Agr. Nat. Resour. 57 (2023) 389–398

AGRICULTURE AND NATURAL RESOURCES

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

Research article

QTL analysis of seed weight and seed dormancy in black gram (*Vigna mungo* [L.] Hepper)

Chalinee Lomlek[a], Sunisa Suamuang[b], Kularb Laosatit[a], Sithichoke Tangphatsornruang[c], Orn-uma Tanadul[a], Prakit Somta[a,*]

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

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

[c] *National Omics Center, National Science and Technology Development Agency, Pathum Thani 12120, Thailand*

Article Info

Article history:
Received 1 April 2023
Revised 16 May 2023
Accepted 23 May 2023
Available online 30 June 2023

Keywords:
Black gram,
Quantitative trait locus (QTL),
Seed dormancy,
Seed size,
Seed weight

Abstract

Importance of the work: Black gram is a scientifically important orphan crop with little research published on the molecular breeding of this legume. Seed weight is a key trait contributing to seed yield. Seed dormancy can be exploited to reduce pre-harvest sprouting.

Objectives: To identify the quantitative trait loci (QTLs) controlling black gram seed weight and seed dormancy.

Materials & Methods: Two F_2 populations developed from a cross between cultivated (Chai Nat 80 [CN80]) and wild black gram (PI213017 or TVNu1076) were grown under field conditions. The F_2 populations were determined for seed weight and seed dormancy. Single nucleotide polymorphism-based linkage maps previously constructed for the two population were used for QTL analysis. Candidate genes were identified using the reference genome sequence of CN80.

Results: The broad-sense heritability (H^2) calculated for seed weight was 65.23–73.71%, while that for seed dormancy was 81.53–99.80%. In total, 10 QTLs on 7 linkage groups (LGs) were detected for seed weight and three QTLs on two LGs were detected for seed dormancy. Depending on the population, QTLs explained between 5.07% (*qSd100wt7.1+*) and 34.20% (*qSd100wt10.2+*) of the seed weight variation, and between 10.18% (*qSdwa6.1-*) and 43.81% (*qSdwa6.2-*) of the seed dormancy variation. Candidate genes of various functions were identified for these two traits.

Main finding: Novel QTLs and candidate genes controlling seed weight and seed dormancy were identified for black gram. One of the candidate genes, *WVD2-LIKE 4* for seed weight, appeared to be an ortholog with a candidate gene for the same trait in mungbean.

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

https://doi.org/10.34044/j.anres.2023.57.3.03

Introduction

Seed dormancy (hardseededness) together with seed/pod shattering are important adaptive trait of wild plant species growing in natural habitats. Dormancy prevents seed of wild plants from germinating in an unsuitable environment to promote successful germination, vegetative growth and reproduction of the plants, thus reducing the risk of plant death and possible species extinction in unfavorable environments, while pod shattering promotes wide dispersion of seeds, and thus supports an increased population size and habitat range (Finch-Savage and Leubner–Metzger, 2006). Plant domestication is the process whereby wild plants are changed and transformed into cultivated plants via conscious and unconscious selections practiced by humans. Seed dormancy and shattering are believed to be the first two traits subjected to selection as they are advantageous for production (cultivation and harvest). In most cereal and legume crops, domestication results in reduced or complete loss of seed dormancy and/or seed/pod shattering (Vaughan et al., 2007).

Black gram (also known as urd bean and urad bean; *Vigna mungo* var. *mungo* [L.] Hepper) is an important legume crop of Asia, mainly grown in South and Southeast Asian countries, including Afghanistan, Bangladesh, India, Pakistan, Nepal, Myanmar, the Philippines, Sri Lanka, and Thailand, whereas elsewhere, it is grown in Australia, Argentina, and Brazil for export to Asian countries (Kaewwongwal et al., 2015). The global cultivation area of black gram is more than 7 million ha, with India being the largest producer (about 5.6 million ha) and consumer, with Myanmar the second largest producer (about 1 million ha) and the biggest exporter (Khine et al., 2021). Black gram is relatively tolerant to drought stress and has a short life cycle (75–90 d), with ability to fix atmospheric nitrogen in association with soil bacteria of the genera *Rhizobium* and *Bradyrhizobium*. Therefore, the crop is popularly grown as a major component in several cropping systems, but mainly after rice, wheat and maize (Kaewwongwal et al., 2015; Khine et al., 2021). Dry seeds of black gram contain high amounts of protein and carbohydrates, being about 20–25% protein and 65–75% (Kaewwongwal et al., 2015); thus, the seeds are important sources of protein for people consuming cereal-based diets and for vegetarians. Black gram seeds 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. Sprouts produced from black gram are popularly consumed as a vegetable source of vitamins and minerals (Kaewwongwal et al., 2015).

Although non-dormant seed is a favorable trait for uniform germination and timely cultivation in modern agriculture, the loss of seed dormancy resulting in pre-harvest sprouting (vivipary) in the field caused by excessive rain or humidity, resulting in yield loss, particularly in cereal and legume crops. Early this century, the estimated annual economic loss globally due to pre-harvest sprouting was USD 1 billion (Black et al., 2006). The pre-harvest sprouting problem is believed to be exacerbated by climate change (Laosatit et al., 2022). Seed dormancy is the state in which viable seed is unable to germinate under favorable conditions within a certain period (Bewley, 1997; Finch-Savage and Leubner–Metzger, 2006). Seed dormancy is a complex adaptive trait of wild plants that is regulated by genetics and environmental factors and is partly mediated by two major plant hormones, abscisic acid and gibberellins (Finch-Savage and Leubner-Metzger, 2006). While seed dormancy is a problematic trait in crop cultivation, seed dormancy in wild species can be exploited to reduce pre-harvest sprouting through genetic improvement. In this regard, understanding the genetic basis of seed dormancy is crucial for breeding for pre-harvest sprouting in crop plants.

Since black gram is mainly grown in the wet season, pre-harvest sprouting is a major problem associated with production of this crop. About 60–70% of yield losses are due to pre-harvest sprouting in black gram (Durga and Kumar, 1997). There have been only a few reports on seed dormancy in black gram. For example, Rao and Mukherjee (1978) noted that black gram has about 3–4 mth of seed dormancy and that the initial dormant seed content of the harvested lot of the landrace cultivar T9 was as high as 90%. Tomer and Kumari (1991) reported that seed dormancy in black gram was in the range 0–76%, depending on the cultivar and environment. To date, it appears that there has been only one study on the genetics of seed dormancy in black gram. Somta et al. (2020) investigated seed dormancy (water absorption) in a recombinant inbred line (RIL) population of a cross between cultivated black gram (var. *mungo*; accession JP219132) and wild black gram (var. *silvestris*; accession TC2210). The results showed that the seed dormancy in this wild black gram was a quantitatively inherited trait with heritability of 58.9% that is controlled by a single major quantitative trait locus (QTL), *qSdwa6.1*+, located on LG6 and accounting for 20.58% of seed dormancy variation in the RIL population. However, dormancy in the wild

black gram TC2210 was not high (only about 25%). Furthermore, the *qSdwa6.1+* has not been confirmed or validated.

Seed weight (or its proxy, size) is the most important trait determining the seed yield of grain crops. In addition, seed weight affects farmer, buyer and consumer preferences. For example, in Thailand, farmers, buyers and consumers prefer black gram cultivars with large seeds, in fact, the larger the better. Hence, seed size is a major target trait in the black gram breeding program. In addition, seed weight may be associated with seed dormancy. In mungbean (*Vigna radiata* [L.] Wilczek), a closely related species to black gram, some QTLs controlling seed weight were colocalized with those regulating seed dormancy (Humphry et al., 2005). Seed weight is a polygenic trait (Humphry et al., 2005). Similar to seed dormancy, there have been only a few studies on the genetics of seed weight in black gram. For example, Ghafoor et al. (2001) reported that the narrow-sense heritability of seed weight in black gram was only 33.0%, while Somta et al. (2020) reported that the heritability of this trait was 58.9%, while also reporting that six QTLs controlled seed weight in black gram. However, those QTLs have not yet been confirmed or validated.

The current study investigated QTL mapping of the genetics of seed dormancy and seed weight traits in black gram. The QTL mapping was conducted using two intraspecific mapping populations derived from crossings between cultivated and wild black gram accessions. The objective of this study was to identify the QTLs controlling seed dormancy and seed weight in black gram.

Materials and Methods

Plant materials

Two bi-parental mapping populations (F2BGA and F2BGB) were used. The populations F2BGA and F2BGB were F_2 generation developed from the crosses Chai Nat 80 × PI213017 and Chai Nat 80 × TVNu1076, respectively, and comprised 153 and 98 individuals, respectively. Chai Nat 80 (CN80) is a popular commercial cultivar in Thailand and has been used for constructing a reference genome sequence for black gram (Pootakham et al., 2021), while both PI213017 and TVNu1076 are wild black gram (*Vigna mungo* var. *silvestris* Lukoki, Marechal & Otoul) from India. These two populations have been used to identify the QTLs controlling days-to-flowering in black gram (Suamuang et al., 2023). In brief, the populations F2BGA and F2BGB and their parents (10 plants each parent) were grown under field condition on the Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. The population F2BGA was grown during November 2020–April 2021, whereas the population F2BGB was grown during March–July 2021. In both populations, the planting distance between plants was 50 cm. At maturity, the pods and seeds of each plant were harvested. The seeds were dried in a hot-air oven at 40°C for 48 hr.

Measurement of seed weight and evaluation of seed dormancy

In both F_2 populations, 100 healthy seeds of each plant were randomly selected and weighed using a digital balance. Then, 50 seeds of each plant were subjected to a germination test following a protocol described by Laosatit et al. (2022). Briefly, the seeds were placed into a hole of a germination tray which had 35–40 holes. Then, tap water was added into each hole until the seeds were submerged. The tray was kept in a germination chamber at 25°C, with a 12 hr light and 12 hr darkness regime for 7 d. Water was added to each hole every day to keep the seeds submerged. The number of seeds of each plant that did not absorb water was counted and calculated as the percentage of seed dormancy (PDS).

Estimation of heritability

Broad-sense heritability (H^2) of 100-seed weight (100SDW) and PSD of the populations F2BGA and F2BGB were calculated using 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^2_{P1}, σ^2_{P2}, and σ^2_{F2} are the variances of CN80, PI213017 or TVNu1076 (depending on the population) and the F_2 population, respectively (Laosatit et al., 2022).

Quantitative trait locus analysis of seed weight and seed dormancy

Linkage maps have been developed for both F2BGA and F2BGB, using single nucleotide polymorphism (SNP) markers (Suamuang et al., 2023). The map for the F2BGA contained 11 linkage groups (LGs) constructed from 2,030 SNP markers. It spanned a total length of 8,119.79 centiMorgans (cM) with

an average distance between adjacent markers of 4.37 cM. The map for the F2BGB comprised 11 LGs, constructed from 1,298 SNP markers with a total map distance of 3,195.68 cM and an average distance between adjacent markers of 4.37 cM. These maps were used for QTL analysis of seed weight and seed dormancy.

QTL analysis was conducted using the inclusive composite interval mapping method (ICIM), according to Li et al. (2007) with the QTL IciMapping 4.2 software package (Meng et al., 2015). ICIM was performed at every 0.1 cM with the probability in the stepwise regression of 0.001. The significant logarithm of odds (LOD) threshold of each trait in each population was computed based on a 1,000 permutations test at a probability of 0.05.

Identification of candidate gene(s)

To identify possible candidate gene(s) for major QTLs (a QTL showing percentage of variance explained by the QTL (R^2) of 10% or higher) controlling seed weight and seed dormancy, locations of flanking markers on the CN80 reference genome (Pootakham et al., 2021) of each QTL were determined using a BLASTN search (Altschul et al., 1990). Then, annotated genes were identified that resided within the genome positions of the flanking markers. Genes having a function that may have been involved in seed weight and seed dormancy were selected and considered as candidate genes.

Results

Variation and heritability of seed weight and dormancy in F_2 populations

Seed weight and dormancy were investigated in the F_2 populations F2BGA (CN80 × PI213017) and F2BGB (CN80 × TVNu1076). The two populations were grown in different environments. CN80 and PI213017 were greatly different in seed weight, with the 100SDW values of these accessions being 7.35 g and 1.72 g, respectively. The range in the 100SDW for the population F2BGA was 2.09–6.19 g, with an average of 3.80 g. Similarly, CN80 and TVNu1076 were strikingly different in seed weight, with the 100SDW values being 6.60 g and 1.74 g, respectively. The range in the 100SDW for the population F2BGB was 2.56–5.16 g, with an average of 3.59 g. In both populations, the frequency distribution of 100SDW was continuous (Fig. 1A), indicating that 100SDW is a quantitative trait. The H^2 values calculated for 100SDW in the F_2 populations F2BGA and F2BGB were 73.71% and 65.23%, respectively.

The seed germination test revealed contrasting seed dormancy for CN80, PI213017 and TVNu1076. The PDS values in these black gram accessions were 0, 30.0 and 99.0%, respectively. The PDS range in the population F2BGA was 0–90.0% with an average of 11.96%, whereas in the population F2BGB, it was 0–100% with an average of 57.67%. In both populations, the frequency PDS distributions were continuous, but distinct (Fig. 1B). In the population F2BGA, most of the plants had low PDS values, while in the population F2BGA, each PDS class had a similar number of plants. The frequency distributions suggested that seed dormancy is a quantitative trait. However, the H^2 values calculated for PDS in both populations were high (81.53% and 99.80%, respectively).

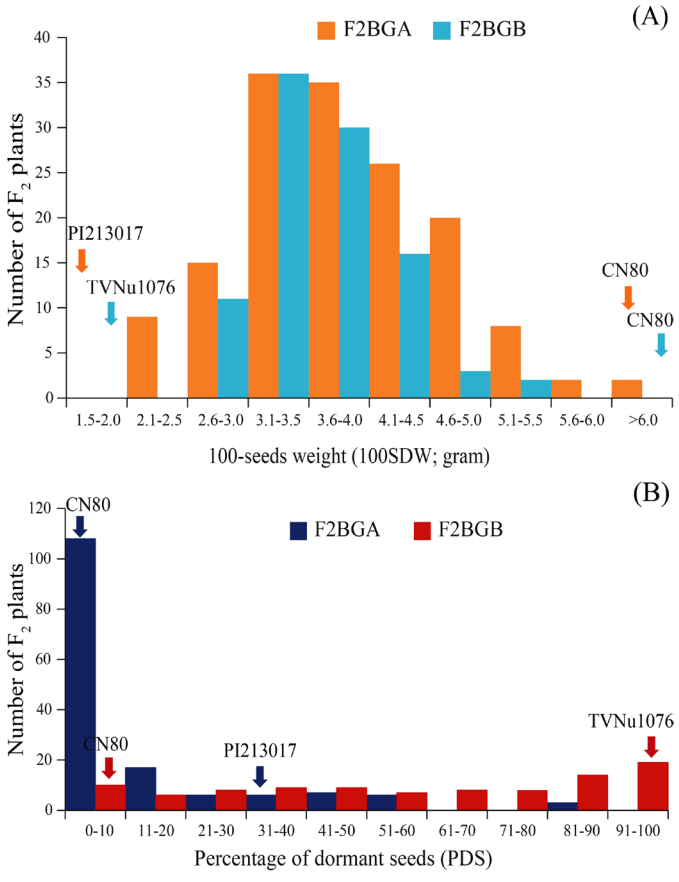

Fig. 1 Frequency distribution of: (A) 100-seed weight and (B) percentage of dormant seeds of the F_2 populations F2BGA (CN80 × PI213017) and F2BGB (CN80 × TVNu1076)

Correlation between seed weight and seed dormancy

Correlation between 100SDW and PDS in the F2BGA was low and not significant (r = -0.13 and p = 0.1135) (Fig. 2A), while that in the F2BGB was moderate and significant (r = -0.37 and p = 0.0002) (Fig. 2B).

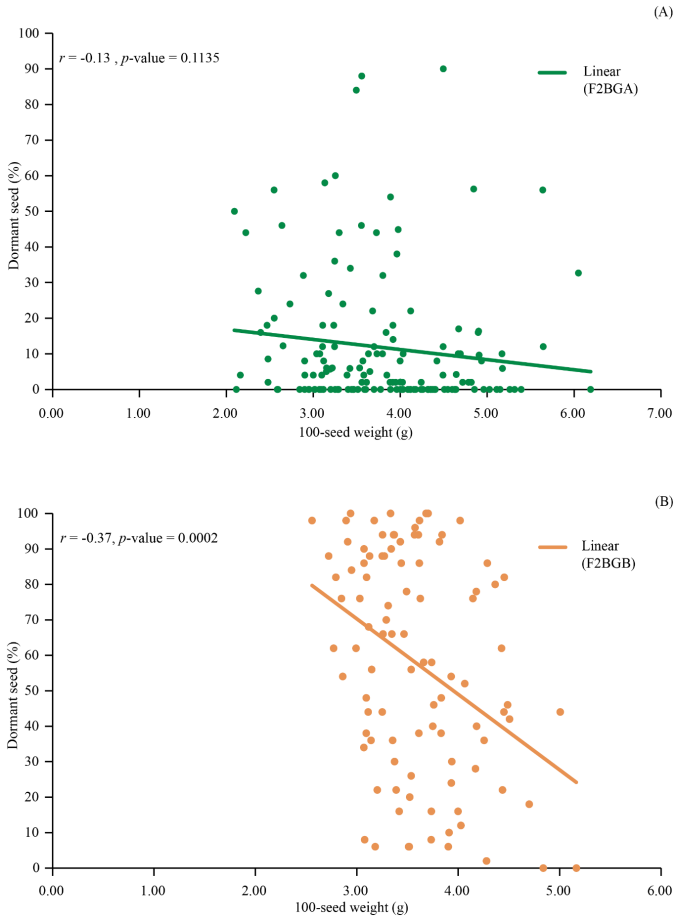

Fig. 2 Scatter plots depicting relationship between 100-seed weight and percentage of dormant seeds in the F_2 populations: (A) F2BGA (CN80 × PI213017); (B) F2BGB (CN80 × TVNu1076, where r = correlation coefficient

Quantitative trait loci for seed weight and seed dormancy

Six QTLs in different LGs were detected for 100SDW in the F2BGA population (Table 1 and Fig. 3A). The QTLs for 100SDW accounted for 5.07–23.13% of the 100SDW variation in the population and had an additive effect of 0.27–0.70 and a dominant effect of -0.21–0.09. The QTL having the largest effect for 100SDW was *qSd100wt4.1+* located on LG4. For all QTLs detected for 100SDW, alleles from CN80 enhanced seed weight.

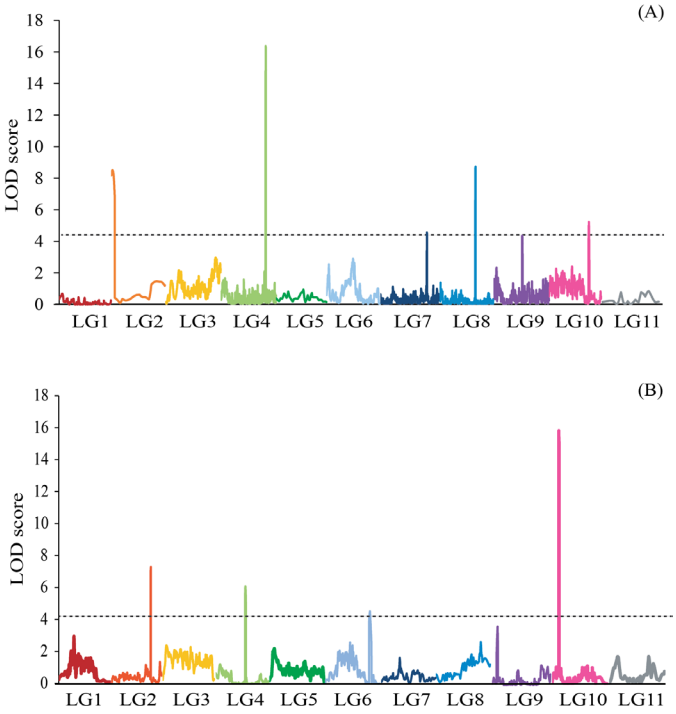

Fig. 3 Logarithm of odds (LOD) graphs of quantitative trait loci for 100-seed weight detected based on inclusive composite interval mapping method in F_2 populations: (A) F2BGA (CN80 × PI213017); (B) F2BGB (CN80 × TVNu1076), where line horizontal to y-axis indicates significant LOD threshold

Table 1 QTLs controlling 100-seed weight identified in F_2 populations F2BGA (CN80 × PI213017) and F2BGB (CN80 × TVNu1076)

Population	LG	QTL	Position (cM)	Interval markers	LOD score	PVE (%)	Additive effect	Dominant effect
F2BGA	2	*qSd100wt2.2+*	2.10	chr02_50750364–chr02_50495327	8.50	11.15	0.49	0.08
	4	*qSd100wt4.1+*	1450.09	chr04_11823009–chr04_9321404	16.41	23.13	0.70	0.09
	7	*qSd100wt7.1+*	998.63	chr07_12457684–chr07_12457757	4.55	5.07	0.27	-0.18
	8	*qSd100wt8.1+*	913.62	chr08_8010331–chr08_8204040	8.73	10.74	0.47	-0.03
	9	*qSd100wt9.1+*	703.48	chr09_8160490–chr09_8739876	4.36	5.6	0.33	-0.21
	10	*qSd100wt10.1+*	558.61	chr10_4279247–chr10_3643004	5.21	6.02	0.35	0.05
F2BGB	2	*qSd100wt2.1+*	283.31	chr02_39126642–ch02_39574548	7.30	12.85	0.28	0.08
	4	*qSd100wt4.2+*	161.30	ch04_20252643–ch04_19545636	6.12	10.81	0.23	-0.02
	6	*qSd100wt6.1+*	230.10	ch06_31215317–ch06_31091390	4.55	7.75	0.19	0.00
	10	*qSd100wt10.2+*	47.40	ch10_5511296–ch10_5176994	15.85	34.20	0.39	-0.12

LG = linkage group; LOD = logarithm of odds; PVE = percentage of variance explained by the QTL

Four QTLs were identified for 100SDW in the F2BGB population (Table 1 and Fig. 3B). The QTLs for 100SDW explained 7.75–34.20% of the 100SDW variation in this population and had an additive effect of 0.19–0.39 and a dominant effect of -0.12–0.08. The QTL with the largest effect for this was *qSd100wt10.1+* on LG10. Again, for all QTLs detected for 100SDW, alleles from CN80 enhanced seed weight.

Two QTLs, *qSdwa5.1+* on LG5 and *qSdwa6.1-* on LG6, were detected for PDS in the population F2BGA (Table 2 and Fig. 4A). These QTLs accounted for 10.59% and 10.18%, respectively, of PDS variation in the population, having additive effects of 9.72 and -10.28, respectively, and expressed dominant effects of -3.43 and -3.95, respectively. Only one QTL, *qSdwa6.2-* located on LG6, was identified for PDS in the population F2BGB (Table 2 and Fig. 4B) and it explained 43.81% of the PDS variation in the population and had an additive effect of -31.35 and a dominant effect of 3.19.

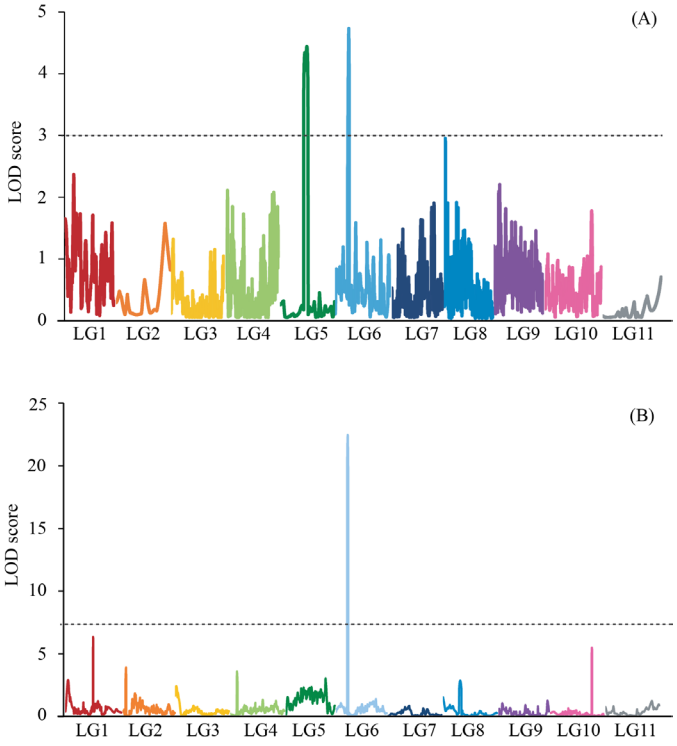

Fig. 4 Logarithm of odds (LOD) graphs of quantitative trait loci for percentage of dormant seeds detected based on inclusive composite interval mapping method in F_2 populations: (A) F2BGA (CN80 × PI213017); (B) F2BGB (CN80 × TVNu1076), where line horizontal to y-axis indicates significant LOD threshold

Candidate genes for seed weight and seed dormancy

Candidate genes were identified for QTLs with PVE ≥10%. In total, 29 genes were identified as the candidates for 9 QTLs (21 for 6 QTLs of 100SDW and 8 for 3 QTLs of PDS), as shown in Table S1. In most cases, 2–4 candidate genes were identified for each QTL, albeit 10 candidate genes were identified for the QTL *qSd100wt4.1+* and only 1 candidate gene was identified for the QTL *qSd100wt4.2+*. In both traits, candidate genes were diverse in functions.

Discussion

Black gram is a scientifically orphaned crop compared to other legume crops with similar socio-economic importance, such as mungbean and cowpea (*Vigna unguiculata* [L.] Walp.). There has been little published research on its genetics and breeding, especially regarding genomics and molecular breeding, and that research is recent. Gene/QTL mapping is the basis for genomics and molecular breeding of crop plants. To date, there have been only six reports on QTL mapping in black gram (Souframanien et al., 2010; Naito to et al., 2017; Somta et al., 2019, 2020; Singh et al., 2022; Suamuang et al., 2023). The current study identified QTLs in black gram for seed weight and dormancy. Since both the F_2 populations (F2BGA and F2BGB) used for mapping in the current study were intraspecific crosses between cultivated and wild forms, the QTLs identified for seed weight and dormancy were domesticated-related QTLs.

Seed weight is one of the most important yield determinants, as well as being one of the most useful traits for farmers, buyers and consumers regarding black gram; thus, it is an important trait in crop production and breeding. However, little is known about the genetics of the seed weight in black gram. The current study calculated the broad-sense for seed weight as moderate, being in the range 65.23–73.71%. Somta et al. (2020) reported

Table 2 QTLs controlling percentage of dormant seeds identified in F_2 populations F2BGA (CN80 × PI213017) and F2BGB (CN80 × TVNu1076)

Population	LG	QTL	Position (cM)	Interval markers	LOD score	PVE (%)	Additive effect	Dominant effect
F2BGA	5	*qSdwa5.1+*	59.60	chr05_42759472–chr05_43096765	4.38	10.59	9.72	-3.43
	6	*qSdwa6.1-*	80.30	chr06_919096–chr06_1356827	4.68	10.18	-10.28	-3.95
F2BGB	6	*qSdwa6.2-*	64.70	chr06_9408783–chr06_9965056	22.35	43.81	-31.35	3.19

LG = linkage group; LOD = logarithm of odds; PVE = percentage of variance explained by the QTL

that the narrow-sense heritability of this trait was 58.9%. These results suggested that both genetic and environment factors are important in determining seed weight. In total, 10 different QTLs (six in population F2BGA and four in F2BGB) were identified for seed weight in the current study (Table 1). For all of the 10 QTLs, alleles from the cultivated black gram (CN80) enhanced seed weight (Table 1). These results were consistent with those reported by Somta et al. (2020), who identified six QTLs controlling seed weight in an RIL population of the cross between cultivated and wild black grams, with all the positive alleles being from the cultivated black gram. However, the number of QTLs identified in the current study and in the study by Somta et al. (2020) were less than in the study by Singh et al. (2022) who identified three QTLs in a set of 100 black gram accessions grown in two environments through GWAS. The contrasting results in the number of QTLs identified by these two studies was possibly due to the method used in identifying the genome regions associated with the traits. Notably, in the study by Singh et al. (2022) a draft genome sequence of mungbean was used to identify the candidate genes, while the current study used the genome sequence of black gram CN80.

Among the candidate genes identified for seed weight (size), five genes encoded for pentatricopeptide repeat (RPP)-containing proteins, four for MYB transcription factors (TFs), three for E3 ubiquitin-protein ligase and two for WRKY TFs. Singh et al. (2022) reported that an *RPP* gene was a candidate for seed weight in black gram. Maize mutants with a defect *RPP* gene had reduced seed size (Dai et al., 2018; Ren et al., 2019b). Li et al. (2021) reported a *PPR* gene was associated with the increased seed size/weight of peanut (*Arachis hypogaea* L.). In the current study, an *RPP* gene was one of the two candidates at *qSd100wt10.2+* which was the QTL having the largest effect on controlling seed weight in the population F2BGB. MYBs are involved in many regulatory processes, developmental processes and responses to biotic and abiotic stresses (Li et al., 2019a). E3 ubiquitin-protein ligase (E3s) are a large and diverse group of proteins that play key roles in many of biological processes (Shu et al., 2017). Zhang et al. (2013) reported that MYB56 controlled seed size in *Arabidopsis thaliana* (L.) Heynh. E3 ubiquitin-protein ligase (E3s) is a large protein family whose members are involved in regulation of many biological processes. For example, studies have revealed that some E3s regulate several other proteins that control seed size (reviewed in Li et al., 2019b; Linden and Callis, 2020). For example, Song et al. (2007) showed that gene encoding RING-type E3 ligase was responsible for grain size and weight in rice (*Oryza sativa* L.) at the QTL *GRAIN WIDTH 2*. WRKYs are one of the largest transcription factor (TF) families in land plants. Some *WRKY* genes have been shown to be involved in regulating seed size (Luo et al., 2005; Kang et al., 2013; Gu et al., 2017; Xiang et al., 2017). In the current study, *WRKY46* was one of the two candidates at *qSd100wt10.2+* which was the largest-effect QTL controlling seed weight in the population F2BGB.

Genes encoding glutathione S-transferase (GTS) U9, NAC domain-containing protein 100, calcineurin B-like protein (CBL)-interacting protein kinases (CBL-CIPK) and cyclin-D6-1 are candidates for seed weight at *qSd100wt4.1+*. Although GTS is generally known to be involved in oxidative stress metabolisms, a recent study in chick pea (*Cicer arietinum* L.) demonstrated that higher expression of GST genes may play an important role in seed development and seed size/weight determination (Ghangal et al., 2020). NAC is a large group of plant-specific TFs that have been found to regulate a number of plant functions, including seed development. Mathew et al. (2016) showed that NAC020, NAC026 and NAC023 were associated with seed weight in rice. CBL-CIPK is involved in plant growth and development and stress response. *CBL2* and *CBL3* affect seed size and embryonic development in *Arabidopsis thaliana* (Eckert et al., 2014), while *CBL3*, *CBL7*, *CIPK2*, *CIPK5* and *CIPK16* might affect seed size and embryo development in quinoa (*Chenopodium quinoa* Willd.), according to Xiaolin et al. (2022). Cyclins are regulatory subunits of cyclin-dependent kinases that control progression through the cell cycle. In *Arabidopsis thaliana*, overexpression of the cyclin gene *CYCB1;4* in transgenic plants resulted in increased seed size (Ren et al., 2019a).

Genes encoding a kinesin and a histidine phosphotransfer (HPt) protein are candidates for seed weight at *qSd100wt8.1+*. Kinesins are a superfamily of microtubular motor proteins in eukaryotes. In rice, *SRS3*, a kinesin gene, regulates seed weight and length (Kitagawa et al., 2010). Notably, a recent study in mungbean, a very closely related species to black gram, identified the gene *LOC106756462* encoding kinesin-like protein KIN-14N as a candidate gene for seed weight (Sandhu and Singh, 2021). HPt acts in multistep phosphorelay signaling pathways, including hormone signal transduction in higher plants. In *A. thaliana*, the quintuple *hpt* (*ahp*) mutant expressed increased seed size (Hutchison et al., 2006). A gene encoding AUXIN RESPONSE FACTOR 2A (ARF2A) TF was the only single candidate at *qSd100wt4.1+* which was the largest-effect QTL controlling seed weight in the population F2BGA. Auxin response factors are the core of auxin signaling;

study in *Arabidopsis* spp. showed that ARF2 was a repressor of cell division and organ growth, including the size of the seed (Schruff et al., 2006). The *evm.TU.Scaffold_3850_HRSCAF_4343.8613* gene encoding the protein WVD2-like 4 was a candidate for seed weight at the QTL *qSd100wt2.1+*. WVD2 (WAVE-DAMPENED2) is a microtubular-associated protein. Notably, *LOC106766914* encoding WVD2-like 4 was also identified as the candidate for seed weight in mungbean (Sandhu and Singh, 2021). A BLASTP search revealed that *evm.TU.Scaffold_3850_HRSCAF_4343.8613* is an ortholog of *LOC106766914*.

Genes encoding the nitrate regulatory gene 2 protein (NRG2), calcium-dependent protein kinase 26 and NDR1/HIN1-like (NHL) 13 are candidates at the QTL *qSdwa5.1+* for seed dormancy. In *A. thaliana*, NRG2 mediates nitrate signaling and interacts with and regulates key nitrate regulators such as NRT1.1 and NLP7 (Xu et al., 2016). In addition, as an important source of nitrogen for plants, nitrate is also a signal molecule that regulates several aspects of plant development. Alboresi et al. (2005) showed that conditions enhancing nitrate accumulation in *A. thaliana* plants and seeds reduced seed dormancy, with nitrate maintaining the dormancy. Calcium-dependent protein kinases (CDPKs or CPKs) are proteins that bind calcium ions and are involved in metabolism, osmosis, hormone response and stress signaling pathways. Studies by Rikiishi et al. (2021) and Anil et al. (2000) have demonstrated that CDPK was involved in seed dormancy in wheat (*Triticum aestivum* L.) and sandalwood (*Santalum album* L.), respectively. NHL plays important roles in plant responses to biotic stress; however, loss-of-function of *NHL6* reduced sensitivity to abscisic acid (ABA) and seed germination (Bao et al., 2016). A gene encoding the HVA22-like protein and two genes each producing ethylene-responsive transcription factor (ERF20 and ERF22) were candidates at the QTL *qSdwa6.1-* for seed dormancy. *HVA22* is an ABA-inducible gene that shares little homology with other ABA-responsive genes. ABA is a key hormone that promotes and maintains seed dormancy (Bewley, 1997; Finch-Savage and Leubner–Metzger, 2006). Study in barley (*Hordeum vulgare* L.) revealed that the expression of *HVA22* in seeds was correlated with seed dormancy (Shen et al., 2001). Ethylene is an important plant hormone known to inhibit ABA-induced promotion of seed dormancy (Bewley, 1997; Finch-Savage and Leubner–Metzger, 2006). In *A. thaliana*, ERF12 acts in the ethylene response pathway and negatively regulates seed dormancy by inhibiting the expression of *DELAY OF GERMINATION1* (*DOG1*), a key gene controlling dormancy (Li et al., 2019c). Sofia (2020) reported that *ERF20* may play a role in the release of dormancy and/or initiation of germination signaling in liverwort (*Marchantia polymorpha*). A gene encoding bZIP16 TF and a gene producing xyloglucan endotransglucosylase/hydrolase protein 30 were candidates at the QTL *qSdwa6.2-*, the only major QTL detected for seed dormancy in the population F2BGB. In *Arabidopsis* spp., *bZIP16* is expressed in seeds and regulates light-, GA- and ABA-responsive genes, thus affecting seed germination (Hsieh et al., 2012). Many studies have revealed that xyloglucan endotransglucosylase (XTH) functions in cell wall remodeling and plays crucial roles in many important processes during plant growth and development (see review in Ishida and Yokoyama, 2022). A recent study in grass weed *Avena fatua* (common wild oat) showed that XTH plays a role in coleorhiza reinforcement through cell wall remodeling to confer coat dormancy (Holloway et al., 2021).

In conclusion, the current study identified QTLs for seed weight and seed dormancy in two F_2 populations developed from crosses between cultivated and wild black grams. For seed weight, six QTLs were identified in one population, while four QTLs were identified in the other population. For seed dormancy, two QTLs were identified in one population, whereas only a single QTL was identified in the other population. For each trait, none of the QTLs detected in the two populations were common. Nonetheless, the major QTLs for seed weight (*qSd100wt4.1+* (PVE = 23.13%) *and qSd100wt10.2+* (PVE = 34.20%)) and the major QTL for seed dormancy (*qSdwa6.2-* (PVE = 43.81%)) detected in the current study have potential to improve seed yield and resistance to pre-harvest sprouting in black gram via marker-assisted breeding.

Conflict of Interest

The authors declare there are no conflicts of interest.

Acknowledgements

This research was supported by the NSRF via the Program Management Unit for Human Resources and Institutional Development, Research and Innovation (grant number B16F640185) and in part by a Graduate Program Scholarship from The Graduate School, Kasetsart University, Bangkok, Thailand. Miss Tarika Yimram assisted with field work.

References

Alboresi, A., Gestin, C., Leydecker, M.T., Bedu, M., Meyer, C., Truong, H.N. 2005. Nitrate, a signal relieving seed dormancy in *Arabidopsis*. Plant Cell Environ. 28: 500–512. doi.org/10.1111/j.1365-3040.2005.01292.x

Altschul, S., Gish, W., Miller, W., Myers, E., Lipman, D. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410. doi.org/10.1016/S0022-2836(05)80360-2

Anil, V.S., Harmon, A.C., Rao, K.S. 2000. Spatio-temporal accumulation and activity of calcium-dependent protein kinases during embryogenesis, seed development, and germination in sandalwood. Plant Physiol. 122: 1035–1044. doi.org/10.1104/pp.122.4.1035

Bao, Y., Song, W-M., Pan, J., et al. 2016. Overexpression of the *NDR1/HIN1-like* gene *NHL6* modifies seed germination in response to abscisic acid and abiotic stresses in *Arabidopsis*. PLoS One 11: e0148572. doi.org/10.1371/journal.pone.0148572

Bewley, J.D. 1997. Seed germination and dormancy. Plant Cell 9: 1055–1066. doi.org/10.1105/tpc.9.7.1055

Black, M., Bewley, J.D., Halmer, P. 2006. Encyclopedia of Seeds: Science, Technology and Uses. CABI. Wallingford, UK.

Dai, D., Luan, S., Chen, X., Wang, Q., Feng, Y., Zhu, C., Qi, W., Song, R. 2018. Maize *Dek37* encodes a P-type PPR protein that affects cis-splicing of mitochondrial *Nad2* intron 1 and seed development. Genetics 208: 1069–1082. doi.org/10.1534/genetics.117.300602

Durga, K.K., Kumar, S.S. 1997. Screening for pre-harvest sprouting in pulses. Legume Res. 20: 193–197.

Eckert, C., Offenborn, J.N., Heinz, T., et al. 2014. The vacuolar calcium sensors CBL2 and CBL3 affect seed size and embryonic development in *Arabidopsis thaliana*. Plant J. 78: 146–156. doi.org/10.1111/tpj.12456

Finch-Savage, W.E., Leubner-Metzger, G. 2006. Seed dormancy and the control of germination. New Phytol. 171: 501–523. doi.org/10.1111/j.1469-8137.2006.01787.x

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

Ghangal, R., Rajkumar, M.S., Garg, R., Jain, M. 2020. Genome-wide analysis of glutathione S-transferase gene family in chickpea suggests its role during seed development and abiotic stress. Mol. Biol. Rep. 47: 2749–2761. doi.org/10.1007/s11033-020-05377-8

Gu, Y., Li, W., Jiang, H., et al. 2017. Differential expression of a WRKY gene between wild and cultivated soybeans correlates to seed size. J. Exp. Bot. 68: 2717–2729. doi.org/10.1093/jxb/erx147

Holloway, T., Steinbrecher, T., Pérez, M., Seville, A., Stock, D., Nakabayashi, K., Leubner-Metzger, G. 2021. Coleorhiza-enforced seed dormancy: A novel mechanism to control germination in grasses. New Phytol. 229: 2179–2191. doi.org/10.1111/nph.16948

Hsieh, W.P., Hsieh, H.L., Wu, S.H. 2012. *Arabidopsis* bZIP16 transcription factor integrates light and hormone signaling pathways to regulate early seedling development. Plant Cell. 24: 3997–4011. doi.org/10.1105/tpc.112.105478

Humphry, M.E., Lambrides, C.J., Chapman, S.C., Aitken, E.A.B., Imrie, B.C., Lawn, R.J., Mcintyre, C.L., Liu, C.J. 2005. Relationships between hard-seededness and seed weight in mungbean (*Vigna radiata*) assessed by QTL analysis. Plant Breed. 124: 292–298. doi.org/10.1111/j.1439-0523.2005.01084.x

Hutchison, C.E., Li, J., Argueso, C., et al. 2006. The *Arabidopsis* histidine phosphotransfer proteins are redundant positive regulators of cytokinin signaling. Plant Cell 18: 3073–3087. doi.org/10.1105/tpc.106.045674

Ishida, K., Yokoyama, R. 2022. Reconsidering the function of the xyloglucan endotransglucosylase/hydrolase family. J Plant Res. 135: 145–156. doi.org/10.1007/s10265-021-01361-w

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

Kang, X., Li, W., Zhou, Y., Ni, M. 2013. A WRKY transcription factor recruits the SYG1-like protein SHB1 to activate gene expression and seed cavity enlargement. Plos Genet. 9: e1003347. doi.org/10.1371/journal.pgen.1003347

Khine, N.A., Kundu, K.K., Malik, D.P., Devi, M. 2021. Production and trade performance of blackgram (*Vigna mungo*) and greengram (*Vigna radiata*) in India and Myanmar. AJAEES 39: 231–243. doi:10.9734/ajaees/2021/v39i1030687

Kitagawa, K., Kurinami, S., Oki, K., et al. 2010. A novel kinesin 13 protein regulating rice seed length. Plant Cell Physiol. 51: 1315–1329. doi.org/10.1093/pcp/pcq092

Laosatit, K., Amkul, K., Yimram, T., Chen, J., Lin, Y., Yuan, X., Wang, L., Chen, X., Somta, P. 2022. A Class II KNOX Gene, *KNAT7-1*, regulates physical seed dormancy in mungbean [*Vigna radiata* (L.) Wilczek]. Front. Plant Sci. 13: 852373. doi: 10.3389/fpls.2022.852373

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, J., Han, G., Sun, C., Sui, N. 2019a._Research advances of MYB transcription factors in plant stress resistance and breeding. Plant Signal Behav. 14: 1613131. doi.org/10.1080/15592324.2019.1613131

Li, N., Xu, R., Yunhai, Li. 2019b. Molecular networks of seed size control in plants. Annu. Rev. Plant Biol. 70: 435–463. doi.org/10.1146/annurev-arplant-050718-095851

Li, X., Chen, T., Li, Y., et al. 2019c. ETR1/RDO3 regulates seed dormancy by relieving the inhibitory effect of the ERF12-TPL complex on *DELAY OF GERMINATION1* expression. Plant Cell 31: 832–847. doi.org/10.1105/tpc.18.00449

Li, Z., Zhang, X., Zhao, K., et al. 2021. Comprehensive transcriptome analyses reveal candidate genes for variation in seed size/weight during peanut (*Arachis hypogaea* L.) domestication. Front. Plant Sci. 12: 666483. doi.org/10.3389/fpls.2021.666483

Linden, K.J., Callis, J. 2020. The ubiquitin system affects agronomic plant traits. J. Biol. Chem. 295: 13940–13955. doi.org/10.1074/jbc.REV120.011303

Luo, M., Dennis, E.S., Berger, F., Peacock, W.J., Chaudhury, A. 2005. *MINISEED3* (*MINI3*), a *WRKY* family gene, and *HAIKU2* (*IKU2*), a leucine-rich repeat (*LRR*) *KINASE* gene, are regulators of seed size in *Arabidopsis*. Proc. Natl. Acad. Sci. U.S.A. 102: 17531–17536. doi.org/10.1073/pnas.0508418102

Mathew, I.E., Das, S., Mahto, A., Agarwal, P. 2016. Three rice NAC transcription factors heteromerize and are associated with seed size. Front. Plant Sci. 7: 1638. doi.org/10.3389/fpls.2016.01638

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

Naito, N., Takahashi, Y., Chaitieng, B., et al. 2017. Multiple organ gigantism caused by mutation in *VmPPD* gene in blackgram (*Vigna mungo*). Breed. Sci. 67: 151–158. doi.org/10.1270/jsbbs.16184

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

Rao, S.P., Mukherjee, R.K. 1978. Dormancy studies in black gram (*Phaseolus mungo* L.). Biol. Plant 20: 81–85. doi.org/10.1007/BF02923264

Ren, D., Wang, X., Yang, M., Yang, L., He, G., Deng, X.W. 2019a. A new regulator of seed size control in *Arabidopsis* identified by a genome-wide association study. New Phytol. 222: 895–906. doi.org/10.1111/nph.15642

Ren, R.C., Lu, X., Zhao, Y.J., et al. 2019b. Pentatricopeptide repeat protein DEK40 is required for mitochondrial function and kernel development in maize. J. Exp. Bot. 70: 6163–6179. doi.org/10.1093/jxb/erz391

Rikiishi, K., Sugimoto, M., Maekawa, M. 2021. Transcriptomic analysis of developing seeds in a wheat (*Triticum aestivum* L.) mutant RSD32 with reduced seed dormancy. Breed. Sci. 71: 155–166. doi.org/10.1270/jsbbs.20016

Sandhu, K., Singh, A. 2021. Strategies for the utilization of the USDA mung bean germplasm collection for breeding outcomes. Crop Sci. 61: 422–442. doi.org/10.1002/csc2.20322

Schruff, M.C., Spielman, M., Tiwari, S., Adams, S., Fenby, N., Scott, R.J. 2006. The *AUXIN RESPONSE FACTOR* 2 gene of *Arabidopsis* links auxin signalling, cell division, and the size of seeds and other organs. Development 133: 251–261. doi.org/10.1242/dev.02194

Shen, Q., Chen, C.N., Brands, A., Pan, S.M., Ho, T.H. 2001. The stress- and abscisic acid-induced barley gene *HVA22*: Developmental regulation and homologues in diverse organisms. Plant Mol. Biol. 45: 327–340. doi.org/10.1023/A:1006460231978

Shu, K., Yang, W. 2017. E3 ubiquitin ligases: Ubiquitous actors in plant development and abiotic stress responses. Plant Cell Physiol. 58: 1461–1476. doi.org/ 10.1093/pcp/pcx071

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

Sofia, D. 2020. The functions of ethylene-signaling in the regulation of gemma dormancy and germination in the liverwort *Marchantia polymorpha*. M.Sc. thesis, Faculty of Science and Technology, Uppsala University. Sweden.

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.org/10.3389/fgene.2020.00635

Somta, P., Chen, J., Yundaeng, C., Yuan, X., Yimram, T., Tomooka, N., Chen, X. 2019. Development of an SNP-based high-density linkage map and QTL analysis for bruchid (*Callosobruchus maculatus* F.) resistance in black gram (*Vigna mungo* (L.) Hepper). Sci. Rep. 9: 3930. doi.org/10.1038/s41598-019-40669-5

Song, X.-J., Huang, W., Shi, M., Zhu, M.Z., Lin, H.X. 2007. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat. Genet. 39: 623–630. doi.org/10.1038/ng2014

Souframanien, J., Gupta, S.K., Gopalakrishna, T. 2010. Identification of quantitative trait loci for bruchid (*Callosobruchus maculatus*) resistance in black gram [*Vigna mungo* (L.) Hepper]. Euphytica 176: 349–356. doi.org/10.1007/s10681-010-0210-3

Suamuang, S., Lomlek, C., Kongkachana, W., Tangphatsornruang, S., Laosatit, K., Tanadul, O., Somta, P. 2023. Identification of quantitative trait loci controlling flowering time in black gram (*Vigna mungo* [L.] Hepper). Agr. Nat. Resour. 57: 43–50. doi.org/10.34044/j.anres.2023.57.1.05

Tomer, R.P.S., Kumari, P. 1991. Hard seed studies in black gram (*Vigna mungo* L.). Seed Sci. Technol. 19: 51–56.

Vaughan, D.A., Balázs, E., Heslop-Harrison, J.S. 2007. From crop domestication to super-domestication. Ann. Bot. 100: 893–901. doi.org/10.1093/aob/mcm224

Xiang, J., Tang, S., Zhi, H., Jia, G., Wang, H., Diao, X. 2017. Loose Panicle1 encoding a novel WRKY transcription factor, regulates panicle development, stem elongation, and seed size in foxtail millet [*Setaria italica* (L.) P. Beauv.]. PLoS One 12: e0178730. doi.org/10.1371/journal.pone.0178730

Xiaolin, Z., Baoqiang, W., Xian, W., Xiaohong, W. 2022. Identification of the CIPK-CBL family gene and functional characterization of *CqCIPK14* gene under drought stress in quinoa. BMC Genomics 23: 447. doi.org/10.1186/s12864-022-08683-6

Xu, N., Wang, R., Zhao, L., et al. 2016. The Arabidopsis NRG2 protein mediates nitrate signaling and interacts with and regulates key nitrate regulators. Plant Cell 28: 485–504. doi.org/10.1105/tpc.15.00567

Zhang, Y., Liang, W., Shi, J., Xu, J., Zhang, D. 2013. MYB56 encoding a R2R3 MYB transcription factor regulates seed size in *Arabidopsis thaliana*. J. Integr. Plant Biol. 55: 1166–1178. doi.org/10.1111/jipb.12094