be further improved.

There have been six reports on the mapping of QTL controlling seed weight in mungbean (Fatokun *et al*., 1992; Humphrey *et al*., 2005; Mei *et al.*, 2009; Isemura *et al*., 2012; Kajonphol *et al.*, 2012; Sompong *et al*., 2012; Chen *et al*., 2013). However, all of them used a population derived from inter-specific crosses between cultivated and wild (*Vigna radiata* var. *sublobata*) mungbeans. In addition, only Mei *et al.* (2009) evaluated seed size in more than one environment (season/location). The number of QTL identified in those studies ranged from 3 to 11. The lowest number of QTL was reported by Chen *et al*. (2013) in which the seeds harvested from three different seasons were mixed and measured for seed weight. The highest number of QTL was reported by Humphrey *et al*. (2005) and Mei *et al*. (2009) using the same population. The number of QTL (4) detected for seed weight in the current study is within the range of the previous reports. Differences in the number of QTL identified in these studies may have resulted from differences in the genetic material (parents) used and the environments studied. Mei *et al.* (2009) evaluated the seed weight of a recombinant inbred line population in four environments (three seasons in one location in Australia and one season in China) and found that the number of QTL varied from three to nine and that several QTL were consistently detected in different seasons, although only 3 QTL were common between the two locations. In the current study, three of the four QTL were common between the two locations indicating that the three seed weight QTL are robust and can be targeted for marker-assisted breeding in Bangladesh.

Using common markers, the mungbean seed weight QTL from the current study can be compared with those reported previously by Fatokun *et al*. (1992), Isemura *et al*. (2012), Kajonphol *et al.* (2012), Sompong *et al.* (2012) and Chen *et al.* (2013). The numbers of QTLs

Table 2 Quantitative trait loci detected for seed weight in an F_2 mungbean population from BM1 × BM6 detected by composite interval mapping.

QTL name	Location	Linkage group	Position (cM)	Interval marker	LOD score	Phenotypic variance explained (%)	Genetic effect	
							additive	dominance
qSDW1.1	Gazipur	1	28.2	CEDG141-MBSSR-179	3.1	7.54	-0.27	0.05
	Madaripur	1	24.2	CEDG141-MBSSR-179	7.7	18.33	-0.34	-0.14
	Combined	1	24.2	CEDG141-MBSSR-179	6.9	16.21	-0.33	-0.09
qSDW6.1	Gazipur	6	47.2	VES0987-cp01225	3.6	9.19	-0.27	0.17
	Madaripur	6	50.0	cp01225-CEDG248	4.4	8.31	-0.26	0.08
	Combined	6	49.0	cp01225-CEDG248	5.1	8.60	-0.25	0.08
qSDW8.1	Madaripur	8	22.6	cp00228-MB-SSR008	7.7	14.00	-0.34	-0.03
	Combined	8	21.8	CEDG286-cp00228	5.4	9.68	-0.27	-0.01
qSDW9.1	Gazipur	9	7.3	CEDG024-VR354	9.0	19.42	-0.38	<0.01
	Madaripur	9	15.3	VR354-CEDG238	11.9	33.72	-0.51	-0.12
	Combined	9	15.3	VR354-CEDG238	13.4	39.39	-0.51	-0.10

LOD = Log of odds.

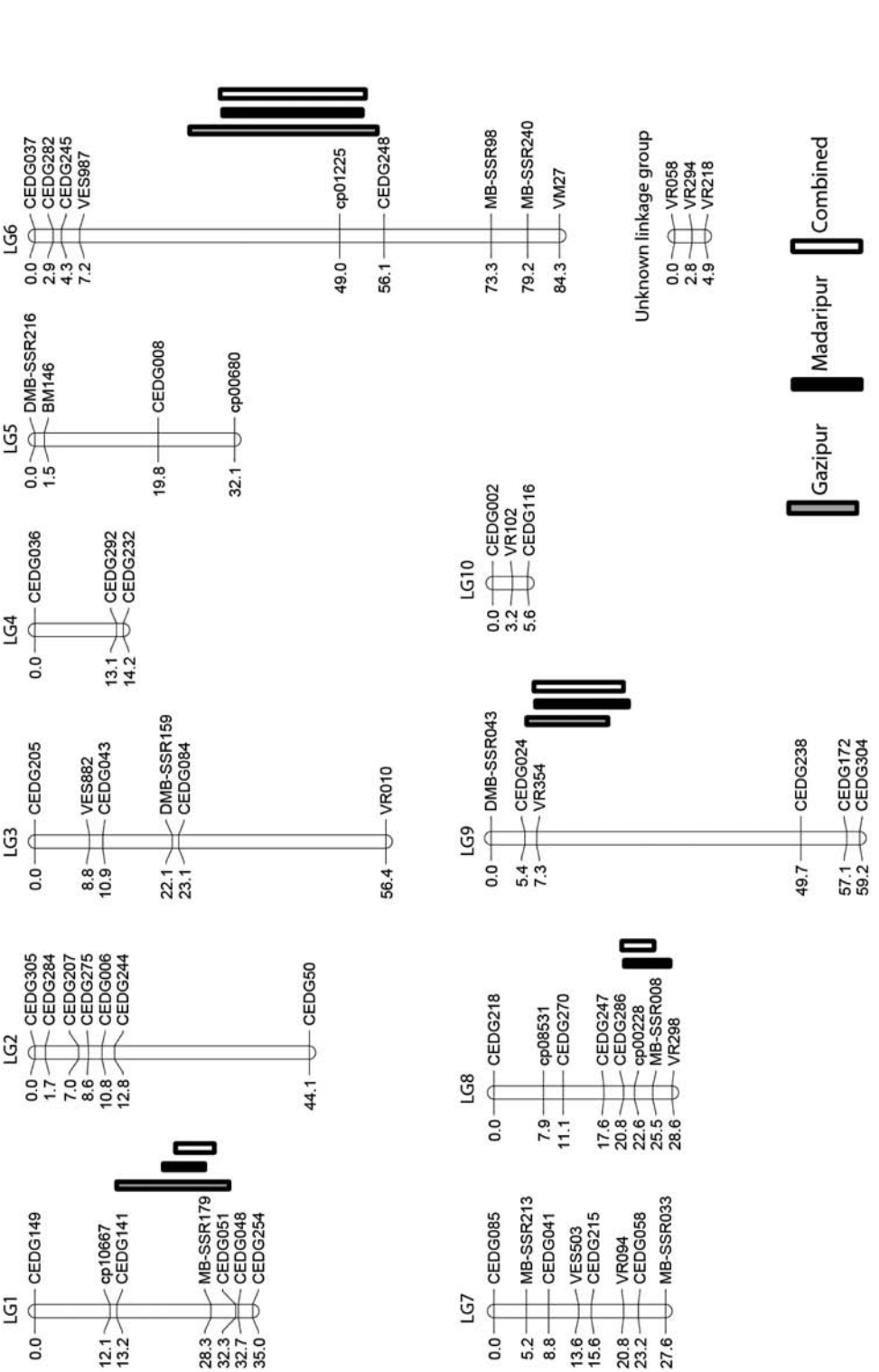

Figure 2 Locations of quantitative trait loci controlling seed weight on a single sequence repeat linkage map of an F_2 mungbean population from BM1 × BM6 detected by composite interval mapping.

in these reports (3, 4, 7, 6 and 5, respectively) and in the current study (4) were more or less comparable. The QTL *qSDWT1.1* and *qSDWT8.1* detected in the current study are common to the QTL *Sd100w5.1.1* and *Sd100w8.1.1* reported by Isemura *et al*. (2012), and appear to be the same as the QTL on LG VI and LG II, respectively, as identified by Fatokun *et al*. (1992). The QTL *qSDWT8.1* and *qSDWT9.1* correspond to the QTL *Sd100wt8* and *Sd100wt9*, respectively, identified by Kajonphol *et al*. (2012). The QTL *qSDWT1.1*, *qSDWT8.1* and *qSDWT9.1* correspond to the QTL *SD100WT1.1*, *SD100WT8.1* and *qSDWT9.1*, respectively, as reported by Sompong *et al*. (2012). These results demonstrated that three out of the four QTL (*qSDWT1.1*, *qSDWT8.1* and *qSDWT9.1*) detected in the current study are resilient, especially *qSDWT8.1*, although this locus was identified in only one season in the current study. It had the largest or second largest effect of QTL for seed weight (Isemura *et al*., 2012; Sompong *et al*., 2012). The current results also revealed that *qSDWT6.1* was a new locus for seed size identified for mungbean.

In addition to the seed weight QTL, the current study was able to compare the QTL identified with the seed weight QTL of other *Vigna* species consisting of azuki bean (Kaga *et al*., 2008), rice bean (Isemura *et al*., 2010) and cowpea (Kongjaimun *et al*., 2012). *qSDWT1.1* was detected in all these crops, *qSDWT6.1* was identified in azuki bean and cowpea, *qSDWT8.1* was found in cowpea and *qSDWT9.1* was detected in azuki bean.

ACKNOWLEDGEMENT

The World Bank financially supported the senior author to undertake Ph.D. study on this work at Kasetsart University. The assistance and support of the staff of the Pulses Research Centre, Bangladesh Agricultural Research Institute, Bangladesh is acknowledged. A part of this research was also funded by the Center of Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University under the National Research University Program of Office of the Higher Education Commission, Ministry of Education, Thailand.

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