THEORETICAL
BIOLOGY FORUM

TBF : 64–70 (2022)
RESEARCH ARTICLE

Identification of QTL Controlling Powdery Mildew Disease Resistance in Blackgram (*Vigna mungo* (L). Hepper)

Mamata Khandappagol*, S. Rangaiah, S. K. Savita, S. Ramesh and Y. M. Somasekhara

Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore-560065, Karnataka, India

ABSTRACT

Background: *Blackgram [Vigna mungo L. Hepper] is one of the most important grain legumes with easily digestible protein and low flatulence content. Powdery mildew (PM) caused by Erysiphe polygoni DC is one of the most devastating biotic production constraints during the late Kharif season. Development PM disease-resistant cultivars are eco-friendly and economical means of mitigating losses due to PM disease. A factor, limiting breeding progress for PM resistance is confined to the cool-dry season. Hence identification of quantitative trait loci (QTL) followed by Marker-assisted selection (MAS) is useful for the genetic improvement of crops. With this background, a study was made for the identification of QTL for PM disease resistance in Blackgram.*

Methods: *Phenotyping was carried out by evaluating 180 F2:3 lines for PM disease resistance along with parents viz., TAU-1 (PM disease susceptible) and LBG-17 (PM disease resistant) and checks (K-5-572 and MASH-114) at 'K' Block, Department of Genetics and Plant Breeding, University of Agriculture Sciences, GKVK, Bengaluru during Kharif 2019 under RCBD Design. Observations were recorded five times at 7 days intervals viz., 40, 47, 54, 61, and 68 DAS using 0 (resistant) to 9 (highly susceptible) scale. A total of 395 SSR primers were used to test polymorphism between parents TAU-1 and LBG-17 and genotyping was carried out for 180 F2 individual plants of the cross TAU-1 x and LBG-17 with 63 polymorphic SSR markers. Linkage and QTL analyses were carried out using QTL IciMapping.*

Result: *A total of 63 polymorphic markers were assigned to eleven chromosomes of Blackgram with a threshold LOD of 3 covering 2726.02 cM with an average density of 43.27 cM. A major QTL for PM resistance was detected on LG 9 at 129.00 cM, flanked by the markers CEDG166 and VrCSSSR3 with an interval of 6.00 cM and explained phenotypic variance of 13.35%. It is proposed to saturate the linkage map, and confirm and validate the identified QTL for use in marker-assisted Blackgram breeding.*

Keywords: *: Blackgram, Powdery Mildew, Yield, QTL, SSR.*

ARTICLE HISTORY:
Received: 25 June 2022
Revised: 06 September 2022
Accepted: 28 October 2022
Available Online: 03 November 2022:

DOI:
https://doi.org/10.5281/zenodo.7466287

CORRESPONDING AUTHOR:
Mamata Khandappagol

E-MAIL ID:
mamatakhandappagol@gmail.com

INTRODUCTION

Blackgram [*Vigna mungo* L. Hepper] is one of the most important grain legumes with easily digestible protein and low flatulence content. It is a self-pollinated diploid (2n=2x=22) crop with a small genome size estimated to be 0.56pg/1C (574Mbp) [8]. It is widely cultivated in India, Burma, and Thailand regions of Asia. It is an ancient and well-known leguminous crop due to its nutritional quality and suitability to the cropping system. It is rich in easily digestible protein (24%), contains vitamin B_1 (0.42mg), B_2 (0.37), Niacin (2.0 mg), iron (8.7 mg), calcium (185 mg), P (345 mg), and Fat (1.2%) per 100 g dry seed. Since it serves as a cheaper source of protein for the poor, it is rightly called the poor man's meat and the plant can also be used as green manure. Blackgram crop is a mini fertilizer factory as it restores soil fertility by fixing atmospheric nitrogen and thus producing nitrogen equivalent of around 22 hectare^{-1} [20]. India is the world's largest producer as well as consumer of blackgram with the production of 24.5 lakh tonnes during 2020-21 from an area of 4.6 million hectares with a productivity of 533kg per hectare (agricoop.nic.in).

Powdery mildew caused by *Erysiphe polygoni* DC is an obligate parasite infecting urdbean leaves. PM is characterized by its greyish, powdery,

superficial growth generally occurring on younger plant material and the upper sides of leaves [2]. It is especially devastating in the cool-dry growing season. The disease usually covers the host leaf surface area reducing photosynthetic activity and attains severe form in *Kharif* season which is also the main season for blackgram cultivation, particularly when the temperature is low (20-25°C) and humidity is high (80-90 %)[18]. The disease can potentially reduce urdbean yield by 40-90 *per cent* if there is no prevention or even cause death to the plants if it occurs at the seedling stage. Grain yield losses have been reported at about 21-26.21 *per cent* [5]. Although this disease can be controlled by chemical spraying, the farmers rarely practice such control measures due to the increase in production cost. Besides, the use of pesticides can negatively affect health and the environment. Therefore, using resistant varieties is the most desirable means of managing the disease [14].

For better identification of the desired genotype in the breeding program, there is a need to identify DNA markers linked to a particular disease. DNA-based markers and linkage maps are the prerequisites for undertaking molecular breeding activities, particularly identifying and localizing important genes controlling both qualitatively and quantitatively inherited traits in a wide range of species [21].The progress of genome research in blackgram is less and the availability of specific markers are also very less so keeping all these points in view, the present study was conducted to identify SSR markers linked to powdery mildew resistance by using cross legume species/genera SSR markers and QTLs implicated in resistance to powdery mildew.

MATERIALS AND METHODS

Development of mapping population: The germplasm line TAU-1 is used as a female parent in developing the F_2 mapping population which is high yielding, popular blackgram variety grown in Karnataka, but highly susceptible to powdery mildew disease. LBG-17 is highly resistance to powdery mildew disease and was used as a male parent and crossed with TAU 1 during *Rabi* 2017 to produce F_1 seeds. The hybrid plants of cross TAU-1 × LBG-17 were confirmed using SSR markers and confirmed plants were advanced to develop F_2 mapping population during *Kharif* 2018. The F_2 seeds were planted during *Rabi* 2018 and the leaf samples from F_2 individual plants were collected 20 days after sowing, for DNA extraction. The same F_2 individuals were advanced to the next generation and the $F_{2:3}$ family progenies were raised during *Kharif* 2019 for phenotyping against powdery mildew disease.

Genotyping of F_2 data-

DNA isolation: Total genomic DNA of parental and F_2 plants was extracted from fresh young leaf tissue of 18-20 days old seedlings using the modified CTAB method [1]. The collected samples were ground in pestle and mortar with pre-warmed (65 °C) 1 µl of CTAB buffer. Extracted samples were taken into eppendorf tubes and were incubated at 65 °C for 30 minutes with intermittent mixing of tubes every 15 minutes. After bringing back tubes to room temperature, equal volume (1ml) of Phenol: chloroform: isoamyl alcohol (PCIA) (25:24:1 v/v) was added to each tube containing the sample and extraction buffer and mixed by inverting. Tubes were then centrifuged at 12000 rpm for 10 minutes. The upper aqueous phase was carefully transferred to a new sterile tube. Then equal volume and pre-chilled ethanol were added and mixed gently. Tubes were kept for 1-2 hours at -20 °C/overnight and centrifuged at 12000 rpm for 10 minutes to pellet the DNA. The supernatant was decanted and the pellet was washed with 1 ml of 70 *per cent* ethanol and then air dried completely for 2-3 hours. A quantity of 50 µl of TE buffer was added to each sample and stored overnight at 4**°C**. RNAse (5 µl) was added to each sample and kept at 37 °C for one hour or at room temperature overnight. The DNA quality and quantity were checked on 0.8% agarose gel and DNA concentration was normalized to 10 ng/ µl.

SSRs and PCR condition: The parents of the F_2 mapping population were genotyped using 395 cross-legume species/genera SSR markers. The polymerase chain reaction (PCR) mixtures, containing 2 µl of 10 ng template DNA, 1.0 µl of 10 X Taq buffer + MgCl2 (1.5 mM), 1.0 µl of dNTPs (2 mM), 1.0 µl of forward and reverse SSR primers (10 pmol/ µl), 0.15 µl of taq polymerase (5 U/ µl) and 3.85µl of sterile double distilled water. The DNA was amplified in a thermocycler under the following conditions: 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 52°C for 45 s, 72°C for

Table 1: Disease severity scale for scoring of Powdery mildew reaction (0-9 scale).

	Scale	Description	Reaction
	0	No symptoms on leaf	Immune
	1	Small scattered powdery specks covering 1 *per cent* or less area	Highly Resistance
	3	Small scattered powdery lesions covering 1-10 *per cent* of leaf area	Resistance
	5	Lesions enlarging with grey colored powdery mass covering 25 *per cent* of leaf area	Moderately Resistance
	7	Grey colored powdery growth covering 26-50 *per cent* of leaf area	Moderately Susceptible
	9	Grey colored patches of powdery growth covering 51 *per cent* or more of leaf area on leaves	Highly Susceptible

Table 2: Analysis of variance in $F_{2:3}$ mapping population for powdery mildew incidence

Sources of variance	df	PDI (%)
Replication	1	1849.82
Genotypes	179	705.63**
Error	179	21.37

*Significant at P 0.05 level ** Significant at P 0.01 level

60 s, with a final extension step of 72°C for 10 min. The PCR products were separated on 3% agarose gel and photographed using a gel documentation unit.

Phenotyping of F_{2-3} data: The material consisted of 180 F_{2-3} populations of the cross TAU-1 and LBG-17 and were evaluated for powdery mildew disease at 'K' Block, Department of Genetics and Plant Breeding, University of Agriculture Sciences, GKVK, Bengaluru during Kharif 2019. The experimental site is located at an altitude of 920 m above mean sea level, 12.97 °N latitude, and 77.59 °E longitudes. One-eighty F_{2-3} progeny families were sown using RCBD design with an inter-row spacing of 30 cm and inter-plant spacing of 10 cm along with the parents TAU-1 and LBG-17. Susceptible genotypes K-5-572 and MASH-114 were sown as disease spreader rows after every eight rows and also around the plots. The observations were recorded at 7 days intervals *viz.,* 40, 47, 54, 61, and 68 DAS, by using 0-9 phenotype disease scoring scale (Table 1) [16].

The phenotypic data on disease incidence was recorded as the percentage of plants showing disease symptoms and expressed as *per cent* disease index (PDI).

Linkage map and QTL analysis: Linkage analysis was performed using QTL Inclusive Composite Interval Mapping (IciMapping) software version 4.0 [22] based on the SSR markers genotypic data on 180 F_2's. A minimum LOD score of 3 was used to test the significance of inter-marker recombination frequencies which were converted into map distance using the Kosambi's mapping function [15]. The QTL analysis was performed

Table 3: Distribution of SSR markers on the linkage map of F_2 population of TAU-1 × LBG-17.

Linkage group (LG)	Length (cM)	No. of markers	Average inter-marker distance (cM)
1	667.66	11	60.70
2	269.23	7	38.46
3	10.74	2	5.37
4	241.94	6	40.32
5	87.88	4	21.97
6	361.15	7	51.59
7	340.27	7	48.61
8	384.03	7	54.86
9	196.91	6	32.82
10	24.73	2	12.36
11	141.47	4	35.37
Total	**2726.02**	**63**	**43.27**

Table 4: QTLs mapping of $F_{2:3}$ progeny of cross TAU-1 × LBG-17 for Powdery Mildew.

QTL name	LG	QTL position (cM)	LOD	Flanking markers (FM)		Distance between FM (cM)	PVE (%)	Genetic effect	
				Right marker	Left marker			Additive	Dominance
qPMR-1-1	1	0	4.03	CEDG023	CEDG059	1.50	2.90	0.647	2.178
qPMR-8-1	8	0	3.98	S16	CESSRDB56	7.50	2.86	-0.810	2.218
qPMR-9-1	9	129	14.81	CEDG166	VrCSSSR3	6.00	13.35	-2.236	9.029

LG-Linkage group; LOD- logarithm of the odds ratio; PVE-Per cent of variation explained

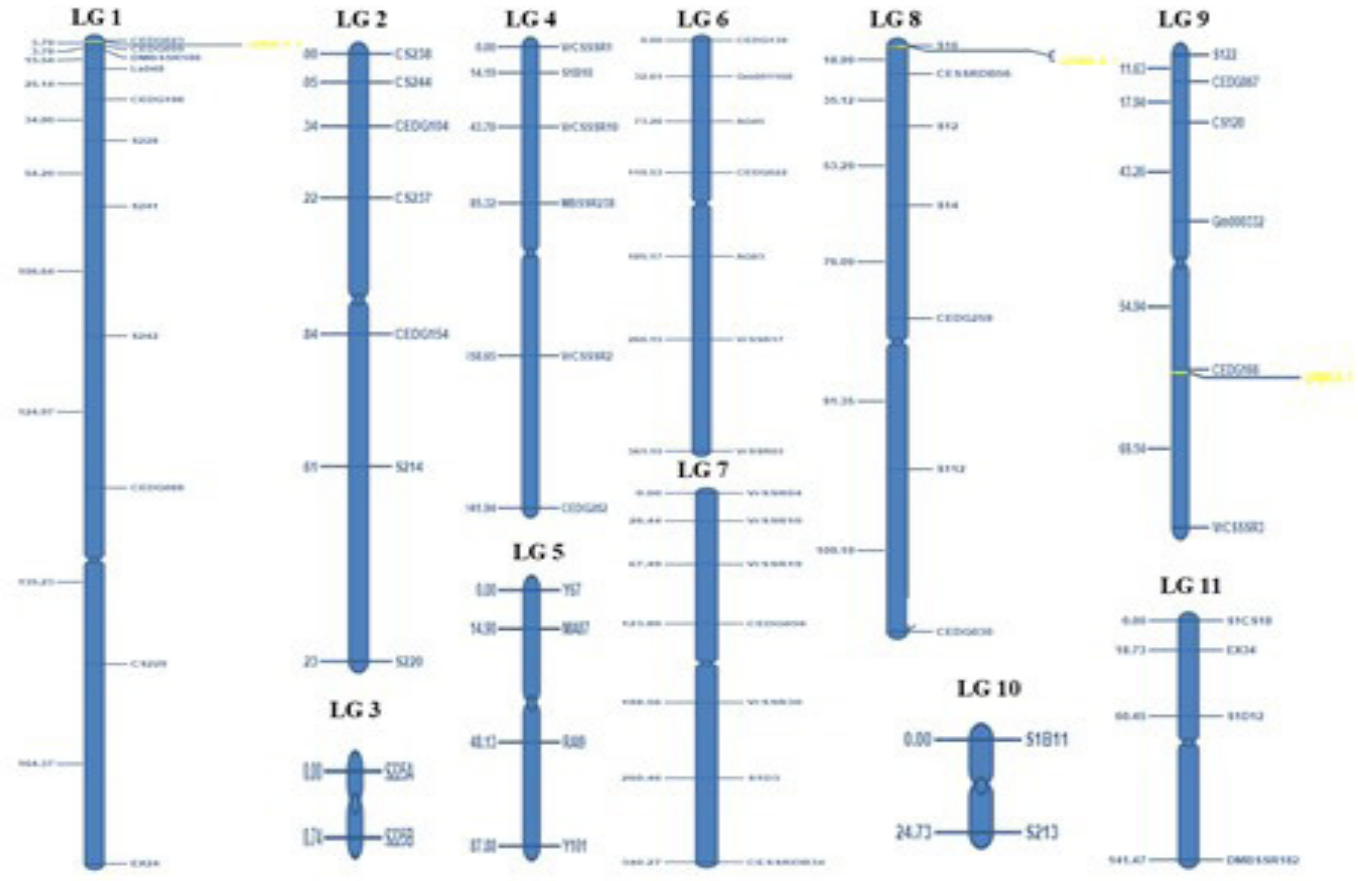

Fig 1: Linkage map of blackgram constructed with $F_{2:3}$ population of TAU-1 × LB

using QTL IciMapping (version 4.0) [22] following composite interval mapping (CIM). ICIM was performed at every 0.1 cM with the probability in stepwise regression (PIN) of 0.001. A 1,000-permutation test at P = 0.05 was carried out to determine the significant LOD threshold for QTLs for powdery mildew disease resistance.

RESULTS AND DISCUSSION

Phenotyping of $F_{2:3}$ families: In the present study, the observation recorded as the percentage of plants showing the disease symptoms are expressed in PDI and these values were transformed using arcsine transformation [7]. Analysis of variance was conducted on arcsine transformed values of the phenotypic data for individual seasons using INDOSTAT statistical software. The components of variance for the $F_{2:3}$ were computed considering all effects in the statistical model as random. Calculated F values resulting from the analysis of variance are given in Table 2. The analysis of variance revealed highly significant differences among the progenies tested indicating the presence of genetic variability in the $F_{2:3}$ progenies for powdery mildew disease.

Development of linkage map: A total of 395 cross-legume species/genera SSR markers includes 65 from Soyabean [19], 12 from *Medicago trunculata* [9], 9 from Chickpea [6], 105 from Adzuki bean [23], 134 from Mungbean [10], and 70 from cowpea (http://cowpeagenomics.med.virginia.edu/CGKB/) to test polymorphism between parents TAU-1 and LBG-17. Among these 395 SSR markers, 315 SSR markers (79.50%) were amplified and only 63 of them (20%) were polymorphic between the parents. Genotyping was carried out for 180 F_2's with 63 polymorphic SSR and linkage analysis was performed using QTL IciMapping version 4.0 [22]. Genotyping data obtained for all 63 loci were checked for segregation ratio using chi-square (^{2}) test and all the 63 markers showed the expected 3:1 segregation ratio (P <0.05) and were used to establish linkage groups (LGs). Using a minimum LOD score of 3.0 and a maximum recombination frequency of 0.30. Eleven linkage groups were established with 63 SSR markers (Fig. 1). The total length of the map spanned was 2726.02 cM with an average marker density of 43.27 cM (Table 3). The high number of markers (11) was mapped onto LG 1 and the least number of markers (2) was mapped on to LG 3 and LG 10. The averages inter marker distance ranged from 5.37 to 60.70 cM (Table 3).

QTLs for powdery mildew disease resistance: Composite Interval Mapping (CIM) was employed to locate QTL for Powdery Mildew disease resistance on the linkage map. Three QTLs, for powdery mildew, were identified with phenotypic variance explained (PVE) 2.86 *per cent* (CEDG023-CEDG059) to 13.35 *per cent* (CEDG166-VrCSSSR3). Among these one major-QTL (M-QTL) detected on LG9 flanked by markers CEDG166 and VrCSSSR3 explained greater phenotypic variance of 13.35 *per cent* with negative additive effects (-2.236) and the dominant effect of 9.029 at LOD score of 14.81 (Fig. 1 and Table. 4). The negative additive effect of -2.236, indicating favourable alleles coming from the resistant parent (LBG-17) and it is showing over dominant gene effect indicating performance of F_2 were better than both the parents for powdery mildew trait. Two minor QTLs were detected on LG1 (one-QTL), and LG8 (one-QTL) with PVE ranging from 2.90 to 2.86 *per cent*, respectively. These QTLs explain lower to moderate phenotypic variance. Hence, minor QTLs need to be validated across different genetic backgrounds and locations.

The advanced breeding line of mungbean VC3890A used as the resistance source to locate RFLP markers associated with the resistance conditioned by three QTLs each on different linkage groups. These QTLs explained between 17.4 to 28.4% of the disease variation and together accounted for a maximum of 58% of the variation [24]. By using the ATF2:3 640 as the resistant source, a single major QTL controlling the resistance was identified [13]. This locus explained as high as 86% of the variation in disease reaction. A major QTL, qPMR-2, associated with the resistance was identified and this LG accounting for 64.9% of the trait variation [3]. In our study, the QTL mapping using SSR markers revealed one major and two minor loci associated with resistance to powdery mildew disease. All the previous gene mapping studies for resistance to this disease and other traits in mungbean have been carried out using RFLP and SSR markers, while ours is the first study of gene mapping using SSR markers in blackgram for powdery mildew disease resistance. Although due to a lack of common markers available, it is difficult

to compare QTL location found in this study with the previously identified QTLs, the major QTL qPMR-3 for disease resistance on LG 9 is possibly the same major locus as reported [13]. The major QTL reported by them was located on LG K, which is equivalent to LG 9 of mungbean linkage map [17]. One RFLP marker, mgM339, was mapped onto LG K [12]. In the genetic linkage maps of adzuki bean [11] and blackgram (*Vigna mungo* (L.) Hepper) [4], the marker mgM339 was linked to SSR marker CEDG024, CEDG166, and CEDG304 on LG 9. These SSR markers were mapped onto LG9 in our study in which the major QTL qPMR-3 is located. These suggested that QTL qPMR-3 and the major QTL may be the same or at least they are linked [13].

Authors' Contribution

Dr. Mamata K performed the field experiments, measurements, data analysis, and Dr. Mamata K. and Dr. Savita K. drafted the manuscript, Dr. S. Rangaiah supervised the work, worked on the manuscript, and aided in interpreting the results, Dr. S. Ramesh and Dr. Y. M. Somasekhara were involved in planning and provided the lab facilities to carry out the molecular work and screening work for powdery mildew disease resistance. All authors provided critical feedback on the research, analysis, and manuscript.

Acknowledgement

Authors are thankful to Dr. Shailaja Hittalamani for providing lab facilities to carry out the work in the Marker Assisted Selection laboratory, Department of Genetics and Plant Breeding, UAS, GKVK, Bangalore.

REFERENCES

[1.] Agbagwa, I. O., Datta, S., Patil, P. G., Singh, P. And Nadarajan, N. (2012). A protocol for high quality genomic DNA extraction from legumes. Genetics and Molecular Research. 11(4): 4632-4639.

[2.] Agrios, G. N. (2005). Plant Pathology. 5th ed., Elsevier Academic Press, San Diego, CA. pp. 922.

[3.] Chaitieng, B., Kaga, A., Han, O. K., Wang, X., Wongkaew, S., Laosuwan, P., Tomooka, N., Vaughan, D. A. (2002). Mapping a new source of resistance to powdery mildew in mungbean. Plant Breeding. 121: 521-525.

[4.] Chaitieng, B., Kaga, A., Tomooka, N., Isemura, T., Kuroda, Y. and Vaughan, D. A., (2006). Development of a black gram [*Vigna mungo* (L.) Hepper] linkage map and its comparison with an adzuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi] linkage map. Theoretical and Applied Genetics.113: 1261-1269.

[5.] Channaveeresh, (2013). Studies on integrated management of powdery mildew of blackgram caused by *Erysiphe polygoni* DC. M.Sc.(Agri.). Thesis, University of Agricultural Sciences Dharwad. pp. 78-85.

[6.] Choudhary, S., Sethy, N. K., Shokeen, B. and Bhatia, S., (2009). Development of chickpea EST-SSR markers and analysis of allelic variation across related species. Theoretical and Applied Genetics. 118:591–608.

[7.] Gomez, K. A. and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research. 2nd ed., New York: John Wiley & Sons.

[8.] Gupta, S. K., Souframanien, J. and Gopalakrishna, T. (2008). Construction of a genetic linkage map of blackgram (*Vigna mungo* L. Hepper), based on molecular markers and comparative studies. Genome. 51(8): 628-637.

[9.] Gupta, S. and Prasad, M. (2009). Development and characterization of genic SSR markers in *Medicago truncatula* and their transferability in leguminous and non-leguminous species. Genome, 52(9):761-71.

[10.] Gupta, S. K., Bansal, R., Vaidya, U. J. and Gopalakrishna, T. (2012). Development of EST-derived microsatellite markers in mungbean [*Vigna radiata* (L.) Wilczek] and their transferability to other *Vigna* species. Indian Journal of Genetics. 72(4): 468-471.

[11.] Han, O. K., Kaga, A., Isemara, T., Wang, X. W., Tomooka, N. and Vaughan D. A. (2005). A genetic linkage map for adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi]. Theoretical and Applied Genetics. 111: 1278-1287.

[12.] Humphry, M. E., Konduri, V., Lambrides, C. J., Magner, T., Mcintyre, C. L., Aitken, E. A. B. and Liu, C. J. (2002). Development of a mungbean (*Vigna radiata*) RFLP linkage map and its comparison with lablab (*Lablab purpureus*) reveals a high level of colinearity between the two genomes. Theoretical and Applied

Genetics.105:160–166.

[13.] Humphry, M. E., Magner, T., Mcintyre, C. L., Aitken, E, A. and Liu, C. L. (2003) Identification of major locus conferring resistance to powdery mildew (*Erysiphe polygoni* D.C.) in mungbean (*Vigna radiate* L. Wiczek) by QTL analysis. Genome. 46: 738-744.

[14.] Kasettranan, W., Somta, P. and Srinives, P. (2010). Mapping of quantitative trait loci controlling powdery mildew resistance in urdbean (*Vigna mungo*L. Hepper). Journal of Crop Science and Biotechnology. 13(3): 155-161.

[15.] Kosambi, D.D. (1944). The estimation of map distances from recombination values. Annals of Eugenics. 12:172-175.

[16.] Mayee, C. D. and Datar, V. V. (1986). Phytopathometry, Marathwada Agricultural University, Parbhani, Technical Bull. 1: 145-146.

[17.] Menancio-hautea, D., Kumar, L., Danesh, D. and Young, N., (1993). A genome map for mungbean (*Vigna radiata* (L.) Wilczek) (2n=2x=22) base on DNA genetic marker. In Genetic Maps 1992: A Compilation of Linkage and Restriction Maps of Genetically Studied Organisms, Cold Spring Harbor Laboratory Press, New York pp 259-261.

[18.] Patil, N. K., Adiver, S. S., Hiremath, P. C., Anilkumar, T. P. and Hegde, R. K. (1989). Field reaction of greengram varieties to powdery mildew. Current Research, UAS, Bangaluru. 18(6): 83.

[19.] Peakall, R., Gilmore, S., Keys, W., Morgante, M. and Rafalski, A. (1998). Cross-species amplification of soybean (*Glycine max*) Simple Sequence Repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. Molecular Biology and Evolution. 15(10): 1275–1287.

[20.] Rachie, K. O. and Roberts, L. M. (1974). Grain legumes of low land tropics. Advances in Agronomy, 26: 1-132.

[21.] Varshney, R. K., Hoisington, D. A. and Tyagi, A. K. (2006). Advances in cereal genomics and applications in crop breeding. Trends in Biotechnology. 24:490–499.

[22.] Wang, J., Li, H., Zhang, L. and Meng, L. (2016). Users' Manual of QTL IciMapping. The Quantitative Genetics Group, Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China and Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico.

[23.] Wang, L. X., Cheng, X. Z., Wang, S. H., Liang, H., Zhao, D., Xu, N. (2009). Genetic diversity of adzuki bean germplasm resources revealed by SSR markers, Acta Agronomica Sinica. 35 1858–186.

[24.] Young, N. D., Danesh, D., Menancio-Hautea, D. and Kumar, L. (1993). Mapping oligogenic resistance to powdery in mungbean with RFLPs. Theoretical and Applied Genetics. 87(1-2): 243-249.