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Research Paper

Mapping of Quantitative Trait Loci (QTLs) for seed weight in blackgram (*Vigna mungo* (L.) Hepper)

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ABSTRACT

The present study was conducted to identify quantitative trait loci (QTLs) governing hundred seed weight in blackgram using 184 recombinant inbred lines derived from the inter-specific cross VBN (Bg) 5 and *Vigna mungo* var. *silvestris* 22/10. The RIL population developed through single seed descent method was grown in three different seasons and the mature dried seeds were taken for observing hundred seed weight. The analysis of variance revealed significant difference for genotype, environment and genotype × environment interaction suggesting the influence of environment over genotypes. In each of the three seasonsand in pooled data, one QTL (*qhswk 1.1*, *qhswr 1.1*, *qhsws 1.1* and *qhswp 1.1*, respectively) was identified which was flanked by the unique markers CEDG 097 and CEDG 198 at LOD ranged from 3.00 to 3.36. The phenotypic variation ranged from 5.68 to 7.54 per cent. The QTL was found to be inconsistent across the seasons. Hence, this QTL could be used to study the interaction between QTL and environmental factors to identify environment-specific QTL. The identified QTL could be further utilized in the genetic enhancement of seed weight in blackgram by increasing the number of associated markers.

Key words: Blackgram, Wild progenitor, Seed weight, QTL

INTRODUCTION

Blackgram (*Vigna mungo* (L.) Hepper) is a self-pollinated diploid legume with chromosome number 2n=2x=22 and a genome size of 574 Mbp (Arumuganathan and Earle 1991). In India, the cultivation of blackgram covers an area of 4.63 million ha with a production of 2.78 million tonnes and productivity of 600 kg/ha (Agricultural Statistics Division of DES, Ministry of Agriculture and Farmers Welfare, 2023-2024) and accounts for fourth largest production of blackgram in the world. It consists of protein (25 – 26%), carbohydrates (60%), fat (1.5%), vitamins, minerals and amino acids (Jegadeesan *et al*. 2021). The requirement for protein source is increasing due to increase in population. Infestation of pests & diseases and cultivation on marginal soil under rainfed conditions affects the productivity of blackgram. As a result of low productivity, the demand for blackgram as a nutritional supplement has been increasing. Seed weight plays an important role in influencing seedling growth, crop stand and yield. Seed weight is positively correlated with seed yield in blackgram (Burris *et al*. 1973). Large seed has an advantage in early germinationand establishment. Seed weight is a complex trait highly influenced by the environment and is quantitatively inherited (Novoselovic *et al.* 2004). The phenotypic selection is impossible due to the interaction effect of genotype and environment. To overcome these difficulties molecular marker technology proved to be accurate, fast and less expensive. Therefore, it is important to identify the QTL controlling seed weight which could be used for genetic improvement in blackgram.

Recent progress in molecular marker technology enabled enormous information about QTLs in different crops. Several SSR markers based linkage map have been developed in blackgram (Chaitieng *et al.* 2006, Gupta *et al.* 2008, Somta *et al.* 2019). To date there is lack of information about mapping of seed weight QTL in blackgram indicating the prerequisite for genetic dissection of seed weight locus. With the development of more advanced techniques, bulk segregant analysis (BSA) helps to find out the markers in specific genomic regions (Michelmore *et al*. 1991). The development of linkage map helps to locate several agronomically important QTLs to know the

complexity of quantitatively inherited characters. The QTL identified would be helpful for marker assisted selection for further improvement of trait.

In BSA, a subset of the population is used in this method rather than the entire population, which saves time and effort. This method was initially employed to find markers linked to disease resistance, which are often qualitative characteristics, and later it was extended to quantitative traits as well, such as drought tolerance in rice (Vikram *et al.* 2012) and QTLs for seed yield in maize (Quarrie *et al.* 1999). In BSA, the two extreme pools were formed from the DNA off springs showing phenotypic extremes. Hence, only two pools of extreme lines along with the parents are genotyped for identification of markers linked with particular trait of interest. The use of BSA eliminates the requirement to create a complete genetic map and this strategy was found to be most effective in the detection of major QTLs which are worthy enough to be used in marker assisted selection.

MATERIALS AND METHODS

Plant material

The mapping population comprising of 184 recombinant inbred lines (F_{10}) developed from the cross between VBN (Bg) 5 and a wild progenitor, *Vigna mungo* var. *silvestris* 22/10 through single seed descent method. VBN (Bg) 5 is a well-adapted, early maturing (65- 70 days) and having high seed weight (4.8 g/100 seeds) which was crossed with wild progenitor (2.5 g/100 seed) to identify the QTL region for seed weight. The parental lines along with 184 RILs were grown during *Kharif,* 2019, *Rabi,* 2019 and summer 2021 with plant to plant spacing of 30 × 10 cm and row length of 4 m distance at the Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The seeds of the plant material were planted in two replications in each season in a randomized block design. All the recommended agronomic practices were followed and the basal application of NPK was applied in the ratio of 25:50:25 kg/ha and the plants were harvested at the time of pod maturity and seeds were threshed separately. The seeds were dried and a hundred seeds of each RIL were counted in three replications in each season and weighed using a weighing balance and average weight of a hundred seeds was taken for further analysis.

DNA extraction and SSR markers

The DNA was extracted from the young leaves of ten days old seedlings using the CTAB method (Murray and Thompson 1980). The DNA concentration was checked using 0.8 percent agarose gel electrophoresis and stained using ethidium bromide. A total of 343 SSR markers specific to specific to adzukibean (Wang *et al.* 2004, Han *et al.* 2005, Chaitieng *et al.* 2006, Wang *et al.* 2009), mungbean (Kumar *et al.* 2002, Somta *et al.* 2006, Tangphatsornruang *et al.* 2009, Seehalak *et al.* 2009, Isemura *et al*. 2012), Commonbean (Blair *et al.* 2003, Blair *et al.* 2010) and cowpea (Li *et al.* 2001) were employed to identify polymorphism between the two parents. The 49 polymorphic markers obtained were used in bulk segregant analysis for map construction. The polymerase chain reaction (PCR) consisted of 11 µl which comprised 2.0 µl of 50 ng DNA, 2.0 µl of 5 µM primer, 3.0 µl of master mix and 4.0 µl of sterile water. The PCR amplification was performed using master cycler gradient PCR (Biorad) as follows: initial denaturation held at 94° C for 3 minutes, denaturation at 94° C for 45 minutes, annealing temperature at 60° C for 1 minute, extension at 72° C for 1 minute, final extension at 72° C for 10 minutes and holding at 4° C which consist totally of 35 cycles. The 10 µl of PCR product was employed for 3 percent gel electrophoresis (80 V for 3 hrs) and documented using a BIO-RAD gel documentation unit.

Bulk segregant analysis and construction of linkage map

Bulk segregant analysis is a trait-based sampling approach that requires sampling and bulking of DNA samples (Michelmore *et al*. 1991). The recombinant inbred lines which fall on both extremes were taken to constitute the seed weight bulk. The six individuals each from two extreme distributions were selected and pooled to form two bulks (B1- >5.0 g/100 seeds and B2- <4.0 g/100 seeds). An equal quantity (10 µl) of DNA from the selected RILs of respective bulk was pooled and a PCR reaction was carried out. The polymorphic markers identified between the parents were used to amplify the DNA bulk to identify markers that were polymorphic between the bulks.The polymorphic markers identified in BSA were used for genotyping of all the RILs. The markers were scored and segregation pattern of SSR markers was analyzed for 1:1 goodness of fit using the chi-square test and a linkage map was constructedusing QTL ICi mapping version 3.2.

QTL mapping

The phenotypic and genotypic data of 184 RILs were subjected to QTL analysis using the software QTL ICi mapping version 3.2 where inclusive composite interval mapping of additive and dominance QTL (ICIM-ADD) was implemented. All the markers were first grouped based on a logarithm of odds (LOD) score of 3.0. The QTLs in a targeted genomic region with LOD values greater than this threshold for each trait were chosen. The threshold LOD value was established using a permutation test at a significance level of p = 0.05. The LOD value of > 3.0 and permutation test at P < 0.05 was considered significant QTL. The markers were ordered by the SER algorithm with a window size of 5 as rippling criteria which re-estimates the recombination frequency without changing the order of the marker. The recombination frequency between the markers was transformed into centimorgan (cM) distance using the Kosambi mapping function (Kosambi 1944). The phenotypic variation (R^2) explained by the QTL was the highest probability peak of the LOD position.

Statistical analysis

In the present study, the observations taken for hundred seed weightswere subjected to statistical analysis. Analysis of variance (ANOVA) was conducted using R studio software (R core Team 2023) using "agricole" package (de Mendiburu and de Mendiburu 2019). Heritability analysis was done using the "variability" package (Singh and Chaudhary 1977). The linkage map was constructed and QTL analysis was done using QTL ICi mapping version 3.2.

RESULTS AND DISCUSSION

Phenotypic evaluation of RIL population

The mapping population comprised of 184 RILs was developed from the parents VBN (Bg) 5 and the wild proginator *Vigna mungo* var. *silvestris* 22/10 exhibited significant phenotypic variation for seed weight. The phenotypic data for hundred seed weight for each of the RILs was assessed in three different seasons separately and the combined data of all the three seasons were presented in Table 1. The average seed weight of VBN (Bg) 5 (4.80 g) was significantly higher than the *Vigna mungo* var. *silvestris* 22/10 (2.50 g). Hundred seed weight showed a significant and positive correlation with single plant yield (0.550). The frequency distribution using the higher order statistics for hundred seed weight for each season and combined data was shown in Figure 1. The frequency distribution suggested the continuous variation explains the normal distribution of the trait hundred seed weight. The skewness and kurtosis values were analysed in three seasons as well as in combined data ranged from -0.04 to 0.41 and -0.04 to 0.52, respectively. This suggested the data of hundred seed weight were relatively close to normal distribution. The heritability values for hundred seed weight for all the seasons and combined data *viz.*,90.81, 96.99, 98.68, and 97.69, respectively. The pooled analysis of variance indicated the significant difference between the genotype and the interaction between genotype and environment suggesting the presence of a wide range of variation for hundred seed weight (Table 2).

Table 1. Mean, range, standard deviation (SD), phenotypic coefficient of variation (PCV),Genotypic coefficient of variation (GCV), heritability values for hundred seed weight in RIL population derived from the cross Vamban (Bg) 5 and *Vigna mungo* var. *silvestris* 22/10.

Environment (year)	Hundred seed weight (g) in RILs							Range of single plant yield (g) (Correlation with Hundred seed weight)
	Mean (g)± SD	Range	Skewness	Kurtosis	PCV (%)	GCV (%)	Heritability (%)	
Kharif, 2019	4.23±0.52	2.84-6.03	0.43	0.19	12.60	12.00	90.81	1.12-4.77
Rabi, 2019	4.66±0.53	3.32-5.94	-0.02	-0.41	11.50	11.33	96.99	5.30-12.63
Summer, 2021	4.20±0.47	3.03-5.53	-0.01	-0.28	11.26	11.19	98.68	2.52-10.88
Pooled data	4.36±0.43	3.19-5.78	0.21	-0.01	9.94	9.82	97.69	3.50-8.17 (0.550**)

** Significant at 1 % level.

Table 2. Pooled analysis of variance for hundred seed weight in RIL population derived from the cross Vamban (Bg) 5 and *Vigna mungo* var. *silvestris* 22/10.

Sources of variation	DF	Mean sum of squares	F calculated
Season	2	23.88	802.23**
Replication within season	3	0.02	2.37**
RILs	183	1.11	88.96**
Season x RILs	366	0.21	17.38**
Pooled error	549	0.01	

** Significant at 1 per cent level

Genetic map

Among the 343 SSR markers screened, 49 markers (14.28%) were found to be polymorphic between parents VBN (Bg) 5 and *Vigna mungo* var. *Silvestris* 22/10 and out of 49 SSR markers eight markers showed polymorphism in bulk segregant analysis (Table 3). These eight markers were subjected to genotyping of the whole RIL population for the construction of a linkage map and all the

Table 3. List of parental polymorphic SSR markers screened between the parents Vamban (Bg) 5 and *Vigna mungo* var. *silvestris* 22/10.

S. No.	Marker	Forward and Reverse sequence (5′-3′)	Annealing temperature (^{0} C)
1	CEDG 006	F:AATTGCTCTCGAACCAGCTC R:GGTGTACAAGTGTGTGCAAG	60
2	CEDG 008	F: AGGCGAGGTTTCGTTTCAAG R: GCCCATATTTTTACGCCCAC	60
3	CEDG 010	F: TGGGCTACCAACTTTTCCTC R: TGAGCGACATCTTCAACACG	60
4	CEDG 014	F: GCTTGCATCACCCATGATTC R: AAGTGATACGGTCTGGTTCC	60
5	CEDG 027	F:ACTTGGGGTTTGAGATGTGG R:TCATTTTGGCCACTCAGTGC	60
6	CEDG 036	F: AGCGTGTTTGTGGTGATAGC R: ACACAGGAACGAACAAACCC	60
7	CEDG 050	F: GGCAGAATCGTACAAGTG R: GTCAGATTCTCGCTTGCATG	60
8	CEDG 020	F: TATCCATACCCAGCTCAAGG R: GCCATACCAAGAAAGAGG	60
9	CEDG 043	F: AGGATTGTGGTTGGTGCATG R: ACTATTTCCAACCTGCTGGG	60
10	CEDG 044	F: TCAGCAACCTTGCATTGCAG R: TTTCCCGTCACTCTTCTAGG	60
11	CEDG 048	F: TCTCTTCCTCTATGGCTTGG R: GCTCCTCTTTTTGCTGCATC	60
12	CEDG 056	F:TTCCATCTATAGGGGAAGGGAG R:GCTATGATGGAAGAGGGCATGG	60
13	CEDC 050	F: TCCCACTTCTCCATTACCTCCAC R: AGATTATCTTCTGGGCAGCAAGG	60
14	CEDG 086	F:GAGTTTACAACAGATGGGGCTAA R:AGGTCTTGATTGACTTTCTGGGT	60
15	CEDG 088	F: TCTTGTCATTTAGCACTTAGCACG R: TTGTTGTTTACTAAGAGCCCGTGT	60
16	CEDG 092	F:TCTTTTGGTTGTAGCAGGATGAAC R:TACAAGTGATATGCAACGGTTAGG	60
17	CEDG 097	F:GTAAGCCGCATCCATAATTCCA R:TGCGAAAGAGCCGTTAGTAGAA	60
18	CEDG 103	F: CACCGCTGTCCATTGAAGTATTA R: TCTTAGAGTGCCCTGTGAGATTG	60
19	CEDG 111	F: TGGAAGTTTCCAAGAGGGTTTTC R: TCTCACCACCTTTTACCTTCTCA	60
20	CEDG 115	F: GGCTCATTGTACCACTGGATAT R: ATGCCTCCTTTCAGGTGATTGT	60
21	CEDG 118	F: AACCCAACCAACCCTTGTGGTAAG R: GCTGGAATCATAATACCGCCTTGT	60
22	CEDG 127	F: GGTTAGCATCTGAGCTTCTTCGTC R: CTCCTCACTTGGTCTGAAACTC	60
23	CEDG 139	F: CAAACTTCCGATCGAAAGCGCTTG R: GTTTCTCCTCAATCTCAAGCTCCG	60
24	CEDG 143	F: GATGAACTCGTCTCGCTCATCG R: CTGGACGCGTCTACTCAGAC	60
25	CEDG 149	F: GGCTGAAGGTGATGACAGAAG R: GGCACTGGTTTTCTAAGGTTGTTG	60
26	CEDG 013	F:CGTTCGAGTTTCTTCGATCG R: ACCATCCATCCATTCGCATC	60
27	CEDG 154	F: GTCCTTGTTTTCCTCTCCATGG R: CATCAGCTGTTCAACACCCTGTG	60
28	CEDG 156	F: CGCGTATTGGTGACTAGGTATG R:CTTAGTGTTGGGTTGGTCGTAAGG	60
29	CEDG 176	F: GGTAACACGGGTTCAGATGCC R: CAAGGTGGAGGACAAGATCGG	60
30	CEDG 180	F: GGTATGGAGCAAAACAATC R: GTGCGTGAAGTTGTCTTATC	60
31	CEDG 204	F: CCTTGGTTGGAGCAGCAGC R: CACAGACACCCTCGCGATG	60
32	CEDG 214	F: CACTCACTGCAAAGAGCAAC R: CTACCTATCTGAGGGACAC	60
33	CEDG 181	F: CGCGAGATCTGGATCGTTGATC R: GCAGTACGGTAACGTCCTTGAC	60
34	CEDG 271	F:GCACTAAAGTTAGACGTGGTTC R:CACTCCCACTGCCAAACAAGG	60
35	CEDGAG001	F:CTCATCAGGGACATCCTCCC R:GATCGTGATCGATCCAACGGTC	60
36	CEDG 090	F: ATAAGTAGAAATTGGTTCAAATG R: GGTTCGTTAAAGTAACTTTTAAT	60
37	CEDG AG002	F:GCAGCAACGCACAGTTTCATGG R:GCAAAACTTTTCACCGGTACGACC	60
38	CEDG225	F: GAGGAAGTGTTGCAGCACC R: GTAGACTCTGCAGAGGGATG	60
39	CEDG 117	F: GTACACTTCCACTAATCCAAAATT R: TGGTACCTTCCTTATCTGAAATTA	60
40	CEDG 015	F : CCCGATGAACGCTAATGCTG R: CGCCAAAGGAAACGCAGAAC	60
41	CEDG 174	F: GAGGGATCTCCAAAGTTCAACGG R: GAAGGCTCCGAAGTTGAAGGTTG	60
42	CEDG 042	F: CACAGTGGTTTGGGCAACAG R: TCAGAGGTTCCCATTTCCCG	60
43	CEDG 108	F: ATCAACTGAGGAGCATCATCGA R: CAACATTTCAACCTTGGGACAG	60
44	CEDG 037	F: GAAGAAGAACCCTACCACAG R: CACCAAAAACGTTCCCTCAG	60
45	CEDG 121	F: CTTTCAAAATAATGTTGAGGCATA R: CAATACATAAATAACCTTTTCTGC	60
46	CEDG 171	F: CTTGAGAACCAACTCGAACTTC R: GGGAAATCGAAGAGGGACAG	60
47	CEDG 198	F: CAAGGAAGATGGAGAGAATC R: CCTTCTAAGAACAGTGACATG	60
48	CEDG 024	F:CATCTTCCTCACCTGCATTC R:TTTGGTGAAGATGACAGCCC	60
49	CEDG 141	F: CCAGGCATCCATGATGACC R:GAAGTTGTTGGTAATGGTTGCCTC	60

Table 4. Segregation pattern for molecular markers linked to seed weight in RIL population of cross Vamban (Bg) 5 and *Vigna mungo* var. *silvestris* 22/10

Marker	Expected segregation ratio	χ^2 value	P value
CEDG 048	1:1	0.08	0.75-0.90
CEDG 214	1:1	0.01	0.90-0.95
CEDG 014	1:1	0.27	0.50-0.75
CEDG 008	1:1	0.02	0.75-0.90
CEDG 171	1:1	1.07	0.25-0.50
CEDG 097	1:1	0.35	0.50-0.75
CEDG 198	1:1	0.30	0.50-0.75
CEDG 111	1:1	0.86	0.25-0.50

Table 5. QTLs identified for seed weight in blackgram

QTL	Year	Inter marker distance (cM)	Position (cM)	LOD	Right marker	Left marker	PVE (%)
qhswk 1.1	*Kharif*, 2019	253.48-313.12	279	3.36	CEDG 097	CEDG 198	7.54
qhswr 1.1	*Rabi*, 2019	253.48-313.12	288	3.29	CEDG 097	CEDG 198	5.72
qhsws 1.1	*Summer* 2021	253.48-313.12	289	3.21	CEDG 097	CEDG 198	5.68
qhswp 1.1	Pooled data	253.48-313.12	289	3.00	CEDG 097	CEDG 198	7.01

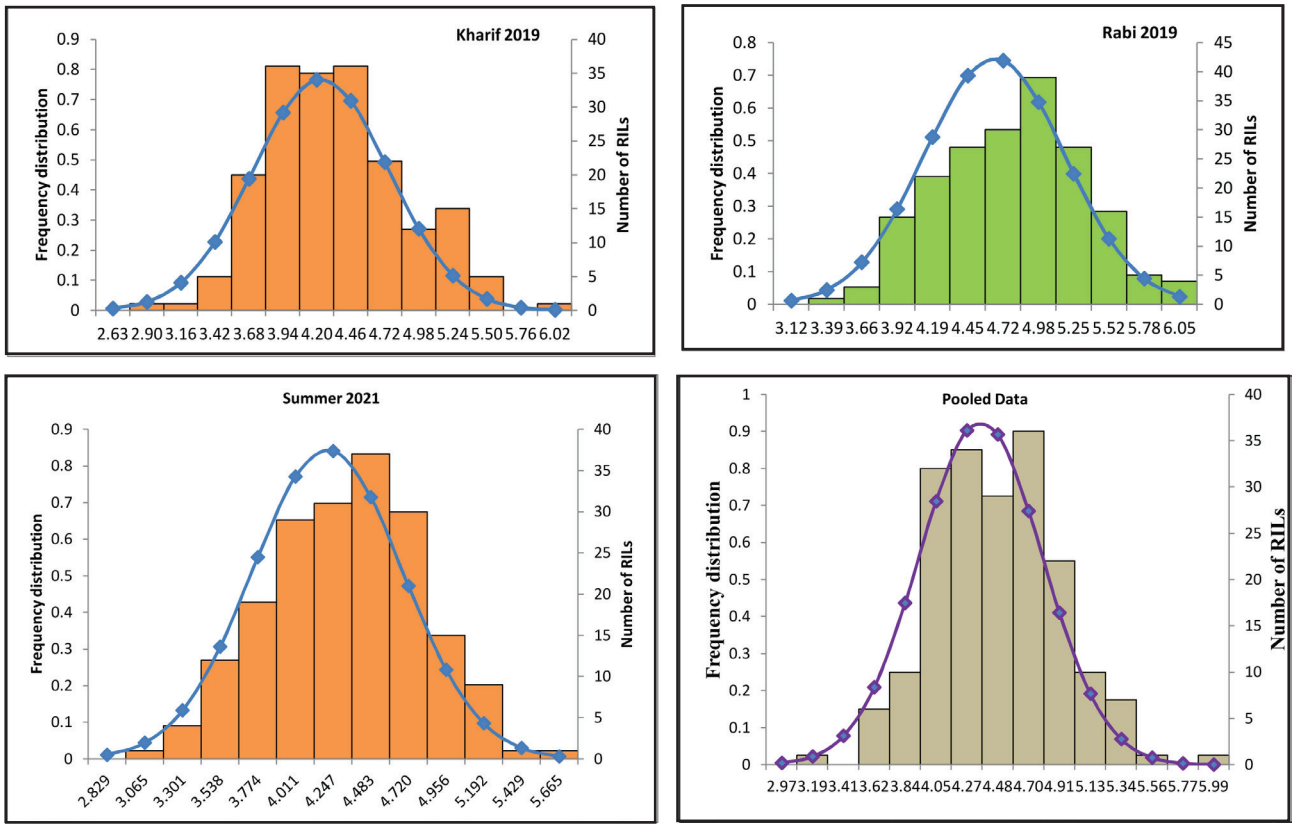

Fig. 1. Frequency distribution patterns of hundred seed weight (g/100 seeds) in a RIL population of cross Vamban (Bg) 5 and *Vigna mungo* var. *silvestris* 22/10

eight markers showed 1:1 goodness of fit through chi-square test (Table 4). The genetic map spans a distance of 366.13 cM and the average distance between the markers was 45.76 cM (Table 5).

Identification of QTL for seed weight

The QTL *qhswk 1.1*, *qhswr 1.1* and *qhsws 1.1*were identified during *Kharif* 2019, *Rabi* 2019 and summer 2022 with an LOD value of 3.36, 3.29 and 3.21, respectively (Figure 2). Each QTL explained the phenotypic variation of 7.54, 5.72 and 5.68 percent, respectively and all three QTLs were flanked by SSR markers CEDG 097 and CEDG 198. Each QTL explained positive additive effect ranged from 0.30-0.48. The location of these QTLs was 279 cM, 288 cM and 289 cM, respectively. The highest proportion of phenotypic variance was explained by the QTL *qhswk 1.1* (*kharif* 2019). QTL *qhswp 1.1*was detected at 289 cM using the pooled data which was also flanked by the marker CEDG 097 and CEDG 198 (LOD=3.00) with the phenotypic variation of 7.01 percent. The expression of the QTL studied in all three seasons as well as in pooled data was found to be perpetual.

Seed weight is directly correlated with seed yield despite its importance; the molecular and genetic basis of this trait is limited in blackgram. Several mapping populations *viz.*, backcross inbred lines, chromosome substitution lines, and near-isogenic lines have been used to study many

quantitative traits. However, recombinant inbred lines are used to locate seed-weight QTLs because of their homozygous and homogeneous nature. Currently, to locate the genes responsible for seed weight, QTL analysis was performed using 184 recombinant inbred lines derived from the cross between VBN (Bg) 5 and *Vigna mungo* var. *silvestris* 22/10. The frequency distribution identifies the type of gene action and a number of genes involved in the trait expression (Robson 1956). Skewness and kurtosis revealed near to normal distribution for seed weight indicating that this population could be further used for identification of QTLs for agronomically important traits. Bulk segregant analysis is one of the effective methods to detect QTL with minor effects that are not identified easily by conventional interval mapping (Michelmore *et al.* 1991). In the present study, 49 polymorphic SSR markers were employed in bulk segregant analysis and of which eight markers were found to be polymorphic between the extreme bulks as well as in parents. This was in accordance with the findings of Masari *et al.* (2017) listed 106 SSR markers as polymorphic among parents from the total of 1417 markers and from those polymorphic markers, only one marker exhibited polymorphism among parents and bulk of high and low content of starch in greengram. The findings are in accordance with Sachdeva *et al.* (2019) who observed 88 SSR markers from a total of 300 markers as polymorphic between parents and bulk consisting of resistant and susceptible lines of grey mould in chickpea. The polymorphic markers in BSA showed goodness of fit to 1:1 ratio through the chi-square test and were used for linkage map construction. Chaitieng *et al.* (2006) constructed a genetic linkage map of blackgram and adzukibean using 123 markers which were tested to the goodness of fit and these markers showed a 1:1 segregation pattern. Gupta *et al.* (2008) observed a segregation pattern for 428 markers and observed a 1:1 segregation pattern for only 237 markers in blackgram. The genetic linkage map obtained in this study generated a distance of 366.13 cM with inter marker interval of 45.76 cM. The large intervals might be due to the liited number of polymorphic markers (Zhang *et al.* 2014). Polymorphic markers can be identified by adding more markers, thus saturating the map. Gupta *et al.* (2008) constructed a linkage map spanning a distance of 865.1 cM with an average marker density of 2 cM in blackgram. Kajonphol *et al.* (2012) observed that the total length of the linkage map was 1174.2 cM with an average of 97.9 cM between the markers in greengram. In this study in each season as well as in pooled data one seed weight QTL (*qhswk 1.1, qhswr 1.1, qhsws 1.1* and *qhswp 1.1*) explained 5.68-7.54 percent of the total phenotypic variation were mapped on to the chromosome through bulk segregant analysis. The explained phenotypic variation was observed to be low and indicates minor QTL, this might be due to the distribution of genes in narrow regions on the linkage group (Isemura *et al.* 2012). The phenotypic

Fig. 2. Map showing SSR markers linked to hundred seed weight

variation explained by the QTL in all three seasons as well as in pooled data was low which is due to the availability of less number of polymorphic markers and found to be governed by many genes with small and cumulative effects (Maughan *et al*. 1996). The phenotypic data obtained in each season was utilized for QTL analysis separately to find out whether the same loci and flanking markers could be detected in three seasons as well as in pooled data. Therefore, each QTL in all seasons was flanked by the markers CEDG 097 and CEDG 198, which may play a crucial role in controlling blackgram seed weight. Andargie *et al*. (2011) identified six QTLs (*qsw1* (18.6 cM), *qsw 2.1* (150.2 cM), *qsw 2.2* (79.8 cM), *qsw 3.1*(167.5 cM), *qsw 3*.2 (152.7 cM) and *qsw*10 (186.0 cM)) for hundred seed weight in cowpea which explained phenotypic variation of 8.9 to 13.8 percent. Kajonphol *et al*. (2012) identified six QTLs (*Sd100wt 2.1, Sd100wt 2.2, Sd100wt 4, Sd100wt 8, Sd100wt 9* and *Sd100wt 11*) for seed weight in greengram with phenotypic variation of 7.22 to 14.56 percent. In the present study, seed weight QTL identified in each season and in pooled data was flanked by the same markers at different positions and found to be inconsistent across environments which is attributed to QTL × environment interaction. Hence, this QTL could be used to study the interaction between QTL and environmental factors to identify environment-specific QTL. This allows for the development of environment-specific MAS strategies, ensuring that the markers used are relevant to the specific growing conditions. Also the identified flanking markers along with the addition of more number of markers, which would reduce the mapping distance and could be further employed in trait improvement breeding in blackgram.The lack of polymorphic markers might be overcome by SNP markers for developing high-density linkage maps in a short period.

The study involves finding markers for traits that are phenotypically complex, provided that there is a suitable population and markers that have known positions on maps. As these QTLs are highly reproducible across different environments and genetic backgrounds, the flanking markers could be used in marker-assisted selection upon further validation for seed weight in blackgram. This preliminary study would be able to identify major QTLs controlling seed weight in blackgram and further deepen our knowledge of the genetic basis of this trait and with enrichment of more polymorphic markers would also be useful for improvement in other *Vigna* species.

AUTHORS CONTRIBUTION

PJ and MK: Planning of the experiment, Supervision and Manuscript reviewing. SM: conduct of the experiment, statistical analysis, compiling data and writing the manuscript

DECLARATION

Consent for Publication

All the authors have given their consent tothe publication

Conflict of Interest

There is no conflict of interest.

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