AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Identification of quantitative trait loci controlling flowering time in black gram (*Vigna mungo* [L.] Hepper)

Sunisa Suamuang[a,b], Chalinee Lomlek[c], Wasitthee Kongkachana[d], Sithichoke Tangphatsornruang[d], Kularb Laosatit[c], Orn-u-ma Tanadul[a,b,c], Prakit Somta[c,*]

[a] *Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand*

[b] *Center of Excellence on Agricultural Biotechnology: (AG-BIO/MHESI), Bangkok 10900, Thailand*

[c] *Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand*

[d] *National Omics Center, National Science and Technology Development Agency, Pathum Thani, Thailand*

Article Info

Article history:
Received 19 September 2022
Revised 26 October 2022
Accepted 20 November 2022
Available online 28 February 2023

Keywords:
Black gram,
Flowering,
Quantitative trait loci,
Single nucleotide polymorphism

Abstract

Importance of the work: Flowering time is a key adaptive trait for expanding cultivation to high-latitude regions. However, little is known about the genetics of this trait.

Objectives: Quantitative trait loci (QTL) controlling days to first flowering in black gram were identified in only the second report published.

Materials & Methods: Two F_2 populations were each developed from a cross between cultivated (Chai Nat 80 (CN80)) and wild black gram (PI213017 and TVNu 1076) accessions and grown in different environments. Two high-density linkage maps were constructed from these populations using single nucleotide polymorphism markers.

Results: Broad-sense heritability for days to first flowering in the F_2 populations was in the range 34.86–61.95%. QTL analysis revealed 11 QTLs controlling days to first flowering in two populations. Four QTLs (*qDFFBGA2.1, qDFFBGA5.1, qDFFBGA8.1* and *qDFFBGA9.1*) were found in the population of CN80 × PI213017 that explained 8.93%, 7.87%, 11.13% and 10.88% of phenotypic variation, respectively. Seven QTLs (*qDFFBGB2.1, qDFFBGB2.2, qDFFBGB4.1, qDFFBGB6.1, qDFFBGB8.1, qDFFBGB8.2* and *qDFFBGB9.1*) were found in the population of CN80 × TVNu 1076 which explained 38.02%, 4.22%, 3.36%, 7.12%, 3.85%, 4.41% and 19.42% of phenotypic variation, respectively. Nonetheless, no QTLs were common between populations, suggesting flowering time in black gram is highly influenced by environmental factors.

Main finding: New QTLs controlling flowering time in natural short daylength and long daylength regimes were identified. None of the candidates were similar with those previously reported in *Vigna* species related to black gram, suggesting that one or more of the flowering pathways in black gram are different from other *Vigna* species.

* Corresponding author.
E-mail address: agrpks@ku.ac.th (P. Somta)

https://doi.org/10.34044/j.anres.2023.57.1.05

Introduction

Black gram (also known as urd bean and urad bean; *Vigna mungo* [L.] Hepper) is an important legume crop of Asia, whose seeds contain high amounts of proteins (20–25%) and carbohydrates (65–75%) and are mainly consumed as soup, while black gram flour and powder are used to prepare several kinds of foods including cakes, biscuits, snacks, cookies and doughnuts (Kaewwongwal et al., 2015). Sprouts produced from black gram are popular as a vegetable source of vitamins and minerals (Choon et al., 2010). Black gram is relatively tolerant to drought stress and has a short life cycle (75–90 d), with the ability to fix atmospheric nitrogen in association with soil bacteria of the genus *Rhizobium* and *Bradyrhizobium* (Somta et al., 2020). Therefore, black gram is grown as a major component in several cropping systems, but mainly after rice, wheat and maize in South and Southeast Asian countries, including Afghanistan, Bangladesh, India, Pakistan, Nepal, Myanmar, the Philippines, Sri Lanka and Thailand (Kaewwongwal et al., 2015). Outside Asia, it is grown in Australia, Argentina and Brazil for export to Asian countries (Joshi and Rao, 2017). More than 7 million ha of black gram are cultivated, with India having the largest area (about 5.6 million ha) and being the largest consumer, with Myanmar having the second largest area (about 1 million ha) and being the biggest exporter (Khine et al., 2021). The seed yield of black gram in major production countries is low (only about 990 kg/ha) due to several production constrains, such as drought, insect infestation, disease infection and photo- and thermo-sensitivity (Sahu et al., 2020; Singh et al., 2022). Therefore, genetic improvement of these traits is necessary to improve the seed yield of black gram.

Flowering time is an important agronomic trait that affects adaption and the agronomic performance (for example plant height) and yield of seed crops (Lin et al., 2021). Although several factors affect flowering time, daylength and temperature are the major ones (Lin et al., 2021). Black gram is a tropical and short-day plant; black gram plants start flowering when the daylength under cultivation is shorter than a critical daylength. Understanding the genetics of flowering time is crucial for breeding new black gram cultivars that flower and mature early and can adapt to new environments (such as higher latitudes). However, little is known about the genetic basis of flowering time in black gram. Ghafoor et al. (2001) evaluated days to 50% flowering in 484 accessions of black gram and reported that the narrow-sense heritability of this trait was 65%. Gene or quantitative trait locus (QTL) mapping is a standard method used to dissect the genetic loci controlling a quantitative trait and is a useful method to reveal the genetic basis of a trait as well as being the basis for marker-assisted selection (Collard and Mackill, 2008), which is a form of molecular breeding. Black gram is an orphan crop and underfunded compared to other legumes of similar economic importance. Consequently, there not been many reports on QTL/gene mapping in black gram. Up to the present, there has been only one published report on the QTLs controlling flowering time in black gram. Singh et al. (2022) identified one QTL, *QDtF.10*, for days to 50% flowering in black gram using a small set of 100 accessions grown in two locations across two seasons in India. *QDtF.10* accounted for 17–18% of the flowering time variation. However, this QTL was identified only in one location. Therefore, this QTL should be confirmed and additional QTLs must be identified for flowering time in black gram. Nonetheless, in other legume crops of the genus *Vigna* closely related with black gram, including mungbean (*Vigna radiata* [L.] Wilczek), azuki bean (*Vigna angularis* [Ohwi] Ohwi and Ohashi), rice bean (*Vigna umbellata* [Thunb.] Ohwi and Ohashi), and cowpea (*Vigna unguiculata* [L.] Walp.), QTL analyses have revealed that flowering time is controlled by several QTLs (Isemura et al., 2007, 2010, 2012; Kaga et al., 2008; Kajonphol et al., 2012; Kongjaimun et al., 2012; Sompong et al., 2012; Somta et al., 2015; Liu et al., 2016; Li et al., 2017; Liu et al., 2017; Yamamoto et al., 2016; Lo et al., 2018).

Genomics research in black gram lags other legume crops of the genus *Vigna* due to the lack of genomic resources, especially DNA markers which are a fundamental tool in genomics research. Nonetheless, recent advances in DNA sequencing technology have revolutionized plant genome research by providing rapid and mass discovery single nucleotide polymorphisms (SNPs) and genotyping of the SNP via the genotyping-by-sequencing technique. Recently, a reference genome sequence was constructed using whole genome sequencing and second and third generation sequencing technologies (Pootakham et al., 2021a).

The current study aimed to identify the QTLs controlling time to flowering (days to first flowering, DFF) in black gram. Two SNP-based linkage maps were constructed from two F_2 populations and the QTLs for flowering time were identified.

Materials and Methods

Mapping populations and phenotyping of days to first flowering

Two F_2 populations (F2BGA and F2BGB) were used in

this study. F2BGA was composed of 153 plants and was developed from a cross between cultivated black gram accession 'CN80' (female) and wild black gram accession 'PI213017' (male). F2BGB was composed of 98 plants which was derived from a cross between CN80 (female) and wild black gram accession "TVNu 1076" (male). CN80 was a commercial cultivar of Thailand and was used to construct a reference genome sequence for black gram (Pootakham et al., 2021a). The wild parents PI213017 and TVNu 1076 from India were used because they were genetically and phenotypically different from CN80 (Pootakham et al., 2021a). The F_2 of each population was derived from the self-pollination of a single F_1 plant.

The populations F2BGA and F2BGB and their parents were grown under field conditions on the Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. The population F2BGA was grown during November 2020–April 2021 (cool-dry season with a short daylength), whereas the population F2BGB was grown during March–July in 2021 (summer with a long daylength). In all cases, the distance between plants was 50 cm. Cultivation practices were performed as described by Park (1978). Days to first flowering was recorded for each plant.

Single nucleotide polymorphism discovery and genotyping

Total genomic DNA was extracted from the young leaves of each plant using a cetyltrimethyl ammonium bromide nucleic acid extraction method (Lodhi et al., 1994). The DNA quantity and quality were determined using gel electrophoresis and spectrophotometric measurements. The two mapping populations (F2BGA and F2BGB) were genotyped by sequencing using the RAD-seq method as described in Pootakham et al. (2021b). DNA libraries were constructed using the MGIEasy RAD library kit and sequenced using the PE150 sequencing chemistry on MGISEQ 2000RS (MGI Tech; China). The sequencing reads were mapped to the reference genome sequence of black gram (CN 80) (Pootakham et al., 2021a) using the Minimap2 version 2.11-r797-v03 software (Li, 2018), and SNP markers were called using GATK HaplotypeCaller 3.8 (McKenna et al., 2010). The SNP loci were filtered based on: minor allele frequency > 0.1, depth coverage 10–200× and < 5% missing data.

Linkage and quantitative trait loci analysis

In each population, a linkage map was constructed using the QTL IciMapping 4.2 software (Meng et al., 2015). Markers showing significant ($p < 0.05$) segregation distortion were excluded from the linkage analysis. The remaining markers were grouped using a minimum logarithm of odds (LOD) value of 3.0. The markers were ordered based on recombination counting and the ordering (RECORD) algorithm function (Van Os et al., 2005). Map distances were calculated using the Kosambi mapping function (Kosambi, 1944).

QTLs were detected based on the inclusive composite interval mapping (ICIM) method (Li et al., 2007) using the QTL IciMapping 4.2 software. ICIM was performed at every 0.1 cM. An LOD score of 3.0 was used as a significant LOD threshold. In addition, a significant LOD threshold for each trait was determined based on a 1,000-permutation test at the 95% confidence level.

Estimation of heritability

The broad-sense heritability (H^2) of DFF of the F_2 population was calculated as described by Amkul et al. (2019) and shown in Equation (1):

$$H^2 = \frac{\left[\sigma^2_{F2} - \left(\frac{\sigma^2_{P1} + \sigma^2_{P2}}{2}\right)\right]}{\sigma^2_{F2}} \quad (1)$$

where σ^2P_1, σ^2P_2 and σ^2F_2 are the variances of the female parental, male parental and F_2 population, respectively.

Identification of candidate gene(s)

Identification of candidate gene(s) was performed for the QTLs based on a percentage of variance (PVE [R^2]) of 10% or higher. The genome region of the flanking markers of each QTL were determined based on a BLASTN search against the CN80 reference genome sequence (Pootakham et al., 2021a). Then, the genes located in such a genome region were identified. Genes with possible roles in controlling flowering were selected and considered as candidate gene(s).

Results

Variation in flowering time

Flowering time was investigated in two F_2 populations, F2BGA and F2BGB, developed from crossing cultivated and wild black grams. The F2BGA population and its parents were grown in 2020; DFF in the cultivated (CN80) and wild (PI213017) parents with a difference of 10 d, being about 42 d and 52 d after planting (DAP), respectively. However, their F_2

progenies showed a wide variation in DFF within the range 32–76 DAP and an average of 50.27 DAP (Fig. 1a). In contrast, the F2BGB population and its parents (CN80 and TVNu 1076) were grown in 2021; CN80 flowered about 10 d earlier than the wild TVNu 1076 at about 35 DAP and 46 DAP, respectively. Their F_2 population exhibited wide variation in flowering time with DFF in the range 29–47 DAP and an average of 36.73 DAP (Fig. 1b). The frequency distributions of DFF in both populations was continuous, implying that the trait was quantitative in nature. In addition, the frequency distribution showed transgressive segregation of the trait, with some F_2 plants having higher and lower DFF values than the parents. H^2 for DFF was 61.95% and 34.86% for the populations F2BGA and F2BGB, respectively.

Genotyping by sequencing

The two black gram populations (F2BGA and F2BGB) were genotyped by sequencing the using RAD–seq method. In each population, RAD-seq libraries of F_2 plants and their parents were constructed using the MGIEasy RAD library kit and sequenced using the PE150 sequencing chemistry on MGISEQ 2000RS. In the F2BGA population (153 F_2 individuals), 396 Gbp were obtained with an average of 2.6 Gbp per sample. Among these

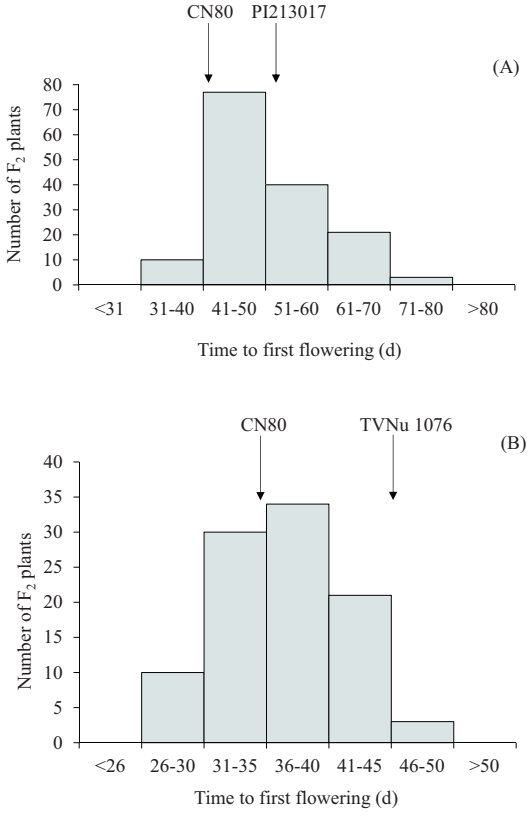

Fig. 1 Frequency distribution of DFF of F_2 population derived from cross between: (A) CN80 and PI213017 (F2BGA); (B) CN80 and TVNu 1076 (F2BGB)

data, 85.60% of bases were high quality (>Q30), and the average guanine-cytosine (GC) content was 38.8%. The sequencing reads were mapped to the CN80 reference genome (Pootakham et al., 2021a) and the percentage of the average mapped read was 94% (87.5–96.2%). The total number of called SNPs in the F2BGA population was 1,423,577 loci which were then filtered to 3,345 loci and used in the linkage analysis. In the F2BGB population (98 F_2 individuals), 43.9 Gbp were obtained, with an average of 0.4 Gbp per sample and 89.3% of the bases were high quality (>Q30) and the average GC content was 33.9%. The total number of called SNPs in this population was 1,211,108 loci which were then filtered to 7,508 loci and used in the linkage analysis.

Linkage map construction

The SNPs with a minor allele frequency >0.1, depth coverage in the range 10–200×, <5% missing data, non-segregation distortion and non-redundant genotypes were used in the linkage analysis. The remainingd markers were constructed into a genetic linkage map. The map of the F2BGA population contained 11 LGs (Fig. 2A) and spanned a total length of 8,119.79 cM. The length of each LG ranged from 94.58 cM (LG11) to 1788.37 cM (LG4), with an average of 738.16 cM. The number of SNP markers in each LG varied from 14markers on LG2 to 436markers on LG4, with an average of 184.55 SNPs per LG. The average distance between adjacent markers across the 11 LGs was 4.37 cM (Table 1).

The map of the F2BGB population contained 11 LGs (Fig. 2B) and spanned a cumulative distance of 3,195.68 cM. The length of each LG ranged from 152.64 cM (LG11) to 395.64 cM (LG4), with an average of 290.52 cM. The number of SNP markers in each LG varied from 55markers on LG8 and LG11 to 170.00 markers on LG1, with an average of 118 SNPs per LG. The average distance between adjacent markers across all the LGs was 2.52 cM (Table 2).

Quantitative trait loci for flowering time

Two LOD thresholds for significance were defined for the QTL analysis: a conventional LOD score of 3.0 and an LOD score computed from 3,000 permutations at $P = 0.05$. At the LOD threshold of 3, four QTLs (*qDFFBGA2.1, qDFFBGA5.1, qDFFBGA8.1* and *qDFFBGA9.1*) were detected for DFF in the F2BGA population (Table 3; Fig. 2a). The QTLs for DFF accounted for 7.88–11.13% of the DFF variation and had an additive effect between -4.22 and 2.02 (Table 3). The QTL with the largest effect for this trait was *qDFFBGA8.1* located on LG8, while *qDFFBGA9.1* on LG9 showed a comparable effect.

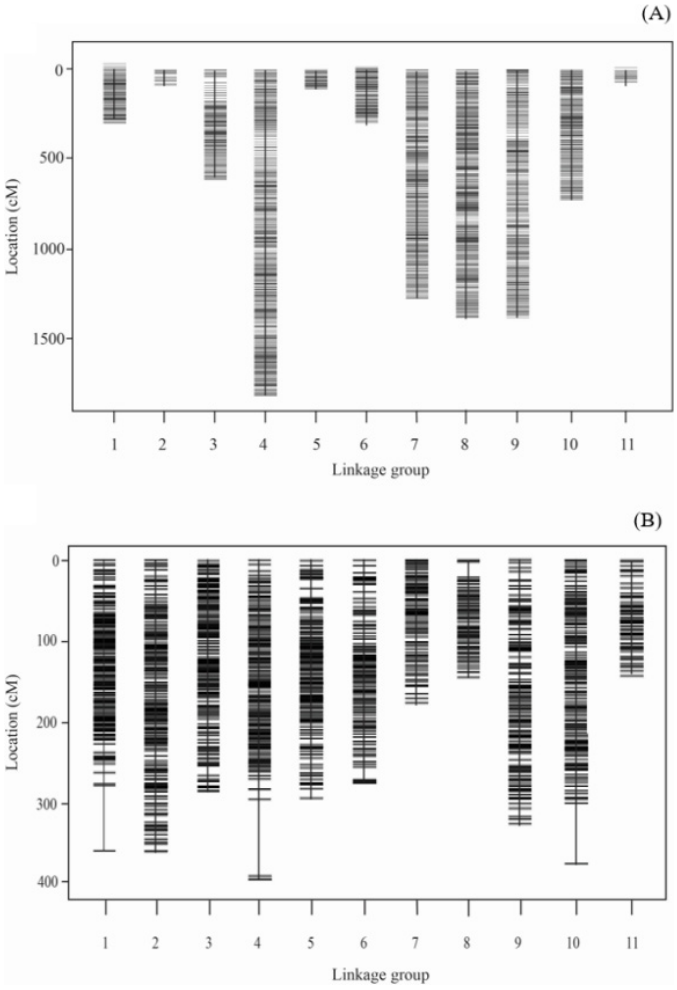

Fig. 2 High-density linkage map of black gram constructed from black gram F_2 population derived from crosses: (A) CN80 and PI213017 (F2BGA) composed of 2,030 single nucleotide polymorphism (SNP) markers; (B) CN80 and TVNu 1076 (F2BGB) composed of 3,675 SNP markers generated from specific-locus amplified fragment sequencing

Table 1 Characteristics of high-density linkage map of black gram (*Vigna mungo*) constructed from F_2 population (F2BGA) derived from cross between CN80 and PI213017 using single nucleotide polymorphism (SNP) markers

Linkage group	Length (cM)	Number of SNP markers	Average distance between adjacent markers (cM)
LG1	345.44	98.00	3.52
LG2	96.99	14.00	6.93
LG3	619.14	136.00	4.55
LG4	1788.37	436.00	4.10
LG5	122.24	33.00	3.70
LG6	323.36	107.00	3.02
LG7	1263.87	298.00	4.24
LG8	1366.56	388.00	3.52
LG9	1369.07	307.00	4.46
LG10	730.17	198.00	3.69
LG11	94.58	15.00	6.31
Total	8119.79	2030.00	-
Average	738.16	184.55	4.37

Table 2 Characteristics of high-density linkage map of black gram (*Vigna mungo*) constructed from F2 (F2BGB) population derived from cross between CN80 and TVNu 1076 using single nucleotide polymorphism (SNP) markers

Linkage group	Length (cM)	Number of SNP markers	Average distance between adjacent markers (cM)
LG1	362.7	170	2.1
LG2	363.0	163	2.2
LG3	291.0	148	2.0
LG4	395.6	146	2.7
LG5	299.7	132	2.3
LG6	280.9	99	2.8
LG7	186.3	76	2.5
LG8	154.2	55	2.8
LG9	331.6	121	2.7
LG10	378.1	133	2.8
LG11	152.6	55	2.8
Total	3,195.7	1,298	-
Average	290.5	118	2.5

Table 3 Quantitative trait loci (QTLs) for DFF in F2BGA and F2BGB population by minimum logarithm of odds (LOD) threshold value = 3, where QTLs in bold font also passed the 1000 permutation threshold

Trait	Population	LG	QTL name	Position (cM)	LeftMarker	RightMarker	LOD score	PVE (%)	Add
Days to first flowering	F2BGA	2	*qDFFBGA2.1*	81.00	Chr02_39620129	Chr02_34622176	3.02	8.93	2.02
		5	*qDFFBGA5.1*	11.00	Chr05_41801356	Chr05_41840399	3.61	7.88	1.22
		8	*qDFFBGA8.1*	557.00	Chr08_33285660	Chr08_33583977	4.07	11.13	-4.22
		9	***qDFFBGA9.1***	**762.00**	**Chr09_7970566**	**Chr09_7495513**	**4.45**	**10.89**	**-3.94**
	F2BGB	**2**	***qDFFBGB2.1***	**114.00**	**Chr02_20493401**	**Chr02_20124343**	**21.55**	**38.02**	**4.16**
		2	*qDFFBGB2.2*	220.00	Chr02_28897593	Chr02_29511196	3.75	4.22	0.06
		4	*qDFFBGB4.1*	44.00	Chr04_7403791	Chr04_8773861	3.01	3.36	-1.23
		6	***qDFFBGB6.1***	**221.00**	**Chr06_9965056**	**Chr06_9146622**	**5.98**	**7.12**	**1.89**
		8	*qDFFBGB8.1*	123.00	Chr08_5221782	Chr08_5084506	3.44	3.85	-1.56
		8	*qDFFBGB8.2*	146.00	Chr08_3060152	Chr08_2070604	3.71	4.41	0.05
		9	***qDFFBGB9.1***	**219.00**	**Scaffold_6909_ HRSCAF_7970**	**Chr09_13263324**	13.72	19.42	-3.33

LG = linkage group; PVE = *phenotypic variance* explained

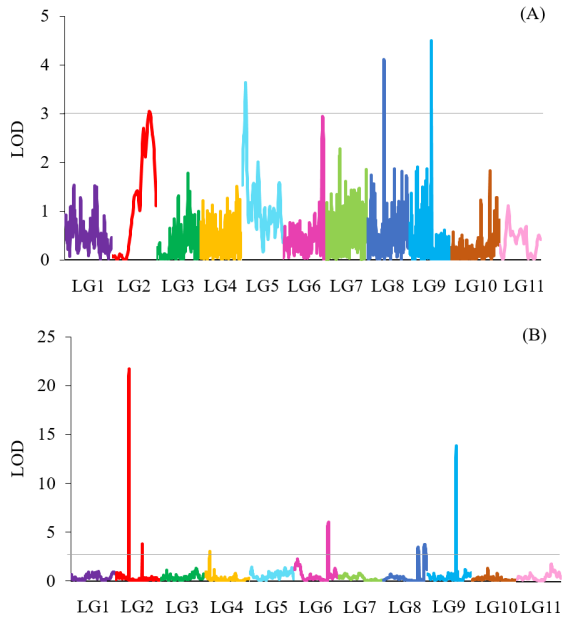

Fig. 3 Logarithm of odds (LOD) graphs of quantitative trait loci for DFF traits based on inclusive composite interval mapping method in F_2 population developed from crosses: (A) CN80 and PI213017 (F2BGA); (B) CN80 and TVNu 1076 (F2BGB), where x axis indicates the linkage group (LG), y axis indicates LOD scores and line horizontal to y axis indicates LOD significance threshold = 3

Seven QTLs were identified for DFF in the F2BGB population (Table 3; Fig. 2B). The QTLs for DFF explained 3.44–38.02% of the DFF variation and had an additive effect between -3.33 and 4.16 (Table 3). The QTL with the largest effect for DFF was *qDFFBGB2.1* on LG2 that explained 38.02% of the phenotypic variation. The QTL *qDFFBGB9.1* on LG9 also had a large effect for this trait, explaining 19.42% of the trait variation.

When the LOD scores based on permutations were applied for QTL analysis, only one QTL, *qDFFBGA9.1*, was identified for DFF in the F2BGA population, whereas three QTLs (*qDFFBGB2.1*, *qDFFBGB6.1* and *qDFFBGB9.1*) were identified in the F2BGB population (Table 3 bold letters).

Candidate genes for flowering time

Candidate genes were identified for the QTLs *qDFFBGB2.1* and *qDFFBGB9.1* which had relatively large effects (PVE higher than 10%), based on the function of the annotated genes. The SNP markers flanking *qDFFBGB2.1* covered a genome region of 369.1 Kb. Twelve genes were annotated in this region (Table S1). Then, the *evm.TU.Chromosome_2.3837* gene encoding zinc finger protein 10 was selected as the only candidate gene at this QTL. The markers delimiting *qDFFBGB9.1* covered a genome region of 369.06 Kb and harbored 24 annotated genes. *Vmun09g001070* encoding the F-box protein SKIP14 and *Vmun09g001078* encoding the NAC domain-containing protein 37–like were considered as the candidate genes at this QTL.

Discussion

Flowering time is one of the key traits of plant domestication and plant adaptation to new environments, with cultivars with day neutral and early flowering and maturity being preferred as they can be grown in all seasons and in several cropping systems. Although flowering time is an important trait in crop production and breeding (Richards, 2006), very little is known about the genetic basis of this trait in black gram.

In the current study, continuous segregation of days to first flowering in both F2BGA and F2BGB populations (Fig. 1) confirmed a result reported by Ghafoor et al. (2001) that days to flowering in black gram is a quantitative trait. The broad-sense heritability of the days to first flowering in the current study was low (34.9%) for the population F2BGB and moderate (61.9%) for the population F2BGA. Ghafoor et al. (2001) reported that narrow-sense heritability of days to 50% flowering is high (65%). Regardless of the materials and calculation method used, these heritability values suggested that days to flowering is largely affected by environmental factors. Notably, the transgressive segregation in the F2BGA and F2BGB populations (Fig. 1) suggested that the cultivated and wild parents possessed alleles/genes that increase and decrease flowering time.

Another QTL study of flowering time in black gram (conducted based on genome-wide association using 100 accessions grown in two locations for two seasons) in India identified an unstable QTL, *Q.DtF.10*, for days to 50% flowering (Singh et al., 2022). By using mungbean as the reference genome, the authors of that study postulated *LOC106774489* encoding the PHD finger-like domain-containing enzyme 5B as the only candidate gene for the *Q.DtF.10*. In the current study, two high-density black gram genetic maps of SNP markers constructed from different parents were used to identify QTLs associated with DFF. In total, 11 QTLs were identified for DFF from the two populations, consisting of four and seven QTLs in the populations F2BGA and F2BGB, respectively (Table 1). These clearly indicated that days to flowering in black gram is a polygenic trait. The difference in the number of the QTLs for flowering detected in the current study and that in Singh et al. (2022) may be due principally to: 1) differences in the methods of QTL mapping (genome-wide association versus linkage-based QTL analysis); and 2) differences in flowering time traits (days to 50% flowering and days to first flowering).

The current study compared the locations of the QTLs for the flowering time detected in the current study with the candidate gene *LOC106774489* at the *Q.DtF.10* and found that the QTLs were located on the CN80 reference chromosomes 2, 4, 5, 6, 8 and 9, while *LOC106774489* was on the CN80 reference chromosome 7. Thus, none of the QTLs detected in the current study matched the *Q.DtF.10* identified by Singh et al. (2022). However, notably, none of the flowering QTLs were common in the F2BGA and F2BGB populations, albeit the two populations shared the same cultivated parent, CN80. This was possibly due to the F2BGA and F2BGB populations were grown in different years. However, contrasts in the wild parents of the F2BGA and F2BGB populations may have also caused the difference in the QTLs detected in these two populations.

The current finding that days to flowering in black gram was a quantitative trait and was controlled by several QTLs (Table 3) was consistent with other findings for other *Vigna* species closely related to black gram, including mungbean (Isemura et al., 2012; Kajonphol et al., 2012; Sompong et al., 2012; Somta et al., 2015; Liu et al., 2017), cowpea (Kongjaimun et al., 2012; Lo et al., 2018), azuki bean (Isemura et al., 2007; Kaga et al., 2008; Liu et al., 2016, Yamamoto et al., 2016; Li et al., 2017) and rice bean (Isemura et al., 2010).

Three genes each encoding the *zinc finger protein*, *F-box protein* and *NAC domain-containing protein* were identified as candidate genes for two QTLs having a large effect on controlling flowering time in black gram (Table S1). In *Arabidopsis thaliana*, the *zinc finger protein* (Yang et al., 2014), *F–box protein* (He et al., 2017) and *NAC domain-containing protein* (Yoo et al., 2007) have been reported to be involved in flowering time. However, none of these genes were the same as the candidate genes for flowering time previously reported for *Vigna* species, such as the *Phytochrome E, Agamous-like MADS-box, Flowering Date1* (*E1*) genes in azuki bean (Li et al., 2017; Imoto et al., 2022), *Phytochrome A* gene in mungbean (Hwang et al., 2017) and *Phytochrome E* and *TCP18* genes in cowpea (Lo et al., 2018). Due to high genome conservation among the crops in the genus *Vigna*, it was expected that some of the QTLs/genes for flowering in black gram in the current study would be the same as for other *Vigna* species. Hence, further study is necessary to clarify the difference.

In conclusion, the current study identified QTLs for days to first flowering in black gram using two F_2 populations of the cross between cultivated and wild black grams grown under different daylengths (short- and long-day conditions). In total, 11 QTLs were identified, with four for the short-day condition and seven for the long-day condition. None of the QTLs were the same for the two conditions. Genes encoding the *zinc finger protein*, *F-box protein* and *NAC domain-containing protein* were identified as candidate genes for the days to first flowering.

Conflict of Interest

The authors declare there are no conflicts of interest.

Acknowledgements

Ms. Tarika Yimram assisted with field work. Other graduate students assisted with this project. This work was supported by the Center of Excellence on Agricultural Biotechnology, Office of the Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation (AG-BIO/MHESI).

References

Amkul, K., Wang, L., Somta, P., Wang, S., Cheng, X. 2019. Construction of a high density linkage map and genome dissection of bruchid resistance in zombi pea (*Vigna vexillate* [L.] A. Rich). Sci. Rep. 9: 11719. doi.org/10.1038/s41598-019-48239-5

Choon, S.Y., Ahmad, S.H., Ding, P., Sinniah, U.R., Hamid, A.A. 2010. Morphological and chemical characteristics of black gram (*Vigna mungo* L.) sprouts produced in a modified atmosphere chamber at four seeding densities. Pertanika J. Trop. Agric. Sci. 33: 179–199.

Collard, B.C., Mackill, D.J. 2008. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Philos. Tran. R. Soc. Lond. B Biol. Sci. 363: 557–572. doi.org/10.1098/rstb.2007.2170

Ghafoor, A., Sharif, A., Ahmad, Z., Zahid, M.A., Rabbani, M.A. 2001. Genetic diversity in blackgram (*Vigna mungo* L. Hepper). Field Crops Res. 69: 183–190. doi.org/10.1016/S0378-4290(00)00141-6

He, R., Li, X., Zhong, M., et al. 2017. A photo-responsive F-box protein FOF2 regulates floral initiation by promoting FLC expression in Arabidopsis. Plant J. 91: 788–801. doi.org/10.1111/tpj.13607

Hwang, W.J., Ha, J., Lee, T., et al. 2017. A candidate flowering gene in mungbean is homologous to a soybean *Phytochrome* A gene. Euphytica 213: 79. doi.org/10.1007/s10681-017-1866-8

Imoto, Y., Yoshikawa, S., Horiuchi, Y., et al. 2022. *Flowering Date1*, a major photoperiod sensitivity gene in adzuki bean, is a soybean floral repressor *E1* ortholog. Breed. Sci. 72: 132–140. doi.org/10.1270/jsbbs.21051

Isemura, T., Kaga, A., Konishi, S., Ando, T., Tomooka, N., Han, O.K., Vaughan, D.A. 2007. Genome dissection of traits related to domestication in azuki bean (*Vigna angularis*) and comparison with other warm-season legumes. Ann. Bot. 100: 1053–1071. doi.org/10.1093/aob/mcm155

Isemura, T., Kaga, A., Tabata, S., et al. 2012. Construction of a genetic linkage map and genetic analysis of domestication related traits in mungbean (*Vigna radiata*). PLoS One 7: e41304. doi.org/10.1371/journal.pone.0041304

Isemura, T., Kaga, A., Tomooka, N., Shimizu, T., Vaughan, D.A. 2010. The genetics of domestication of rice bean, *Vigna umbellata*. Ann. Bot. 106: 927–944. doi.org/10.1093/aob/mcq188

Joshi, P.K., Rao, P.P. 2017. Global pulses scenario: Status and outlook. Ann. N.Y. Acad. 1392: 6–17.

Kaewwongwal, A., Kongjaimun, A., Somta, P., Chankaew, S., Yimram, T., Srinives, P. 2015. Genetic diversity of the black gram (*Vigna mungo* [L.] Hepper) gene pool as revealed by SSR markers. Breed. Sci. 65: 127−137. doi.org/10.1270/jsbbs.65.127

Kaga, A., Isemura, T., Tomooka, N., Vaughan, D.A. 2008. The genetics of domestication of the azuki bean (*Vigna angularis*). Genetics 178: 1013–1036. doi.org/10.1534/genetics.107.078451

Kajonphol, T., Sangsiri, C., Somta, P., Srinives, P. 2012. SSR map construction and quantitative trait loci (QTL) identification of major agronomic traits in mungbean (*Vigna radiata* [L.] Wilczek). Sabrao J. Breed. Genet. 44: 71–86.

Khine, N.A., Kundu, K.K., Malik, D.P., Devi, M. 2021. Pulses production and trade performance in India and Myanmar. Asian J. Agric. Ext. Economics Soci0l. 39: D-5479. doi: 10.18805/ag.D-5479

Kongjaimun, A., Kaga, A., Tomooka, N., Somta, P., Vaughan, D.A., Srinives, P. 2012. The genetics of domestication of yardlong bean, *Vigna unguiculata* (L.) Walp. ssp. *unguiculata* cv.-gr. *sesquipedalis*. Ann. Bot. 109: 1185–1200. doi.org/10.1093/aob/mcs048

Kosambi, D.D. 1944. The estimation of map distance from recombination values. In: Ramaswamy, R. (Ed.). D.D. Kosambi. Springer, New Delhi, India, pp. 172–175. doi.org/10.1007/978-81-322-3676-4_16

Li, H. 2018. Minimap2: Pairwise alignment for nucleotide sequences. Bioinformatics 34: 3094–3100. doi.org/10.1093/bioinformatics/bty191

Li, H., Ye, G., Wang, J. 2007. A modified algorithm for the improvement of composite interval mapping. Genetics 175: 361–374. doi.org/10.1534/genetics.106.066811

Li, Y., Yang, K., Yang, W., et al. 2017. Identification of QTL and qualitative trait loci for agronomic traits using SNP markers in the adzuki bean. Front. Plant Sci. 8: 840. doi.org/10.3389/fpls.2017.00840

Lin, X., Liu, B., James, L., Weller, J.A., Fanjiang, K. 2021. Molecular mechanisms for the photoperiodic regulation of flowering in soybean. J. Integr. Plant Biol. 63: 981–994. doi.org/10.1111/jipb.13021

Liu, C., Fan, B., Cao, Z., Su, Q., Wang, Y., Zhang, Z., Tian, J. 2016. Development of a high-density genetic linkage map and identification of flowering time QTLs in adzuki bean (*Vigna angularis*). Sci. Rep. 6: 39523. doi.org/10.1038/srep39523

Liu, C., Wu, J., Wang, L., et al. 2017. Quantitative trait locus mapping under irrigated and drought treatments based on a novel genetic linkage map in mungbean (*Vigna radiata* L.). Theor. Appl. Genet. 130: 2375–2393. doi.org/10.1007/s00122-017-2965-6

Lo, S., Muñoz-Amatriaín, M., Boukar, O., et al. 2018. Identification of QTL controlling domestication-related traits in cowpea (*Vigna unguiculata* L. Walp). Sci. Res. 8: 6261. doi.org/10.1038/s41598-018-24349-4

Lodhi, M.A., Ye, G.N., Weeden, N.F., Reisch, B.I. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol. Biol. Rep. 12: 6–13. doi.org/10.1007/BF02668658

McKenna, A., Hanna, M., Banks, E., et al. 2010. The genome analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20: 1297–1303. doi.org/10.1101/gr.107524.110

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. doi.org/10.1016/j.cj.2015.01.001

Park, H.G. 1978. Suggested Cultural Practices for Mungbean. International Cooperator's Guide. Asian Vegetable Research and Development Center. Shanhua, Taiwan.

Pootakham, W., Nawae, W., Naktang, C., et al. 2021a. A chromosome-scale assembly of the black gram (*Vigna mungo*) genome. Mol. Ecol. Resour. 21: 238–250. doi.org/10.1111/1755-0998.13243

Pootakham, W., Sonthirod, C., Naktang, C., et al. 2021b. De novo assemblies of *Luffa acutangula* and *Luffa cylindrica* genomes reveal an expansion associated with substantial accumulation of transposable elements. Mol. Ecol. Resour. 21: 212–225. doi.org/10.1111/1755-0998.13240

Richards, R.A. 2006. Physiological traits used in the breeding of new cultivars for water-scarce environments. Agric. Water Manag. 80: 197–211. doi.org/10.1016/j.agwat.2005.07.013

Sahu, B., Thakur, C.L., Markam, S.K., Nag, U.K. 2020. Performance of frontline demonstrations on productivity and profitability of black gram (*Vigna mungo*) in rainfed condition of Uttar-Bastar Kanker. Int. J. Curr. Microbiol. App. Sci. 9: 1898–1903. doi.org/10.20546/ijcmas.2020.904.224

Sompong, U., Somta, P., Raboy, V., Srinives, P. 2012. Mapping quantitative trait loci for phytic acid and phosphorus content in seed and seedlings of mungbean (*Vigna radiata* (L.) Wilczek). Breed. Sci. 62: 87–92. doi.org/10.1270/jsbbs.62.87

Somta, P., Chankaew, S., Kongjaimun, A., Srinives, P. 2015. 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). Agrivita 37: 159–168.

Somta, P., Chen, J., Yimram, T., Yundaeng, C., Yuan, X., Tomooka, N., Chen, X. 2020. QTL mapping for agronomic and adaptive traits confirmed pleiotropic effect of *mog* gene in black gram (*Vigna mungo* [L.] Hepper). Front. Genet. 11: 635. doi: 10.3389/fgene.2020.00635

Singh, L., Dhillon, G.S., Kaur, S., et al. 2022. Genome-wide association study for yield and yield-related traits in diverse blackgram panel (*Vigna mungo* L. Hepper) reveals novel putative alleles for future breeding programs. Front. Genet. 13: 849016. doi.org/10.3389/fgene.2022.849016

Van Os, H., Stam P., Visser, R.G.F., Van Eck, H.J. 2005. RECORD: A novel method for ordering loci on a genetic linkage map. Theor. Appl. Genet. 112: 30–40. doi.org/10.1007/s00122-005-0097-x

Yoo, S.Y., Kim, Y., Kim, S.Y., Lee, J.S., Ahn, J.H. 2007. Control of flowering time and cold response by a NAC-domain protein in Arabidopsis. PLoS One 2: e642. doi.org/10.1371/journal.pone.0000642

Yang, Y., Ma, C., Xu, Y., et al. 2014. A zinc finger protein regulates flowering time and abiotic stress tolerance in chrysanthemum by modulating gibberellin biosynthesis. Plant Cell. 26: 2038–2054. doi.org/10.1105/tpc.114.124867

Yamamoto, H., Horiuchi, Y., Ogura, R., Sakai, H., Sato, H., Kato, K. 2016. Identification and molecular mapping of *Flowering Date1* (*FD1*), a major photoperiod insensitivity gene in the adzuki bean (*Vigna angularis*). Plant Breed. 135: 714–720. doi.org/10.1111/pbr.12412