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Quantitative Trait Loci (QTL) Detection and Validation of Linked Markers Associated with Yellow Mosaic Virus Resistance in Urdbean [*Vigna mungo* (L.) Hepper] in Andhra Pradesh

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ABSTRACT

Background: The YMD is one of the serious viral diseases in blackgram and is being transmitted by whitefly (*Bemisia tabaci*). The development of YMV resistant blackgram varieties is one of the important aspects for sustainable blackgram production. Marker assisted selection and genetic transformation could be utilized in developing YMV resistant blackgram genotypes.

Methods: We attempted to map the QTLs governing YMV resistance in blackgram employing parents *i.e.*, PBG32 (susceptible) and PU31 (resistant). Parental polymorphic studies were conducted using 147 SSR markers and genotyping and phenotyping data were used for QTL mapping. The markers, CEDG097 and CEDG172 that flanked to *qYMV1* QTL were validated in 25 blackgram genotypes.

Result: SSR markers showed 16% polymorphism between the parents. By using F_2 genotypic data, genetic linkage map was constructed and total genetic map length observed was 335.47 cm. Single QTL (*qYMV1*) for YMV tolerance was detected and located between markers CEDG097 and CEDG172 with LOD score value of 2.76. The PVE by *qYMV1* is 13.10% and the QTL is tightly linked (0 cM) to the left flanking marker CEDG97, which denotes a major QTL. Genotyping of the 25 known blackgram genotypes employing CEDG097 and CEDG172 markers, CEDG097 showed 91% linkage in known resistant and susceptible varieties to the YMV disease reaction and CEDG172 exhibited 74% linkage in known varieties to the YMV disease reaction. The resistance alleles (PU31 allele) of both markers *i.e.* CEDG097 and CEDG172 were recorded in VBN7, LBG922, LBG933 and TBG130-1 hence, these genotypes can be considered as potent YMV resistant varieties and also can be used as donor parents in resistance breeding programmes.

Key words: Blackgram, Genetic linkage map, QTL detection, SSR markers, Validation, Yellow mosaic disease.

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] or urdbean is an important short duration self pollinated pulse crop belongs to family *Leguminaceae* cultivated in almost all parts of India. In term of area and production, blackgram is the fourth most important cultivated grain legume crop in India after chickpea, pigeonpea and mungbean. In blackgram, yellow mosaic disease (YMD) cause severe yield loss, it is caused by yellow mosaic virus (YMV) belongs to family Geminiviridae, genus begomovirus and YMV is transmitted by whitefly (*Bemisia tabaci*) from infected to healthy plants. In India, the YMD on blackgram was first reported by Williams *et al*. (1968). Association of *Mungbean yellow mosaic India virus* (MYMIV) with YMD of blackgram in Andhra Pradesh was reported by Reddy *et al*. (2015) for the first time from South India. Among the begomoviruses, MYMV and MYMIV cause severe yield loss up to 85 per cent (AVRDC, 1998), causes the yellow mosaic disease (YMD) on all pulse crops such as greengram, blackgram, soybean, mothbean, cowpea, pigeon pea, common bean, horsegram and dolichos (Mishra *et al*., 2020).

Management of YMD is often linked with control of the *Bemisia tabaci* population by spraying insecticides, which is sometimes ineffective because of high population pressure. The vector management using chemicals is expensive and numbers of sprayings are required to control whitefly. Repeated sprayings cause health hazards so, cultivation of virus resistant varieties are the most economical, efficient and environment friendly. Molecular marker technology can enhance the efficiency of breeding through marker-assisted selection (MAS), in which phenotypic selection is carried out together with molecular markers that are associated with the target trait. MAS can accelerate the development of new cultivars by reducing the number of generations and increasing the accuracy in phenotypic evaluations (Collard and Mackill, 2008).

Identification of molecular markers tightly linked with the trait of interest is one of the prerequisites for transfer of

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QTL(s)/gene(s) for YMV resistance employing marker assisted breeding. As of now, a few QTLs have been identified for YMV resistance in urdbean, their direct utility in the MAS programs is limited by their co-inheritance ability with the resistant phenotype in the segregating mapping populations.

The identified molecular markers that are linked with the trait of interest in one population should be validated by testing for the presence of the marker in a range of genotypes. Hence, before using reported markers directly in MAS programs, it is better to validate them in known resistant and susceptible genotypes. Validation of molecular marker linked to YMV disease resistance genes has been reported in urdbean (Prasanthi *et al*., 2013; Sowmini and Jayamani, 2014; Gupta *et al*., 2015). Molecular markers linked to YMV resistance also have been reported in mungbean (Kabi *et al*., 2018). The objectives of this study were construction of genetic linkage map using SSR markers, detection of QTLs associated to YMV resistance and validation of QTL linked markers that are associate with YMV resistance in blackgram.

MATERIALS AND METHODS

Parental polymorphic study and development of F_1 individuals

The F_1 individuals were developed from the cross between the susceptible genotype (PGB32) and the resistant genotype (PU31) during *kharif*, 2016 under net house at RARS, Tirupati. Parental polymorphic study was done by employing a total of 147 markers developed from azukibean and greengram genome sequences (Chaitieng *et al*., 2006; Gupta *et al*., 2008).

Phenotyping and genotyping of F_2 mapping population

The F_1s seeds were selfed to produce F_2 population during *rabi,* 2017 after confirming their heterozygosity using CEDG180 marker. In total, 180 F_2 individuals of PU31×PBG32 cross along with the parents were evaluated for YMV resistance at RARS, Tirupati during summer, 2018 under natural field conditions. The data for the presence or absence of the YMV disease was recorded from initial flowering to harvesting with 10 days intervals.

The total genomic DNA was isolated from tender leaves of parents, F_1 and F_2 mapping population at 30-40 DAS (days after sowing) using modified CTAB method (Murray and Thomson, 1980). DNA was subjected to PCR by using microsatellite markers and the PCR product was loaded on to 3% agarose gel by mixing with 3 µl of 6 × loading dye, banding pattern was analyzed using gel documentation system (Biorad Gel Doc × R^+ Imaging Systems). The genotyping of the F_2 mapping population was carried out using the polymorphic markers identified between the parents. The allele sizes of PCR products were resolved in agarose gel and assigned an allele code of 'A' for parent 1 allele (PU31), 'B' for parent 2 allele (PBG32) and 'H for heterozygote to score the entire F_2 mapping population.

QTL analysis

The genotypic data of cross PU31 × PBG32 generated by polymorphic markers were used for construction of genetic linkage map using ICI mapping software ver. 4 (http://www.isbreeding.net).The generated linkage map and phenotypic data were subjected to Composite Interval Mapping (CIM) for detection of QTLs employing ICImapping software ver. 4 (http://www.isbreeding.net).

Validation of QTL linked markers in known blackgram resistant and susceptible genotypes

The identified markers flanked to *qYMV* were validated in 25 diverse resistant and susceptible blackgram genotypes made available from Pulses Breeding Scheme, RARS, Tirupati. These were grown at RARS Farm, Tirupati during summer, 2020. The phenotypic data of blackgram genotypes *i.e*., susceptible and resistant reaction to YMV disease was recorded. The genomic DNA was isolated from each genotype using CTAB method. For validation of linked markers, the total DNA was amplified in PCR with linked markers that are identified in this study.

RESULTS AND DISCUSSION

Parental polymorphism study using SSR markers

A total of nine F_1 seeds of crosses PU31 × PBG32 were obtained and sown in the field of RARS, ANGRAU, Tirupati for development of F_2 population. Of 147 SSR markers used, 24 showed polymorphism (16%) between resistant and susceptible parents *viz*., PU31 and PBG32. Gupta *et al*. (2013) recorded 8.6% of polymorphism between DPU88-31 (YMV resistant) and AKU9904 (YMV susceptible) and Rambabu *et al*. (2018) reported 28% polymorphism between the resistant (T9) and susceptible (LBG759) parents of blackgram. Based on these reports it can be implied that low polymorphism is common due to the existence of narrow genetic base in blackgram (Kalaria *et al*., 2014). However, in our study the SSR markers showed 16% polymorphism that implies availability of higher variation among the parents when compared to the earlier studies.

Phenotyping and genotyping of F_1 and F_2 mapping population

Screening of F_1 plants (PU31 × PBG32) showed susceptibility to YMV indicating recessive nature of YMV tolerance. In contrast, Thamodhran *et al*. (2016) studied inheritance pattern of YMV resistance, wherein three susceptible blackgram genotypes *i.e.,* MDU 1, CO6 and LPG-752 were crossed with four resistant genotypes *i.e.,* VBN (Bg) 6, PU 31, Mash-114 and Uttara. The F_1s of all crosses showed resistance to YMV, which means resistance is dominant over the susceptible.

The phenotypic data of F_2 population, for the YMV disease reaction *i.e.,* resistance or susceptible nature of each plant was recorded from 30 DAS to harvesting.Of 186 F_2 plants of PU31 × PBG32 cross, 152 plants showed

disease symptoms, absent in 31 plants and 3 plants were died. The segregation ratio in F_2 population of PU31 × PBG32 cross was 5 susceptible: 1 resistant indicated that MYMV resistance was governed by several genes. Genotypic analysis of F_2 population derived from the cross PU31×PBG32 was performed employing 24 polymorphic SSR markers. The total DNA was amplified in PCR using polymorphic primers, which produced 3 types of alleles among F_2 individuals.

Lekhi *et al*. (2018) investigated the mode of inheritance of MYMV resistance in the cross SML668 × Mash114 of mungbean reported segregation ratio in F_2 population was 3 resistant: 1 susceptible and Muraleedhar *et al*. (2015) reported the segregating ratio 9 S: 3 MS: 3 MR: 1 R in F_2 population of mungbean.

Mapping of YMV resistance genomic regions/QTLs

The genotypic data generated employing 18 polymorphic markers was used for construction of genetic linkage map using ICImapping software ver. 4 (http://www.isbreeding.net) at LOD threshold score of 3.0. Of these 17 markers clustered into a single linkage group while one marker namely CEDG225 was unlinked. The total map length observed was 335.47 cM. The highest interval *i.e.,* 41.13 cM observed between the markers CEDG006 and CEDG180 while the lowest interval of 13.62 cM observed between CEDG172 and BM170 markers. Gupta *et al*. (2008) worked on construction of blackgram genetic linkage map using a total of 428 markers including 254 AFLP, 47 SSR, 86 RAPD and 41 ISSRs at a LOD score of 5.0 and distributed into 11 linkage groups with 865.1cM of total length. In the current study four markers *viz*., CEDG006, CEDG044, CEDG097and CEDG271 were also mapped onto the single linkage group similar to Gupta *et al*. (2008) using the F_2 population of the cross PU31 × PBG32. Chaitieng *et al*. (2006) constructed a genetic linkage map of blackgram using JoinMap ver. 3 at LOD score of 3.0, which has a length of 783 cM. The linked markers CEDG006, CEDG008, CEDG020, CEDG044, CEDG048, CEDG097, CEDG180, CEDG271 and MB170 that identified in the current study were also reported by Chaitieng *et al*. (2006). Hence, based on the current study and also from Gupta *et al*. (2008) and Chaitieng *et al*. (2006) reports, it can be assumed that the marker regions of CEDG006, CEDG044, CEDG097 and CEDG271 are highly variable across blackgram genomes warranting detailed mapping study with more number of markers.

QTL analysis

Single QTL, '*qYMV1*'for YMV tolerance was detected by using CIM that located between the markers CEDG097 and CEDG172 with LOD score value of 2.76. The phenotypic variation explained (PVE) by *qYMV1* is 13.10%, which denotes a major QTL (Table 1) and the YMV tolerance allele comes from the resistant parent, PU31. The QTL is located very closer (0 cM) to the left flanking marker CEDG97, thus the marker can be used to screen YMV resistance/ susceptibility among blackgram genotypes. Ver few reports are there on QTL mapping studies in blackgram. Among them, Ratanakorn *et al*. (2013) detected five QTLs resistant to MYMIV in India and Pakistan, the QTL *qYMIV1* (CEDG100-cp02662), *qYMIV2* (DMB-SSR008-VR113) and *qYMIV3* (CEDG166-CEDG304)were showed resistant to MYMIV in India and the QTLs *qYMIV4* (CEDG100-cp02662) and *qYMIV5* (CEDG121-CEDG191)were resistant to MYMIV in Pakistan. Similarly, Mathivathana *et al*. (2019) reported five QTLs resistant to MYMV in cross of *Vigna radiata* × *Vigna umbellata,* two QTLs *i.e., qMYMV4-1* and *qMYMV5-1* were detected in the year 2015 on chromosomes 4 and 5, respectively and also three QTLs *i.e.,qMYMV4-1*, *qMYMV6-1* and *qMYMV10-1* were detected in the year 2016 on chromosomes 4, 6 and 10, respectively. They found common QTL *i.e., qMYMV4-1* between the same markers VigSNP_04_32 and VigSNP_04_36 with phenotypic variance of 20.04% and 18.97% in 2015 and 2016, respectively, hence, it was concluded that the QTL is stable and is a major QTL for resistance to MYMV. The QTL region identified in the present study can be considered as novel as it is not reported in earlier studies.

Validation of QTL linked markers in diverse blackgram genotypes

The two markers, CEDG097 and CEDG172 that flanked to *qYMV1* QTL were validated in known 25 resistant and susceptible blackgram genotypes. The phenotypic screening of 25 blackgram genotypes revealed, 15 genotypes showed YMV resistant while 10 showed susceptibility (Table 2). Genotyping of the 16 resistant blackgram varieties employing CEDG097 amplified the resistant allele (allele observed in PU31) of 100 bp fragment in 10 varieties, 135bp fragment (Susceptible parent allele) was amplified in one variety (TBG104), both fragments were recorded in 3 varieties and amplification was not occurred in two varieties *i.e.,* TBG140 and VBN4. Among the susceptible blackgram varieties, the 135 bp fragment was observed in 8 out of 9 varieties and one resistant allele (100 bp) was observed in MBG1058 (susceptible variety). The marker CEDG097 showed 91% linkage in known resistant and susceptible varieties to the YMV disease reaction, when excluding the heterozygotic loci. Similarly, genotyping of the 16 resistant blackgram varieties employing marker CEDG172 was amplified the resistant allele of the 130 bp fragment in 6 varieties, 140 bp (Susceptible allele) fragment was recorded in 4 varieties and no amplicon was observed in 3 genotypes. In the susceptible varietal group, the 140 bp fragment was

Table 1: The details of the QTL governing YMV resistance in F_2 population.

Trait name	Position (cM)	Left marker	Right marker	LOD	PVE(%)	Additive effect	Dominance	YMV tolerance
YMV resistant	0	CEDG097	CEDG172	2.76	13.10	-0.1274	0.1703	PU31

Table 2: Validation of blackgram genotypes with QTL linked markers.

Blackgram varieties	YMV disease reaction	Genotypic data			
		CEDG097		CEDG172	
		100 bp (R)	135 bp (S)	130 bp (R)	140 bp (S)
Resistance varieties					
PU31	Resistant	✓	-	✓	-
TBG104	Resistant	-	✓	-	✓
TBG130-1	Resistant	✓	-	✓	-
TBG139	Resistant	✓	-	-	✓
TBG140	Resistant	-	-	-	-
TU40	Resistant	✓	-	-	-
LBG787	Resistant	✓	-	✓	✓
VBN4	Susceptible	-	-	-	-
VBN7	Resistant	✓	-	✓	-
LBG884	Resistant	✓	✓	✓	-
LBG904	Resistant	✓	✓	✓	✓
LBG932	Resistant	✓	✓	✓	-
LBG918	Resistant	✓	-	-	✓
LBG922	Resistant	✓	-	✓	-
LBG933	Resistant	✓	-	✓	-
LBG946	Moderately resistant	✓	-	-	✓
Susceptible varieties					
LBG685	Susceptible	-	✓	-	✓
LBG709	Susceptible	-	✓	✓	-
MBG207	Susceptible	-	✓	-	✓
LBG752	Susceptible	-	✓	-	✓
LBG645	Susceptible	-	✓	-	✓
MBG1058	Susceptible	✓	-	-	✓
VBG11-031	Moderately susceptible	-	✓	✓	-
MBG1061	Susceptible	-	✓	✓	-
PBG32	Susceptible	-	✓	-	✓

Note: Presence of both alleles represents heterozygotic condition for that locus.

recorded in 6 susceptible varieties, 130 bp fragment (Resistant allele) in 3 susceptible genotypes. The primer CEDG172 exhibited 74% linkage in known varieties to the YMV disease reaction.

The genotype TBG104 showed resistant at field screening however it showed susceptible fragment alleles 135 bp (CEDG097) and 140 bp (CEDG172) at both loci, suggest that TBG104 has different gene controlled YMV resistance. Interestingly, the resistance alleles (PU31 allele) of both markers *i.e.,* CEDG097 and CEDG172 were recorded in VBN7, LBG922, LBG933 and TBG130-1 hence, these genotypes can be considered as potent YMV resistant varieties and also can be used as donor parents in resistance breeding programmes. Similar results were reported by Gupta *et al*. (2015) in blackgram by validation of 20 blackgram genotypes with 10 markers. The genotype PU31 was amplified with three markers which means PU31 carrying both resistant genes and it could be used as a donor of YMV resistance in blackgram breeding programme for incorporating YMV resistance.

CONCLUSION

From the current study *viz*., QTL mapping and *qYMV1* validation, it can be concluded that the QTL linked markers CEDG097 and CEDG172 would be employed for identification of blackgram genotypes for YMV resistance and in marker-assisted breeding approaches. However, further in depth investigation is warranted before being employed to develop YMV resistant varieties using these YMV linked markers in marker-assisted selection.

Conflict of interest: None.

REFERNCES

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 azuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi] linkage map. Theoretical and Applied Genetics. 113(7): 1261-1269.

Collard, B.C and Mackill, D.J. (2008). Marker assisted selection: An approach for precision plant breeding in the twenty first century. Philosophical Transactions of the Royal Society of London. 363(1491): 557-72.

Gupta, S., Gupta, D.S., Anjum, T.K., Pratap, A and Kumar, J. (2013). Inheritance and molecular tagging of MYMIV resistance gene in blackgram (*Vigna mungo* L. Hepper). Euphytica. 193: 27-37.

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: 628-637.

Kabi, M., Das, T.R., Baisakh, B and Swain, D. (2018). Validation of molecular markers linked to yellow mosaic virus disease resistance in diverse genotypes of greengram (*Vigna radiata*). International Journal of Current Microbiology and Applied Sciences. 7(2): 3457-3465.

Kalaria, R.K., Chauhan, D., Mahatma, M.K. and Mahatma, L. (2014). Identification of RAPD and ISSR makers for resistance against Mungbean yellow mosaic virus in mungbean (*Vigna radiata* L.) under south Gujarat agro climatic condition of India. The Bioscan. 9(3): 1177-1182.

Lekhi, P., Gill, R.K., Kunal and Kaur, S. (2018). Identification of molecular marker linked to Mungbean yellow mosaic virus (MYMV) resistance in *Vigna radiata* (L.) Wilczek. Electronic Journal of Plant Breeding. 9(3): 839-845.

Mathivathana, M.K., Murukarthick, J., Karthikeyan, A., Jang, W., Dhasarathan, M., Jagadeeshselvam, N., Sudha, S., Vanniarajan, C., Karthikeyan, G., Yang, T.L., Raveendran, M., Pandiyan, M. and Senthil, N. (2019). Detection of QTLs associated with *Mungbean yellow mosaic virus* (MYMV) resistance using the interspecific cross of *Vigna radiata* × *Vigna umbellata*. Journal of Applied Genetics. 60: 255-268.

Mishra, G.P., Dikshit, H.K., Ramesh, S.V., Tripathi, K., Kumar, R.R., Aski, M., Singh, A., Roy, A., Priti., Kumari, N., Dasgupta, U., Kumar, A., Praveen, S and Nair, R.M. (2020). Yellow mosaic disease (YMD) of mungbean (*Vigna radiata* (L.) Wilczek): Current status and management opportunities. Frontiers in Plant Science. 11: 1-24.

Muraleedhar, A., Dikshit, H.K., Singh, D., Singh, A. and Prapti, P. (2015). Inheritance of resistance to *Mungbean Yellow Mosaic India Virus* (MYMIV) in mungbean (*Vigna radiata*). Indian Journal of Agricultural Sciences. 85 (9): 1144-1147.

Murray, M.G and Thompson W.F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 8: 4321-4326.

Naimuddin, Akram, M. and Singh, N.P. (2016). Yellow mosaic of mungbean and urdbean: Current status and future strategies. Journal of Food Legumes. 29(2): 77-93.

Prasanthi, L., Reddy, B.V.B., Geetha, B., Jyothi, R. and Abhishek. (2013). Molecular marker for screening yellow mosaic disease resistance in blackgram [*Vigna mungo* (L.) Hepper]. Electronic Journal of Plant Breeding. 4(2): 1137-1141.

Rambabu, E., Anuradha, Ch., Sridhar, V and Sokka R.S. (2018). Genetics of Mungbean yellow mosaic virus (MYMV) resistance in blackgram (*Vigna mungo* (L.) Hepper). International Journal of Current Microbiology and Applied Sciences. 7(4): 3869-3872.

Ratanakorn, K., Somta, P., Orawan, C., Akhtar, K.P., Tariq, M.S., Ramakrishnan, M.N., Bains, T.S., Sirari, A., Kaur, L. and Srinives, P. (2013). Detection of quantitative trait loci for *Mungbean yellow mosaic India virus* (MYMIV) resistance in mungbean [*Vigna radiata* (L.) Wilczek] in India and Pakistan. Breeding Science. 63: 367-373.

Reddy, B.V.B., Obaiah, S., Prasanthi, L., Sivaprasad, Y., Sujitha, A. and Krishna, T.G. (2015). Mungbean yellow mosaic India virus is associated with yellow mosaic disease of blackgram (*Vigna mungo* L.) in Andhra Pradesh, India. Archives of Phytopathology and Plant Protection. 48(4): 345-353.

Thamodhran, G., Geetha, S and Ramalingam, A. (2016). Genetic study in urdbean [*Vigna mungo* (L.) Hepper] for inheritance of Mungbean yellow mosaic virus resistance. International Journal of Agriculture, Environment and Biotechnology. 9(1): 33-37.

Williams, F.J., Grewal, J.S. and Amin, K.S. (1968). Serious and new diseases of pulse crops in India in 1966. Plant Disease Reporter. 52: 300-304.