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QTLs CONTROLLING SEED WEIGHT AND DAYS TO FLOWERING IN MUNGBEAN [*Vigna radiata* (L.) Wilczek], THEIR CONSERVATION IN AZUKI BEAN [*V. angularis* (Ohwi) Ohwi & Ohashi] AND RICE BEAN [*V. umbellata* (Thunb.) Ohwi & Ohashi]

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ABSTRACT

Mungbean (*Vigna radiata* (L.) Wilczek) is a socio-economically important legume crop of Asia. Varieties with large seed size and early maturity are preferred in commercial production. This study was to identify quantitative trait loci (QTL) controlling seed weight and days to flowering in mungbean. The mapping population comprises 155 F_2-derived lines from a cross between Kamphaeng Saen 1 (large-seeded and early flowering) and V4718 (small-seeded and late flowering). The F_2 population was analyzed with 67 simple sequence repeat markers. The F2:3 families were evaluated for 100-seed weigh and days to flowering in two years, 2008 (one season) and 2011 (two seasons). Composite interval mapping identified six QTLs for 100-seed weight and 5 QTLs for days to flowering. Three genomic regions harbored QTLs for both seed weight and days to flowering, revealing association between the two traits. Comparison of QTLs for both traits found in this study with those reported in azuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] and rice bean [*Vigna umbellata* (Thunb.) Ohwi & Ohashi] revealed that several QTLs are conserved among the three *Vigna* species.

Keywords: comparative mapping, flowering, mungbean, quantitative trait loci, seed size

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is an important legume crop of Asia. The crop is grown principally for its dry seeds, although young pods are sometimes consumed and other plants parts can be used as fodders. Mungbean seeds contain 20-25% of proteins and 65-70% of carbohydrates (Poehlman, 1990). It is a major inexpensive source of dietary proteins and amino acids for people in South, East and Southeast Asia. Seed and flour of mungbean are used to prepare several kinds of food. Mungbean sprout is consumed as a popular vegetable containing high vitamin C and iron. Mungbean is grown in the total production area of about 5.5 million ha of which 90% is in Asia (Somta and Srinives, 2007), although the real production area is believed to be much higher. Major producing countries include India, China, Myanmar, Thailand and Viet Nam where India is the largest producer in an area of about 3 million ha annually. China, Myanmar, Thailand and Viet Nam are also the chief exporters of mungbean grain and products.

Mungbean is usually grown after rice or other cereal crops, or sometimes intercropped with other field and tree crops. It is relatively tolerant to drought and can be harvested within 60-90 days depending on varieties (Fernandez and Shanmugasundaram, 1988). This makes mungbean suitable for several cropping systems. Most commercial mungbean cultivars are large-seeded with 100-seed weight being 0.60 to 0.75 gram and early maturity (55 to 75 days after sowing). Seed size is positively correlated with yield. Large seed has advantage in field germination and establishment. It is also desirable for sprout industry in that large seeds always yield larger sprouts than small seeds. Very early maturing cultivars are desirable because they can escape drought condition. Therefore, large seed size and early maturity are major goals in mungbean breeding programs (Fernandez and Shanmugasundaram, 1988).

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Better understanding of the genetic control of these two traits will be useful for improving new mungbean cultivar(s).

Like most seed crops, genetics of seed size and flowering time have been well studied in mungbean. Seed size is a polygenic trait with high heritability (>80%) (Imrie *et al*., 1985; Humphry *et al*., 2005; Yimram *et al*., 2009). Similarly, days to flowering is controlled by polygenes which has moderate to high heritability (43% to 93%) (Yimram *et al*., 2009). Quantitative trait loci (QTL) controlling seed weight of mungbean has been identified (Fatokun *et al*., 1992; Humphry *et al*., 2005; Sompong *et al*., 2012). Fatokun *et al.* (1992) reported that seed weight is controlled by one major and three minor QTLs. Humphry *et al*. (2005) found that the trait is controlled by as many as 11 QTLs with moderate to minor effect. Sompong *et al*. (2012) reported that five QTLs with moderate to minor effect govern seed size. In contrast to seed weight, there is only a single report on QTL for days to flowering in mungbean. Sompong *et al.* (2012) identified three QTLs, two major and one minor, for days to flowering.

In this paper, we report QTLs for seed weight and days to flowering in mungean and conservation of these QTLs with other related species.

MATERIALS AND METHODS

Plant Materials

An F_2 population comprising 155 lines derived from a cross between Kamphaeng Saen 1 (here after called as KPS1) and V4718. KPS1 is a popular commercial mungean cultivar from Thailand. It has large seed size (about 6.4 g per 100 seeds) and is early flowering (30-35 days after planting). V4718 is a mungbean germplasm originated from India with small seed size (about 3.3 g per 100 seeds) and moderate flowering (40-50 days after planting).

Measurement of Seed Weight and Days to Flowering

The parents and F_2 progenies were individually grown in pots during February to April 2007. DNA of each entry was extracted from leaves using a CTAB method (Lodhi *et al*., 1994). The parents and $F_{2:3}$ progenies were sown in a randomized complete block design (RCBD) with three replicates in two seasons, viz. rainy (August to December, 2008) and dry (January to March 2011). Each replicate contained 20 to 25 plants. In each entry, days to first flowering was recorded, seeds were harvested and weighed for 100 seed weight (g). The parents and $F_{2:4}$ progenies were sown in a RCBD with three replicates in dry season during September 2011 to January 2012. Seed weigh and days to flowering were recorded as described in the $F_{2:3}$ generation.

SSR Marker Analysis

KPS1 and V4718 were screened for DNA polymorphism using 753 primer pairs of simple sequence repeat (SSR) markers [433 from mungbean (Gwag *et al*., 2006; Tangphatsornruang *et al*., 2009; Somta *et al*., 2008; Seehalak *et al*., 2009; Somta *et al*., 2009), 188 from azuki bean [*V. angularis* (Ohwi) Ohwi & Ohashi] (Wang *et al*., 2004), 46 from cowpea [*V. unguiculata* (L.) Walp.] (Li *et al*., 2001), and 86 from common bean (*Phaseolus vulgaris* L.) (Gaitán-Solís *et al*., 2002; Blair *et al*., 2003; Guerra-Sanz, 2004; Buso *et al*., 2006)]. Polymorphic markers were then used to analyze the F_2 population. DNA marker analysis was carried out as per Somta *et al.* (2008).

Linkage Map Construction and QTL Analysis

Genetic linkage maps were constructed using Join Map 3.0 with the minimum logarithm of the odds (LOD) at 3.0 and the maximum recombination frequency (*r*) at 0.5. The recombination frequency was converted into genetic distance (centimorgan; cM) using Kosambi mapping function (Kosambi, 1943). Linkage groups were named after azuki bean linkage map (Han *et al*., 2005) based on the common markers. QTL for seed weight and days to flowering was detected by composite interval mapping (CIM) using WinQTL Cartographer version 2.5 (Wang *et al*., 2011). Genome-wise significant LOD threshold of 3 was used to declare QTL.

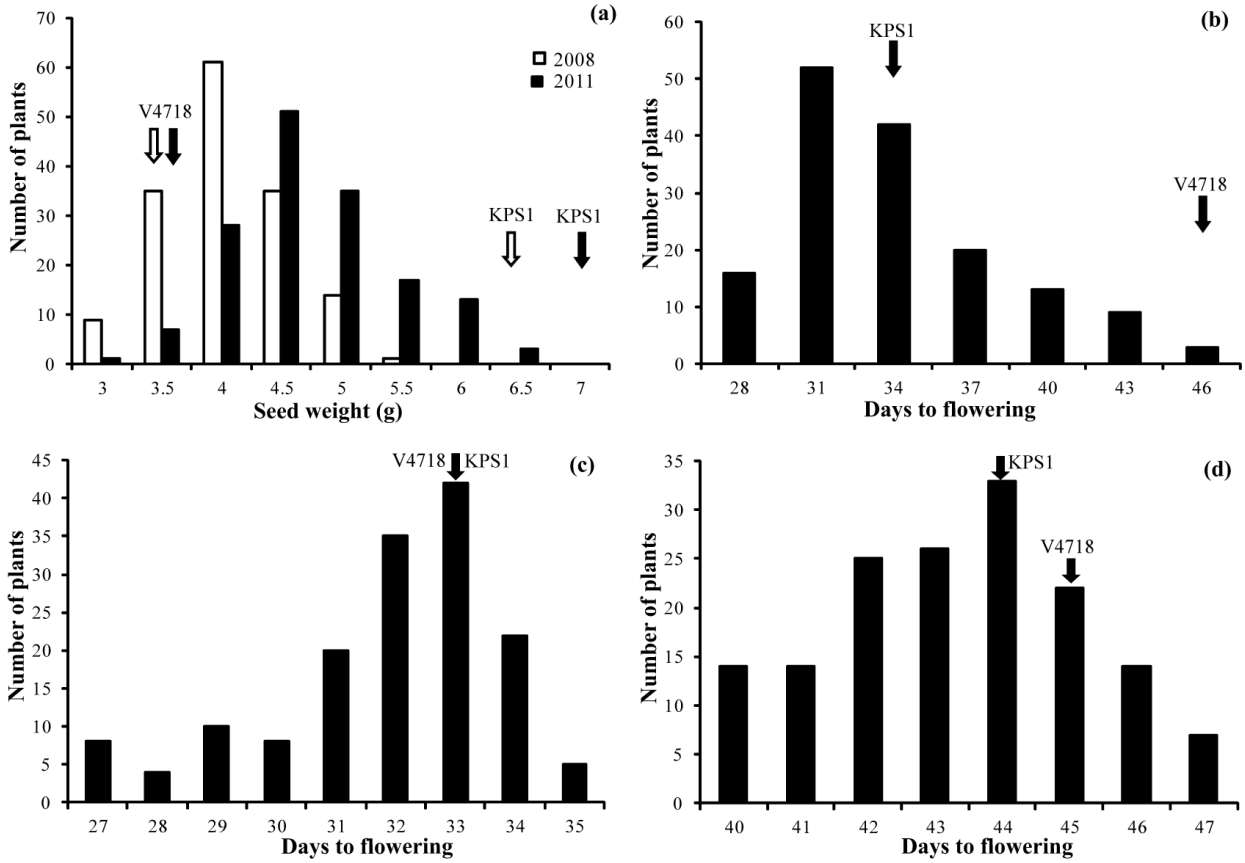

Figure 1. Frequency distribution of seed weight of $F_{2:3}$ population grown in rainy season 2008 and early dry season of 2011 (a), and of days to flowering grown in rainy season 2008 (b), and early (c) and late (d) dry seasons of 2011

RESULTS AND DISCUSSION

Seed Weight

Seed of KPS1 was about one fold larger than V4718. One hundred-seed weight of KPS1 and V4718 grown in the rainy season of 2008 were 6.62 g and 3.27 g in that order, and those grown in the dry season of 2011 were 6.75 g and 3.30 g, respectively. In the $F_{2:3}$ population grown in the rainy season of 2008, their 100-seed weights varied between 2.77 g and 5.36 g with an average of 3.79 g. For the population grown in dry season of 2011, 100-seed weight ranged from 2.78 g to 6.30 g with an average of 4.50 g. As expected, frequency distributions of seed weight in both seasons were continuous (Figure 1). A few progenies had smaller seed weight than V4718, but none of them were larger than KPS1.

Days to Flowering

KPS1 flowered earlier than V4718 when grown in rainy season (long photoperiod condition) of 2008, but they showed almost the same flowering time when grown in the dry season of 2011 (short photoperiod condition). In the rainy season of 2008, days to flowering of KPS1 and V4718 were 33 and 44, respectively. Days to flowering of both KPS1 and V4718 in the early dry season of 2011 were 33, while those in the late dry season of 2011 were 44 and 45 days, respectively. Days to flowering of the $F_{2:3}$ population grown in the rainy season of 2008 was between 26.7 and 45.3 with an average of 32.7 days. For the F_3 population grown in early dry season of 2011, days to flowering varied from 26.5 to 35.5 with an average of 31.6 days. In case of the $F_{2:4}$ population which was grown in the late dry season of 2011, days to flowering ranged from 40 to 47 with an average of 43.2 days. The

transgressive segregation suggests that both KPS1 and V4718 possess both positive and negative alleles for seed weight. There was a positive correlation between days to flowering measured in different populations and seasons. Frequency distribution of days to flowering in all generations and seasons demonstrated continuous segregation (Figure 1). Transgressive segregation was found in all seasons, suggesting that the parents possess both positive and negative alleles for days to flowering.

Correlation between Traits and between Seasons

There were positive correlations of seed weight or days to flowering measured in different seasons (data not shown). The correlation was moderate for seed weight and relatively low for days to flowering, suggesting different degrees of environmental effect on the two traits. Correlation between seed weight and days to flowering was significant and negative being -0.48 ($P < 0.0001$) and -0.24 ($P = 0.0023$) for the $F_{2:3}$ population grown in 2008 and 2011, respectively. Negative correlation between seed weight and earliness (days to flowering and maturity) in mungbean have been reported previously (Siddique *et al.,* 2006).

Mungbean Linkage Map

There was low polymorphism between genomes of V4718 and KPS1. Out of 753 SSR markers screened, 496 (65.87%) amplified DNA of the two genotypes (data not shown), but only 69 (13.91%) of the amplifiable markers were polymorphic. Only 56 polymorphic markers could be assigned to the linkage map (Figure 2), while 13 markers were unlinked. The linkage map consisted of 11 linkage groups (LGs). The total map distance was 995.6 cm with and an average density of 17.78 cm per marker. Comparison of our linkage map with azuki bean map reported by Han *et al.* (2005) based on 30 common markers revealed that all of the markers were conserved on both maps with a few inversions of marker order (data not shown). This indicates high genome homology between mungbean and azuki bean.

QTLs for Seed Weight and Days to Flowering

Six QTLs locating on different LGs were identified for seed weight (Figure 2; Table 1). Five and two QTLs for this trait were found in $F_{2:3}$ population planted in the rainy season of 2008 and dry season of 2011, respectively, in which only one QTL, *qSW5.1*, was common between the seasons. The phenotypic variance explained (PVE) by these QTLs varied between 5.9 (*qSW2.2*) to 22.1% (*qSW2.1*). PVE of the *qSW5.1* in both seasons were very similar. The number of QTLs for seed weight found in this study was only about a half of those reported by Humphry *et al.* (2005) who reported 11 QTLs for seed weight in a RIL from a cross between wild and cultivated mungbeans. The lower number of QTLs found in our study was possibly stem from narrow difference in seed size of the parents used. However, the number of QTLs for seed size found in our study was very similar to those reported by Sompong *et al.* (2012). Although there is small number of common markers between the two studies, the QTLs on LG2 and LG10 appear to be common, especially *qSW2.2* in this study and *SD100WT2.1* reported by them were both linked to the same marker VR413.

Five QTLs were detected for days to flowering (Figure 2; Table 1). All the QTLs were detected in the $F_{2:3}$ population grown in rainy season of 2008. The PVE of these QTLs ranged from 18.2 for *qDFL4.1* to 32.0% for *qDFL5.1*. Only one QTL, *qDFL2.2*, was detected in the $F_{2:3}$ population grown in dry season of 2011, and only QTL *qDFL2.1* was identified in the $F_{2:4}$ population grown in the same season. The PVE of *qDFL2.1* and *qDFL2.2* found in these two seasons was only about a half of that in the same QTL in the rainy season of 2008. The marked difference in number of QTLs for days to flowering found in rainy and dry seasons is very likely due to effect of photoperiod. Mungbean is a short-day plant (Poehlman, 1990). This caused high and low variation in days to flowering in the rainy season (long photoperiod; 19 days) and dry season (short photoperiod; 7 or 9 days), respectively (data not shown). This resulted in difference number of QTLs detected in such different environments.

Table 1. QTL controlling seed weight and days to flowering in the F_2 (KPS1 x V4718) population as detected by composite interval mapping

Trait	Plant generation-Season	QTL name[1]	LG[2]	Marker interval	LOD score	PVE[3] (%)	Additive effect	Dominant effect
Seed weight	$F_{2:3}$-rainy, 2008	*qSW2.1*	2A	DMBSSR160 - GBssrMB087	10.4	22.1	-0.33	-0. 08
		qSW2.2	2B	VR413 - CEDG284	3.6	5.9	-0.17	-0.08
		qSW4.1	4	CEDG088 - CEDG232	3.2	7.8	-0.19	0.08
		qSW5.1	5	CEDG014 - MBSSR39	6.7	11.6	-0.19	-0.19
		qSW10.1	10 B	CEDG198 - VR293	3.4	8.1	-0.18	-0.17
	$F_{2:3}$-dry, 2011	*qSW5.1*	5	CEDG014 - MBSSR39	3.2	9.1	-0.24	-0.15
		qSW11.1	11	MBSSR164 - VR304	3.8	13.0	-0.30	-0.27
Days to flowering	$F_{2:3}$-rainy, 2008	*qDFL2.1*	2A	CEDAAG002 - DMBSSR160	7.4	29.6	3.50	-2.05
		qDFL2.2	2A	DMBSSR160 - GBssrMB087	10.9	29.1	2.75	-2.20
		qDFL4.1	4	DMBSSR167 - VRSSR035	6.7	18.2	2.00	-2.26
		qDFL5.1	5	CEDG014 - MBSSR39	4.5	32.0	0.42	7.27
		qDFL6.1	6	VR095 - CEDG037	5.0	31.0	-0.08	7.02
	$F_{2:3}$-dry, 2011	*qDFL2.2*	2A	CEDAAG002 - DMBSSR160	5.1	12.6	1.10	0.21
	$F_{2:4}$-dry, 2011	*qDFL2.1*	2A	DMBSSR160 - GBssrMB087	4.3	23.2	5.14	-0.65

Remarks: [1] QTLs of the same trait that showed similar position and effect in the different seasons were assigned the same name; [2] Linkage group; [3] Percentage of phenotypic variance explained by the QTL

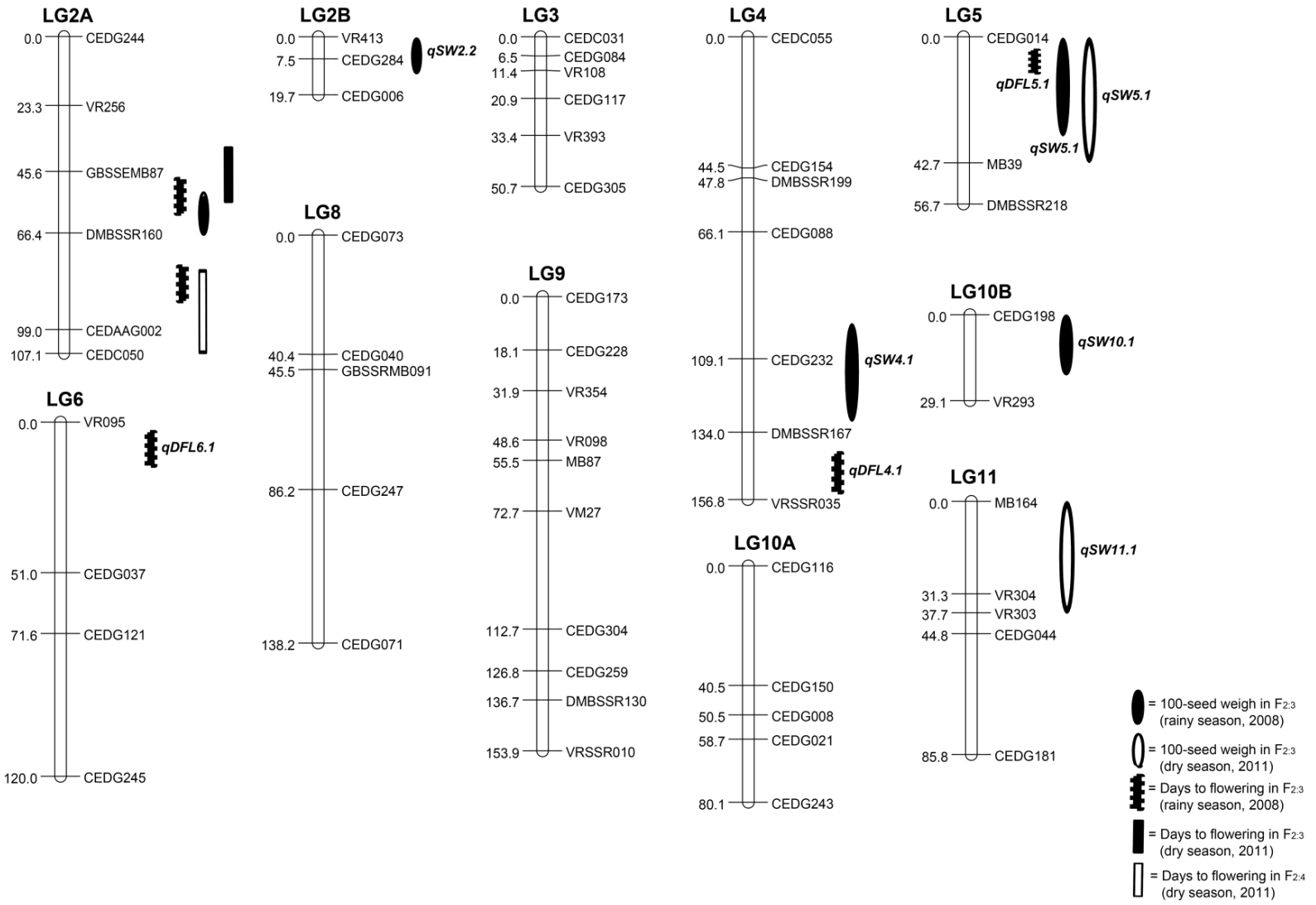

Figure 2. Linkage map and locations of QTLs controlling seed weight and days to flowering of F_2 population developed from a cross between KPS1 and V4718 mungbeans

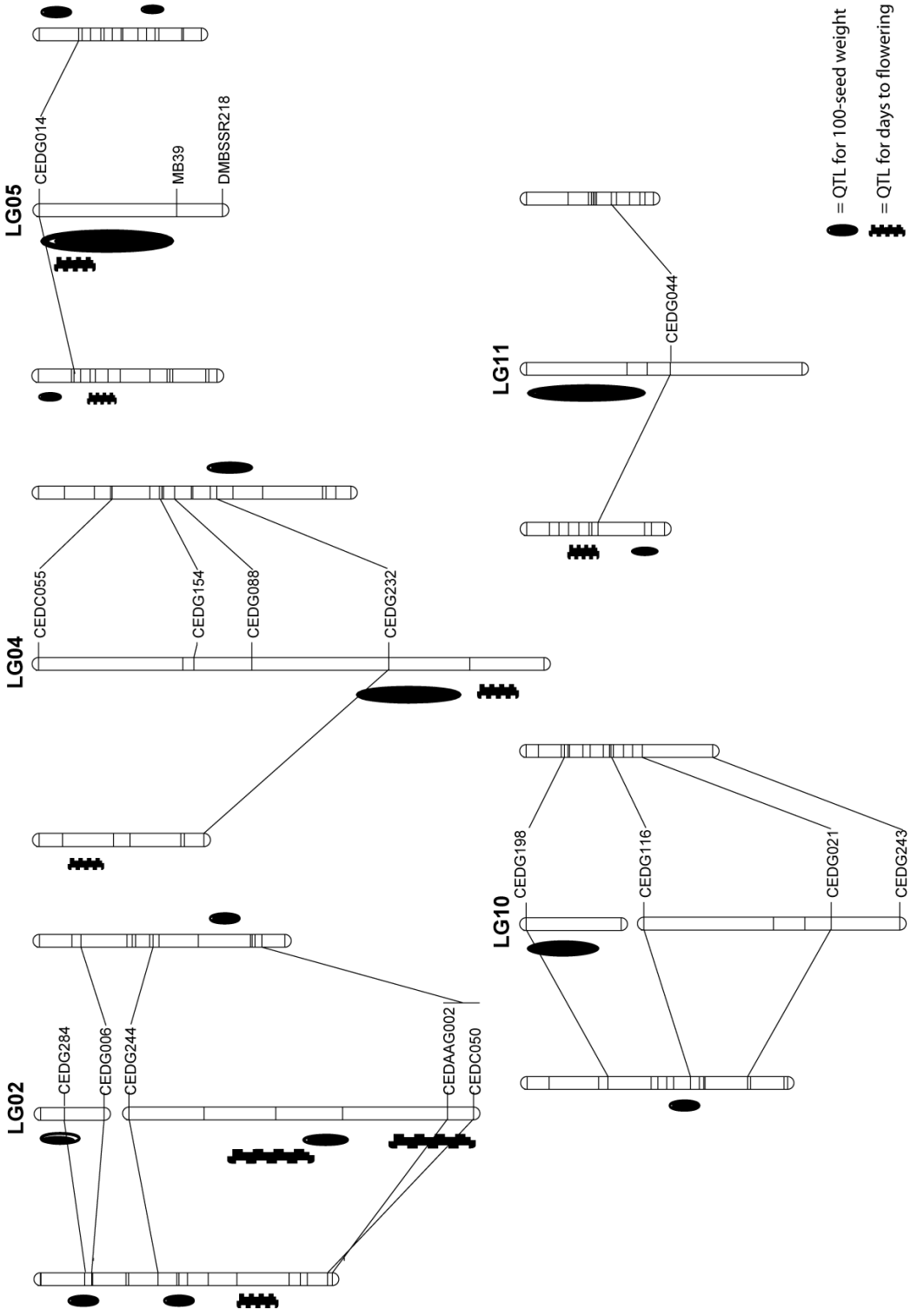

Figure 3. A comparative QTL map for seed weight and days to flowering of azuki bean, mungbean and rice bean. In each linkage group, common markers between maps are connected by lines. Linkage maps of azuki bean, mungbean and rice bean are on the right, middle and left, respectively. Size of QTL symbol does not relate to genetic effect

Since the *qDFL2.1* and *qDFL2.2* were found in both photoperiod conditions, they can be considered important loci controlling flowering time in mungbean. *qDFL4.1, qDFL5.1 and qDFL* which were identified in rainy season may be specific to long photoperiod regime in mungbean. Nonetheless, the number of QTLs for days to flowering found in this study is higher than those reported by Sompong *et al.* (2012) who found only three QTLs for days to flowering. Based on common markers, only QTL on LG4 are likely the same QTL in the two studies (data not shown).

Co-localization or linkage of QTLs for seed weight and days to flowering was found on LGs 2A, 4 and 5. On LG2A and 5, QTLs for seed weight and days to flowering were overlapped (*qSW2.1* vs. *qDFL2.2* and *qSW5.1* vs. *qDFL5.1*). *qSW4.1* and *qDFL4.1* were linked at the lower part of LG4. These support the significant correlation between the two traits.

Comparison of Seed Weight and Days to Flowering QTLs between Mungbean and Azuki Bean, and Rice Bean

High genome conservation among species in the genus *Vigna* has been demonstrated (Fatokun *et al*., 1992; Somta *et al*., 2006; Isemura *et al*., 2007, 2010; Kongjaimun *et al*., 2012). Mungbean, azuki bean and rice bean belong to the subgenus *Ceratotropis* which are known as Asian *Vigna* because they are all originated in Asia. QTLs for seed weight and flowering time have been reported in azuki bean (Isemura *et al*., 2007; Kaga *et al*., 2008) and rice bean (Isemura *et al*., 2010). Comparison of QTL locations for seed weight and flowering time of mungbean in the present study with those of azuki bean and/or rice bean (Figure 3) further supports previous findings. Several QTLs for seed weight and days to flowering of the three *Vigna* species were found on the same LG. Some of them are linked to the same markers or located in the similar genomic regions. For example, a seed size QTL for azuki bean, rice bean and mungbean on LG5 are all linked by marker CEDG014 (Figure 3). These QTLs are possibly the same locus. On the same LG, a QTL for flowering time of mungbean and azuki bean were located in the similar genomic region (Figure 3). It is worth noting that although the QTLs for flowering time on LG4 of azuki bean and mungbean appear to far apart, they are both major QTLs for this trait (Table 1; Kaga *et al*., 2008). The high genome conservation among *Vigna* species is useful for comparative and candidate gene mappings of important traits among species in this taxon.

CONCLUSION

Nine genomic regions harboring six QTLs for 100-seed weight and 5 QTLs for days to flowering was identified in $F_{2:3}$ families of KPS1 x V4718 grown in one rainy season (long day condition) and two dry seasons (short day condition) of two years. Only two of the nine genome regions harbored both QTLs for seed weight and days to flowering detected in rainy season, while the other regions each harbored only a single QTL for a trait. This results suggested substantial effect of day length on the two traits. Therefore, breeding for seed weight and days to flowering simultaneously for different day length conditions in mungbean would be difficult. When the QTLs detected in this study were compared with those QTLs detected previously in azuki bean and rice bean, two genome regions each on linkage groups 2 and 5 containing QTLs for seed weight were found to be common among the three legumes. This suggested that those QTLs can be used as candidates for map-based cloning of seed weight QTLs.

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