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# Mapping of molecular markers linked with MYMIV and yield attributing traits in mungbean

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**Abstract**

**The present study employed recombinant inbred lines (RILs) derived from the cross between a susceptible cultivar Sonali and resistant wild relative of mungbean (*Vigna radiata* var. *sublobota* to map molecular markers linked with mungbean yellow mosaic Indian virus (MYMIV) resistance and yield attributing traits in mungbean. Resistance to the virus was evaluated in RIL population under field conditions during two consecutive years 2013 and 2014. A set of 224 molecular markers were employed for the identification of polymorphism between parents. Only 46 markers showed polymorphism between Sonali and *V. radiata* var. *sublobota*. Twenty two polymorphic markers were used to construct a linkage map comprising 11 linkage groups. QTL analysis identified molecular markers linked with MYMIV resistance and agronomic traits *viz*., no. of pods per plant, no. of seeds per pod and 100-seed weight. Molecular markers identified to be linked with MYMIV were confirmed in 93 diverse mungbean accessions screened for yellow mosaic disease. The molecular markers linked to the MYMIV and yield attributing traits identified in this study will be useful in marker assisted breeding for development of high yielding mungbean varieties resistant to MYMIV.**

**Key words**: Mungbean, mungbean yellow mosaic Indian virus, molecular markers, mapping.

## Introduction

Mungbean (*Vigna radiata* L. Wilczek) is a self pollinated diploid (2n=2x=22) warm season grain legume crop with a small genome size of 579 Mb/1C having short life span (Arumuganathan and Earle 1991; Van et al. 2013). Mungbean is a cheap source of easily digestible proteins, vitamins, minerals and carbohydrates (Rishi 2009). Biotic stresses in mungbean are major constraints limiting its production. Among the viral diseases, yellow mosaic disease (YMD) is one of the major destructive disease of mungbean caused by different species of Geminiviruses belonging to the genus Begomovirus and family Geminiviridae such as Mungbean Yellow Mosaic Virus (MYMV), Mungbean Yellow Mosaic India Virus (MYMIV), Horsegram Yellow Mosaic Virus (HYMV) and *Dolichos* Yellow Mosaic Virus (Nariani 1960; Iqbal et al. 2011). The disease can reduce great yield even sometimes results in cent percent losses in mungbean. Main pathogens causing YMD in mungbean in India are MYMV and MYMIV (Malathi and Jones 2009). Successful infection and transmission of this virus occurs by a whitefly (Nariani 1960). Host species and susceptibility of each plant affect the development of disease symptoms after virus infection (Singh et al. 2014). Higher incidence of disease has been observed during spring and rainy seasons due to favorable condition for multiplication of the vector, *Bemisia tabaci* (Singh and Gurha 1994). In mungbean first symptoms of the disease appear on the young leaves in the form of mild scattered yellow specks or spots. The leaf size is generally not much affected but sometimes the green areas are slightly raised and the leaves show slight puckering and reduction in size. The size of yellow areas goes on increasing in the new growth and ultimately some of the apical leaves turn completely yellow. The diseased plants usually mature late and bear very few flowers and pods, the size of the pod is reduced and more frequently immature and small sized seeds are obtained from the pods of diseased plants. Mungbean genotypes have wavering ability for tolerance against YMD depending upon location and environment. Chemical control of the vector white fly is not very effective and nor environment friendly. Development

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of YMV-tolerant variety is of prime importance for stabilizing the yield levels. The field screening for the disease presents major constraints in breeding YMD resistant mungbean, because of non-uniform development of the disease due to fluctuation of the whitefly population in different locations and different seasons. Since conventional breeding is time consuming hence selection based on molecular markers associated with the target trait will help in decreasing the number of phenotypic evaluations and thus reducing time and cost and increasing gain from selection. A very few reports on development and identification of molecular markers linked to the disease are available in literature. Random amplified polymorphic DNA (RAPD) markers (Selvi et al. 2006), SCAR markers (Dhole and Reddy 2012), RGA derived markers (Maiti et al. 2011) were developed and reported to be linked with disease however there is no reports on successful application of these markers till now. QTLs associated with MYMIV have been identified by Chen et al. (2013), Alam et al. (2014) and Kitsanachandee et al. (2013). Even though mungbean genome has been sequenced by Kang et al. (2014) but still genomic resources are lacking in mungbean. Mungbean genetic linkage map revealing QTLs linked with yield attributing traits (Kajonphol et al. 2012; Isemura et al. 2012), seed weight and other agronomic traits have been identified earlier in mungbean (Fatokun et al. 1992; Humphrey et al. 2005; Isemura et al. 2012; Kajonphol et al. 2012; Chen et al. 2013). Successful application of marker assisted selection (MAS) requires highly efficient and stable molecular markers linked to the trait of interest. Among multifarious marker systems such as Restriction Fragment Length Polymorphism (RFLPs), Random Amplified Polymorphic DNA (RAPDs), Sequence Tagged Sites (STSs), Amplified Fragment Length Polymorphism (AFLPs), Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphism (SNPs) SSRs have occupied a pivotal place because of their reproducibility, multiallelic nature, codominant, inheritance, relative abundance and good genetic coverage. To date there is only one report on QTL mapping for YMD resistance in mungbean (Kitsanachandee et al. 2013) hence objective of this study is to identify and locate molecular markers linked with the MYMIV resistance and yield attributing traits in greengram.

## Materials and methods

### *Plant material and DNA extraction*

Mapping population comprised of 100 RILs was developed from a cross between a cultivar Sonali (@&) and an accession of *Vigna radiata* var. *sublobata* (B&). Sonali is a popular small seeded cultivar of West Bengal susceptible against MYMIV while *sublobata* a wild relative of *Vigna radiata* and a progenitor of cultivated greengram is resistant to MYMIV. Total genomic DNA of parents, RILs and 93 mungbean germplasm lines was extracted from fresh young tissue following the modified method of Dellaporta et al. (1983) with slight modification as proposed by Chattopadhyay et al. (2008). Purification was done by RNase treatment and quantification was done in 0.8% agarose gel comparing with the known standard.

### *Phenotyping for agromorphological traits and screening for resistance to MYMIV infection*

Parents together with RILs and mungbean accessions were screened for MYMIV resistance under natural environment at infection hot spot zone at experimental farm, Bidhan Chandra Krishi Viswavidyalaya, Kalyani Simanto over two consecutive years during pre-*kharif* in the years, 2013 ($F_7$ RIL) and 2014 ($F_8$ RILs). Sonali was planted as a susceptible check and spreader in every third row, and *sublobata* was planted every 10$^{th}$ row as resistant check. No insecticide was sprayed in order to maintain the natural whitefly population in the field. Weeding and harvesting were done manually. Disease scoring was done following 0-5 scale as per the methods described earlier by Kitsanachandee et al. (2013). Yield attributing traits in mungbean were observed of parents and in RILs *viz*., number of seeds/pod, number of pods/plant and 100-seed weight for two consecutive years (2013 and 2014) and their mean values were used for statistical analysis.

### *Molecular marker analysis*

Two hundred and thirteen SSR markers, two STS markers and a few RGA and SCAR markers retrieved from the already published literatures on yellow mosaic disease in mungbean and employed for polymorphism survey among parental genotypes, Sonali and *V. radiata sublobata* (Supplementary Table S1). A sample of 25µl PCR mixture comprising of 50ng of template DNA, 10ng of forward and reverse primers, 1µl 2.5mM dNTP mixture, 10X Taq buffer and 1.0 unit Taq polymerase (Genie, Bangaluru) was used for amplification of markers using GeneAmp PCR System 9700 (Applied Biosystem) with reaction condition of 94$^{o}$C for 5 min (preheat), 94$^{o}$C for 45s, annealing temperature for 45s, 72$^{o}$C for 1 min (35 cycles) with final extension step at 72$^{o}$C for 7 min (one cycle) and

then final storage at 4$^{o}$C. Amplified product analyzed in metaphor agarose (Lonza) visualized in Gel Documentation Unit (UVP Ltd, UK) under the UV light.

### *Linkage map construction and QTL analysis*

A linkage map was constructed using QTL IciMapping (Meng et al. 2015). Marker scores were subjected to goodness of fit test using Chi squared analysis. Twenty two polymorphic markers were assigned to linkage groups (LGs) using maximum logarithm of an odds (LOD) score of 3.0 and maximum recombination frequency (r) of 0.5. The genetic distance was estimated using Kosambi mapping function (Kosambi 1944). QTL analysis was done using single marker analysis to identify molecular markers linked with MYMIV tolerance and yield attributing factors. Only markers showing significance at P = 0.001 were considered as linked markers.

## Results and discussion

### *MYMIV reaction in the RILs*

RIL population, the parents and mungbean accessions were evaluated for MYMIV resistance under field condition over two consecutive years during pre-*kharif* in the years, 2013 ($F_7$ RILs) and 2014 ($F_8$ RILs). In both the years, Sonali and *V. radiata sublobata* showed moderately susceptible (score, 3) and highly resistant (score, 0) reactions to MYMIV, respectively, while the RILs and mungbean accessions expressed disease scores varying degree from 0 to 5. The mean value of disease score in the RILs were 1 and 2 in 2013 and 2014, respectively (Table 1). Hence, infection of the disease in the year 2014 was more severe than the year 2013 suggesting dependence of disease expression on various factors among which most potential one is presence of sufficient population of

**Table 1.** MYMIV reaction in parents and RIL population derived from Sonali x *Vigna radiata* var. *sublobata*

| Disease score | Disease reaction | Screening on 24.5.2013 | Screening on 20.5 2014 | Over two years (2013 and 2014) |
|---|---|---|---|---|
| 0 | Highly tolerant | BS21, BS23, BS25, BS28, BS30, BS32, BS36, BS39, BS41, BS45, BS46, BS48, BS55, BS57, BS64, BS66, BS68, BS69, BS72, BS73, BS74, BS80, BS82, BS83, BS91, BS96, BS99, BS100 | NIL | *Vigna radiata* var. *sublobata*, BS13, BS14, BS15 , Bs16, BS17, BS18, BS19, BS20, BS24, BS26, BS27, BS35, BS47, BS49, BS55, BS57 |
| 1 | Tolerant | BS90, BS98 | BS64, BS70 | BBS1, BS4, BS9, BS10, BS11, BS12, BS22, BS33, BS34, BS37, BS38, BS40, BS42, BS43, BS44, BS50, BS51, BS52, BS53, BS60, BS61, BS62, BS63, BS67, BS71, BS76, BS77, BS87, BS89, BS92, BS93, BS94, BS95 , BS97, |
| 2 | Moderately tolerant | NIL | BS21, BS23, BS25, BS28, BS30, BS32, BS36, BS39, BS41, BS45, BS46, BS48, BS66, BS68, BS69, BS72, BS73, BS74, BS80, BS82, BS91, BS96, BS99 | BS8, BS56, BS59, BS65, BS78, BS86, BS88 |
| 3 | Moderately susceptible | BS70 | BS79.BS90 and BS98 | Sonali, BS3, BS5, BS7, BS29, BS31, BS54, BS58, BS79 and BS84 |
| 4 | Susceptible | NIL | BS83 and BS100 | BS75, BS81 and BS85 |
| 5 | Highly susceptible | | NIL | BS2 and BS6 |

vector (virus causing the disease) and environment affecting the vector population. Environmental dependence of disease has been suggested by several researchers (Bashir et al. 2006; Islam et al. 2008; Akhtar et al. 2011). The correlation coefficient (r) of the disease score and coefficient of determination ($r^2$) between the two years was moderate, being 0.65 and 0.446, respectively and significant ($P < 0.0001$). Although, frequency distribution plotted as bar from the Sonali x *V. radiata* ssp. *sublobata* (Fig. 1) showed

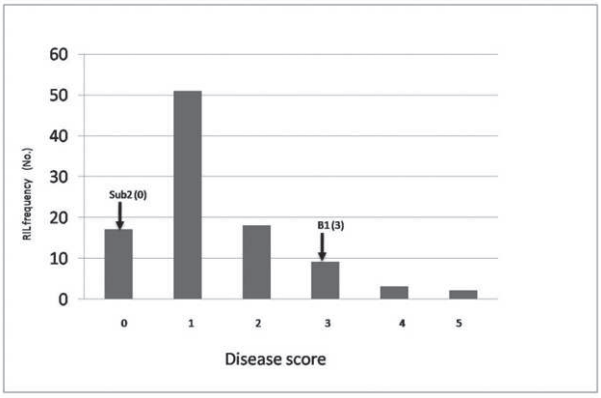


**Fig. 1. Frequency distribution of disease score for resistance to mungbean yellow mosaic India virus in the mungbean RIL population over two years**

continuous variation but broadly classified into two major classes comprising 16 highly resistant (scale 0) and 24 moderately susceptible (scale 2-3) lines.

### *Variation in yield attributing traits*

The RILs were classified into different ranges according to number of pods/plant, number of seeds/pod, and 100-seed weight (Figs. 2A, B and C). All these traits showed continuous variation and presence of transgressive segregants in RILs. The positive transgressive segregation was observed in the number of pods per plant when compared with both Sonali and *sublobata* (Fig. 2A). Pod number is an important yield component and directly proportional to crop yield. Miah and Bhadra (1989) reported a difference in pod production among mungbean cultivars. The transgressive segregation for 100-seed weight has also been reported in mungbean (Khattak et al. 2003; Chen et al. 2013).

### *Parental polymorphism survey*

A set of 224 molecular markers comprising of SSRs,

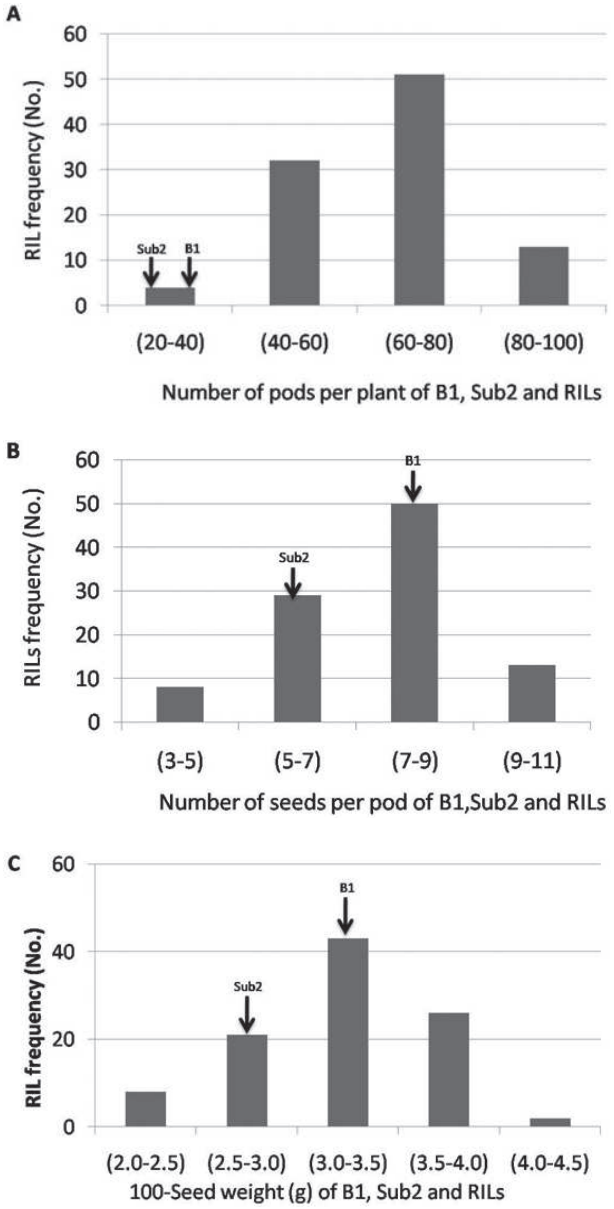


**Fig. 2. Frequency distribution of mungbean RIL population with respect to A) Number of pods/ plant, B) Number of seeds/pod and C) 100-Seed weight**

RGA, SCAR and STS were employed for identification of polymorphism among parents (Supplementary Table S1). Only 45 SSR markers and one STS marker were found polymorphic among the parents. SCAR marker (Dhole and Reddy 2013) and most of the RGA markers (Maiti et al. 2011) did not show any polymorphism between resistant and susceptible parents. Only one RGA (RGA22F2/24R2) primer carrying CCNBS- LRR domain amplified 400bp fragment from the resistant parent, *V. radiata* ssp. *sublobata* but 470bp from

susceptible parent, same primer pair amplified a fragment of 1236 bp from the resistant parent as observed earlier (Maiti et al. 2011), which may be due to the use of different parent(s) by earlier workers. However, the reproducibility of same primer, RGA22F2/ 24R2 was not good enough in mapping population. STS based primer pair linked with the bruchid tolerance (Sarkar et al. 2011) and a QTL for MYMIV tolerance (Kitsanachandee et al. 2013) showed polymorphism between Sonali and *V. radiata* ssp. *sublobata.*

### *Mapping and QTL analysis*

Out of 46 polymorphic molecular markers among parental genotypes, 24 markers for which goodness of fit test showed significant deviation from a segregation ration of 1:1 were excluded from mapping. Twenty two polymorphic markers were assigned to cover 11 linkage groups of 727.1 cM in length with 33.05 cM/marker average density at LOD score of 3.0 (Fig. 3) using QTL IciMapping (Meng et al. 2015). QTL

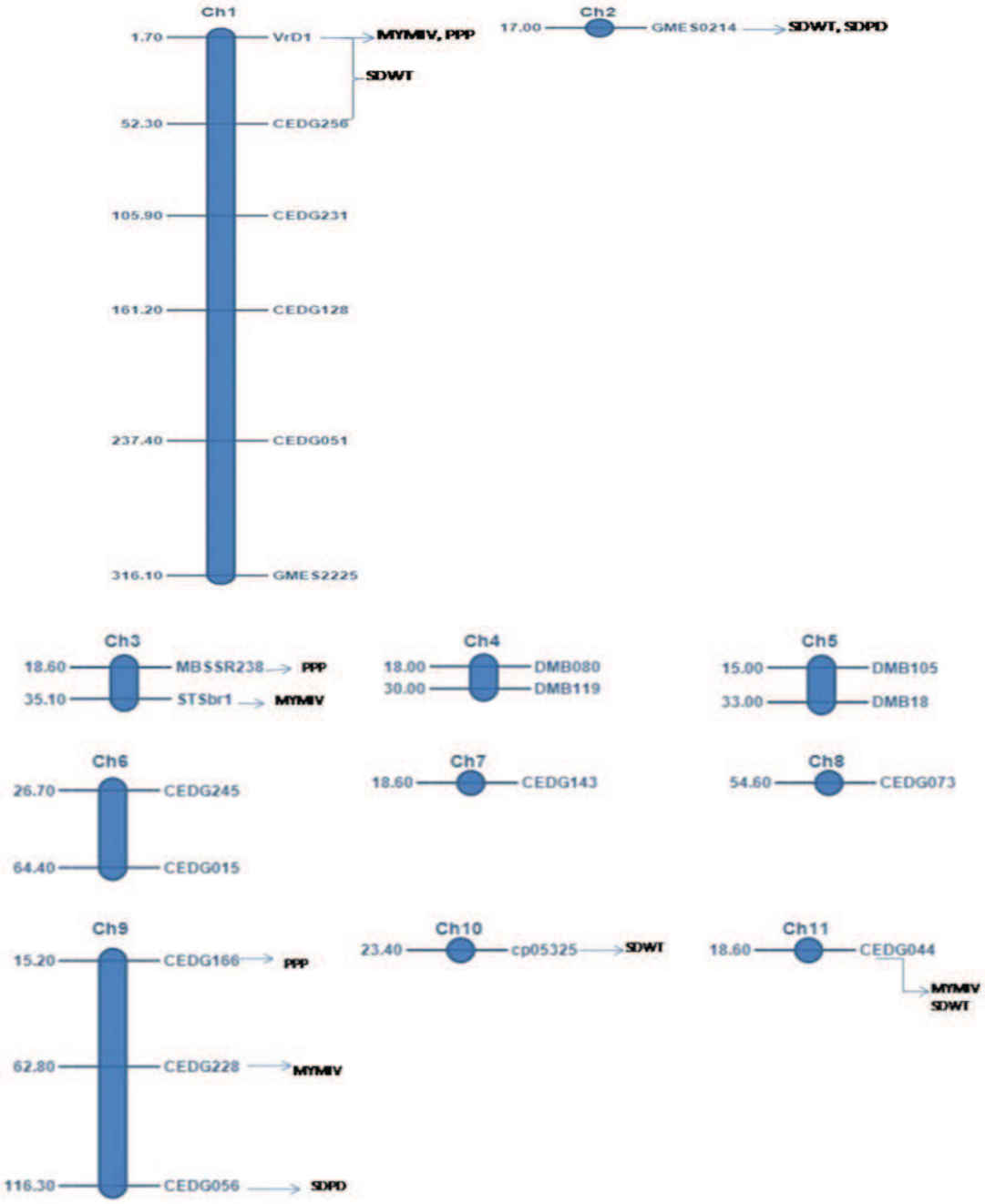


**Fig. 3. Linkage map of RILs of mungbean population and location of QTLs linked with number of pods/ plant (PPP), 100-seed weight (SDWT), Number of seeds/pod (SDPD) and Mungbean Yellow Mosaic Indian Virus (MYMIV)**

analysis was done using single marker analysis to identify molecular markers linked with MYMIV tolerance and yield attributing factors (Table 2). Five molecular markers *viz*., CEDG044, CEDG256, cp05325, GMES0214 and VrD1were found to be linked with 100-seed weight, while three molecular markers, namely, CEDG166, VrD1 and MBSSR238 were found to be linked with number of pods/plant. Molecular markers, CEDG056 and GMES0214 were linked with number of seeds/pod. QTL analysis by single marker analysis revealed linkage of molecular marker VrD1, CEDG228, CEDG044 and STSbr1 with MYMIV tolerance in mungbean (Table 2). Three QTLs *viz.,* qMYMIV1, qMYMIV2 and qMYMIV3 for the MYMIV were identified by Kitsanachandee et al. (2013) in mungbean from India. In the same study SSR marker CEDG166 was linked with QTL qMYMIV3 exhibiting MYMIV resistance, however in the present study same marker was not found to be linked with MYMIV. This may be due the differences in parental genotypes. In the present study, we located molecular markers linked with MYMIV resistance. Based on the earlier research SSR marker CEDG180 was reported to be associated with a major gene controlling MYMIV resistance in blackgram (Gupta et al. 2013) however, in the present study same marker showed no polymorphism between our mapping population parents. A molecular marker CEDG180 is mapped to LG10 according to the linkage map of mungbean reported by Isemura et al. (2012). This suggests that the genetic control of resistance to MYMIV in mungbean and blackgram is different.

### *Validation of MYMIV linked molecular markers*

Ninety three mungbean germplasm were evaluated for MYMIV to validate the molecular markers linked with MYMIV identified in the present study (Table 3). Single marker analysis revealed association of CEDG228 and CEDG044 with the disease at particular allelic size (Table 4), however, STSbr1 and VrD1 amplified all germplasm lines but no polymorphism was observed.

**Table 2.** Molecular markers linked with MYMIV and yield attributing traits

| Traits | Molecular markers | LG | $R^2$ (%) | LOD Score | Source |
|---|---|---|---|---|---|
| **MYMIV resistance**(MYMIV) | VrD1 | 1 | 6.00 | 3.9 | B1 |
| | CEDG228 | 9 | 8.00 | 4.2 | Sub2 |
| | CEDG044 | 11 | 11.33 | 3.5 | Sub2 |
| | STSbr1 | 3 | 18.00 | 2.6 | Sub2 |
| **100-Seed weight** (SDWT) | CEDG044 | 11 | 8.11 | 2.8 | B1 |
| | CEDG256 | 1 | 8.00 | 3.6 | B1 |
| | cp05325 | 10 | 9.44 | 3.2 | B1 |
| | GMES0214 | 2 | 12.55 | 3.4 | B1 |
| | VrD1 | 1 | 8.12 | 4.5 | B1 |
| **Number of pods/plant** (PPP) | CEDG166 | 9 | 6.00 | 4.8 | Sub2 |
| | VrD1 | 1 | 5.12 | 3.6 | Sub2 |
| | MBSSR238 | 3 | 11.80 | 3.8 | B1 |
| **Number of seeds/pod** (SDPD) | CEDG056 | 9 | 7.12 | 3.7 | Sub2 |
| | GMES0214 | 2 | 6.49 | 4.5 | B1 |

B1= Sonali; Sub2= *Vigna radiata* ssp. *sublobata*

Hence, it has been confirmed that SSR markers CEDG228 and CEDG044 linked with yellow mosaic disease resistance in mungbean. Bruchid resistance markers, Vrd1 (Isemura et al. 2012) and STSbr1 (Sarkar et al. 2011) linked with the MYMIV resistance in mapping population were found monomorphic among mungbean germplasm. Hence, both Vrd1 and STSbr1 were not confirmed to be linked with yellow mosaic disease resistance and needs further validation.

This is the first report of mapping of molecular linked with MYMIV in India. Different DNA markers linked to the MYMIV resistance identified in the present population may not show association with the same

**Table 3.** Disease reaction to MYMIV in mungbean germplasm

| Disease score | Disease reaction | Screening on 24.5.2013 | Screening on 20.52014 | Over two years (2013 and 2014) |
|---|---|---|---|---|
| 0 | Highly resistant | TLM-24 (KM11-558), SM12-33, PM5, NDM11-301 (KM11-579), NDM-1 (KM11-552), TARM-18, SM13-32, Pusa-9531, KM11-564, SM13-03, SPM13-12, Sukumar, Pusa-1171 (KM11-587), RMG -62, RBS-M-14, SM13-10 and Uttakarsh | KM11-574 (PM09-6), GM08-09 (KM11-560, KM553, Meha, SPM2, SM12-18, SM13-42, GM08-01, SPM13-29, SM13-06, SM13-19, SPM13-18, SPM13-25, SM13-23, SPM13-24, KM11-582, SM13-45, SPM13-27, SM13-21, SPM13-21 and SPM13-40 | SM12-78, Sonali, SPM-13-5, SM12-80, SM13-10, SM13-46, SPM13-34, KM11-557(KM-11-PM4), TGM-3 and SM12-56 |
| 1 | Tolerant | SPM13-05, TMB-37, SM12-63, SM13-14, KM11-571,(PM09-6), SM12-25 (K-15-4), Meha, PM2, SM12-18, SM13-42, SM13-16, KM12-28, SM12-79, GM08-01, Ousa-0932, SM12-57, SPM13-29, SM13-06, SM13-19, SPM13-18, SPM13-25, SM13-23, SPM 13-19, SM13-22, SPM13-20, SM12-35, IPM02-03, SM13-45, SM13-21 and SPM 13-21 | TLM-24 (KM11-558), SM12-33, PM5, NDM11-301 (KM11-579), NDM-1 (KM11-552), NDM11-302 (KM11-585), KM11-586 (ML-1464), SM13-32, Pusa 9531, KM11-564, SM13-03, SPM13-12, SM13-01, SM13-02, SM12-13, SM13-08, SM12-68, SM12-76 and SM13-44 | Bireshwar, KM12-08, KM12-56, SPM13-38, SPM13-1, MH539-1 (KM11-551), KM11-55 (Pusa 1172), SM12-26, NBL-638, SM12-32, SPM13-13, RMG-375, SM12-64, SPM13-17 and SPM13-8 |
| 2 | Moderately tolerant | Pusa Vishal, NDM11-302,(KM11-585), KM11-586 (ML-1464), SPM13-24, SPM13-2, SM13-02, SM12-13, SM12-68, SPM13-08, SPM13-27, SPM13-32 and SM13-44 | SM13-05, TARM-18, TMB-37, SM12-63, SM12-29, SM12-25 (K-15-4), SM13-16, KM12-28, Sukumar, Pusa-1171 (KM11-587), Pusa-0932, SM12-57, SM13-19, SPM13-18, SPM13-25, SM12-35 and IPM02-03 | SM12-28 and AKLM09-2 |
| 3 | Moderately susceptible | SM12-19, SM13-01, SM12-76 and SM13040 | SM12-79, SM13-2 and SPM13-32 | Nil |
| 4 | Susceptible | Nil | Pusa Vishal and SM13-14 | Nil |
| 5 | Highly susceptible | RM553, and KM11-532 | RMG-62 and PVSM-14 | Nil |

**Table 4.** Validation of MYMIV linked markers in mungbean germplasm

| Marker | Allelic size (bp) | P-value | $R^2$ (%) |
|---|---|---|---|
| CEDG228 | 180 | <0.001* | 8.4 |
| | 200 | >0.001 | 3.84 |
| | 250 | >0.001 | 0.0000919 |
| CEDG044 | 133 | >0.001 | 3.34 |
| | 150 | <0.001* | 8.77 |
| | 200 | >0.001 | 3.22 |
| VrD1 | Monomorphic | - | - |
| STSbr1 | Monomorphic | - | - |

Note: *- P value significant

trait in the other population. The SSR marker CEDG044 (Table 1) linked with both MYMIV resistance and 100-seed weight can be used in marker assisted selection programmes for development of high yielding and yellow mosaic disease resistant mungbean cultivar.

**Authors' contribution**

Conceptualization of research (SB, NS); Designing of the experiments (SB, NS); Contribution of experimental materials (SB); Execution of field/lab experiments and data collection (NS, JM, DS); Analysis of data and interpretation (NS, SB, NM); Preparation of manuscript (NS, NM, SB).

**Declaration**

The authors declare no conflict of interest.

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## Reference


Akhtar K. P., Sarwar G., Abbas G., Asghar M. J., Sarwar N. and Shah T. M. 2011. Screening of mungbean germplasm against mungbean yellow mosaic India virus and its vector *Bemisia tabaci*. Crop Prot., **30**:1202-1209.

Alam A. K. M. M., Somta P., Muktadir M. A. and Srinives P. 2014. Quantitative Trait Loci Associated with Seed Weight in Mungbean (*Vigna radiata* (L.) Wilczek). Kasetsart J. Nat. Sci., **48**: 197-204.

Arumuganathan K. and Earle E. D. 1991. Nuclear DNA content of some important plant species. Plant Mol. Bio. Rep., **9**: 208-218.

Bashir M., Ahmad Z. and Mansoor S. 2006. Occurrence and distribution of viral disease of mungbean and mashbean in Punjab and Pakistan. Pak J. of Bot., **38**: 1341-1351.

Chattopadhyay K., Bhattacharyya S., Mandal N. and Sarkar H. K. 2008. PCR based characterization of Mungbean genotypes from Indian subcontinent at intra and inter specific level. J. Plant Biochem. Biotechnol., **17**: 141-148.

Chen H. M., Ku H. M., Schafleitner R., Bains T. J., Kuo C. G., Liu C. A. and Nair R. 2013. The major quantitative trait locus for mungbean yellow mosaic Indian virus resistance is tightly linked in repulsion phase to the major bruchid resistance locus in a cross between mungbean (*Vigna radiata* (L.) Wilczek) and its wild relative *Vigna radiata* ssp. s*ublobata*. Euphytica, **192**: 2015-216.

Dellaporta S. L., Wood J. and Hicks J. B. 1983. A plant DNA mini preparation version II. Plant Mol. Bio. Rep., **1**: 19-21.

Dhole V. J. and Reddy K. 2012. Genetic analysis of resistance to Mungbean Yellow Mosaic Virus in mungbean (*Vigna radiata*). Plant Breed., **131**:414-417.

Dhole V. J. and Reddy K. S. 2013. Development of a SCAR marker linked with a MYMV resistance gene in mungbean (*Vigna radiata* L. Wilczek). Plant Breed., **132**: 127-132.

Fatokun C. A., MenancioHautea D., Danesh D. and Young N. D. 1992. Evidence for orthologous seed weight genes in cowpea and mungbean based on RFLP mapping. Genetics., **132**: 841-846.

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.

Humphrey M. E., Lambrides C. J., Chapman S. C., Aitken E. A. B., Imrie B. C., Lawn R. J., McIntyre C. L. and Liu C. J. 2005. Relationships between hard-seededness and seed weight in mungbean (*Vigna radiata*) assessed by QTL analysis. Plant Breed., **124**: 292-298.

Iqbal U., Iqbal S. M., Afzal R., Jamal A., Farooq M. A. and Zahid A. 2011. Screening of mungbean germplasm against mungbean yellow mosaic virus (MYMV) under field conditions. Pak. J. Phytopath., **23**(1): 48-51.

Isemura T., Kaga A., Tabata S., Somta P., Srinives P., Shimizu T., Jo U., Vaughan D. A. and Tomooka N. 2012. Construction of a genetic linkage map and genetic analysis of domestication related traits in mungbean (*Vigna radiata*). PLoS One, **7**: e41304.

Islam M. S., Latif M. A., Ali M. and Hossain M. S. 2008. Pop-ulation dynamics of white fly on some recommended mung-bean varieties in Bangladesh and its impact on incidence of mungbean yellow mosaic virus disease and yield. Int. J. Agri. Sci. Tech., **2**: 41-47.

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

Kang Y. J., Kim S. K., Kim M. Y. et al. 2014. Genome sequence of mungbean and insights into evolution within *Vigna* species. Nat. Commun., **5**: 5443.

Karuppanapandian T., Karuppudurai T., Sinha T. P. M., Hamarul A. H and Manoharan K. 2006. Genetic diversity in green gram [*Vigna radiata* (L.)] landraces analyzed by using random amplified polymorphic DNA (RAPD). Afr. J. of Biotechnol., **5:** 1214 -1219.

Khattak G. S. S., Ashraf M., Elahi T. and Abbas G. 2003. Selection for large seed size at the seedling stage in mungbean (*Vigna radiata* (L.) Wilczek). Breed. Sci., **53**: 141-143.

Kitsanachandee R., Somta P., Chatchawankanphanich O., Akhtar K. P., Shah T. M., Nair R. M., Bains T. S., Sirari A., Kasur 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. Breed Sci., **63**: 367-373.

Kosambi D. D. 1944. The estimation of map distances from recombination values. Ann. Eugen. **12**: 172-175.

Lavanya G. R., Srivastava J. and Ranade S. A. 2008. Molecular assessment of genetic diversity in mungbean germplasm. J. Genet., **87**: 65-74.

Maiti S., Basak J., Kundagrami S., Kundu A. and Pal A. 2011. Molecular marker assisted genotyping of mungbean yellow mosaic India virus resistance germplasm of mungbean and urdbean. Mol. Biotechnol., **47**: 95-104.

Malathi V. G. and Jones P. 2009. Mungbean yellow mosaic viruses In: Mahy BWJ, Van Regenmortal MHV (eds)

Desk encyclopedia of plant and fungal virology. Academic Press London, 217-226.

Meng L., Li H., Zhang L. and Wang J. 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop J., **3**(3): 269-283.

Miah A. J. and Bhadra S. K. 1989. Genetic variability in the F2 generation of mungbean. Bangl. J. Agri. Research, **14:** 72-75.

Nariani T. K. 1960. Yellow mosaic of mung (*Phaseolus aureus*). Indian Phytopathol., **13**: 24-29.

Rishi N. 2009. Significant plant virus diseases in India and a glimpse of modern disease management technology. J. Genet. Plant Pathol., **75**: 1-18.

Sarkar S., Ghosh S., Chatterjee M., Das P., Lahari T., Maji A., Mondal N., Pradhan K. K. and Bhattacharyya S. 2011. Molecular markers linked with bruchid resistance in an Indian Sublobata (*Vigna radiata* var. Sublobata) and its validation. J. Plant Biochem. Biotechnol., **20**: 155-160.

Selvi R., Muthiah A. R. and Manivannan N. 2006. Tagging of RAPD marker for MYMV resistance in mungbean (*Vigna radiata* (L.) Wilczek). Asian J. Plant Sci., **5**: 277-280.

Singh N., Mallick J., Murmu S., Sagolsem D., Sarkar S., Mandal N and Bhattacharyya S .2014. Inheritance of MYMIV tolerance in two RIL population of greengram on lower Gangetic alluvial zone during summer and their parental diversity by SSR. Indian J. Genet., **74**: 584-588.

Singh R. A. and Gurha S. N. 1994. Influence of cropping seasons on the incidence of yellow mosaic disease in mungbean genotypes. Indian J. Pulse Res., **7**(12): 206-208.

Van K., Kang Y. J., Han K. S., Lee Y. H., Gwag J. G. and Moon J. K. *et al*. 2013. Genome-wide SNP discovery in mungbean by Illumina HiSeq. Theor. Appl. Genet., **126**: 2017-2027.

**Supplementary Table S1.** A list of molecular markers used for polymorphism survey in RILs and mungbean germplasm

| S.No. | Marker name | Marker type | Expected product size(bp) | Linkage group | Tm (°C) | Reference |
|---|---|---|---|---|---|---|
| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
| 1 | CEDG225 | SSR | 118 | 2 | 53 | Han et al. (2005) |
| 2 | CEDG204 | SSR | 153 | 1 | 55 | |
| 3 | CEDG115 | SSR | 91 | 5 | | |
| 4 | CEDG048 | SSR | 200 | 1 | 52 | |
| 5 | CEDG006 | SSR | 120 | 2 | 52 | |
| 6 | CEDG026 | SSR | 142 | 10/2 | 52 | |
| 7 | CEDG088 | SSR | 120 | 4 | 54 | |
| 8 | CEDG013 | SSR | 92 | 11 | 52 | |
| 9 | CEDG151 | SSR | 184 | 8 | 50 | |
| 10 | CEDG173 | SSR | 124 | 9 | 50 | |
| 11 | CEDG168 | SSR | 147 | 11 | 50 | |
| 12 | CEDG282 | SSR | 133 | 6 | 50 | |
| 13 | CEDG010 | SSR | 189/196 | 3 | 50 | |
| 14 | CEDG248 | SSR | 112/108 | 6 | 50 | |
| 15 | CEDG133 | SSR | 218/212 | 1 | 50 | |
| 16 | CEDG050 | SSR | 135/131 | 2 | 52 | |
| 17 | CEDG143 | SSR | 124/121 | 7 | 53 | |
| 18 | CEDG156 | SSR | 186/184 | 8 | 52 | |
| 19 | CEDGAG001 | SSR | 175/172 | 9 | 52 | |
| 20 | CEDG118 | SSR | 191 | 6 | 52 | |
| 21 | CEDG181 | SSR | 158 | 4 | 52 | |
| 22 | CEDG304 | SSR | 86/82 | 9 | 56 | |
| 23 | CEDG228 | SSR | 200 | 9 | 52 | Wang et al. (2004) |
| 24 | CEDG056 | SSR | 241/209 | 9 | | |
| 25 | CEDG020 | SSR | 143/149 | 9 | 50 | |
| 26 | CEDG180 | SSR | 119/113 | 10 | 50 | |
| 27 | CEDG214 | SSR | 158/148 | 1 | 51 | |
| 28 | BM146 | SSR | | x | 51 | |
| 29 | CEDG086 | SSR | 126/130 | 4 | 52 | |
| 30 | CEDG275 | SSR | 254/260 | 2 | 52 | |
| 31 | CEDG139 | SSR | 196\200 | 4 | 54 | |
| 32 | CEDG103 | SSR | 110 | 4 | 55 | |
| 33 | CEDG111 | SSR | 191/193 | 7 | 53 | |
| 34 | CEDG141 | SSR | 209/179 | 1 | 54 | |
| 35 | CEDG115 | SSR | 91/89 | 5 | 53 | |
| 36 | CEDG001 | SSR | 118 | 1 | 56 | |
| 37 | CEDG002 | SSR | 128 | 11 | 58 | |
| 38 | CEDG003 | SSR | 250 | 1 | 58 | |
| 39 | CEDG008 | SSR | 138 | 5 | 58 | |
| 40 | CEDG012 | SSR | 117 | 1 | 58 | |
| 41 | CEDG015 | SSR | 174 | 6 | 58 | |
| 42 | CEDG024 | SSR | 132 | 9 | 58 | |
| 43 | CEDG030 | SSR | 105 | 1 | 58 | |
| 44 | CEDG035 | SSR | 162 | 8 | 58 | |
| 45 | CEDG037 | SSR | 127 | 6 | 55 | |
| 46 | CEDG041 | SSR | 102 | 6/7 | 55 | |
| 47 | CEDG051 | SSR | 250 | 1 | 55 | |
| 48 | CEDG053 | SSR | 110 | 1 | 55 | |
| 49 | CEDG059 | SSR | 217 | 8 | 52 | |
| 50 | CEDG060 | SSR | 51 | 5 | 52 | |
| 51 | CEDG064 | SSR | 124 | 7 | 53 | |
| 52 | CEDG067 | SSR | 64 | 5 | 55 | |
| 53 | CEDG070 | SSR | 209 | 9 | 54 | |
| 54 | CEDG071 | SSR | 263 | 8 | 54 | Wang et al. (2004) |
| 55 | CEDG072 | SSR | 106 | 11 | 55 | |
| 56 | CEDG073 | SSR | 207 | 8 | 55 | |
| 57 | CEDG074 | SSR | 208 | 1/4 | 55 | |
| 58 | CEDG075 | SSR | 211 | 11/10 | 52 | |
| 59 | CEDG076 | SSR | 176 | 11 | 54 | |
| 60 | CEDG139 | SSR | 196 | 4 | 54 | |
| 61 | CEDG268 | SSR | 177 | 5 | 55 | |
| 62 | CEDG128 | SSR | 196 | 1 | 55 | |
| 63 | CEDG231 | SSR | 196 | 1 | 55 | |
| 64 | CEDG291 | SSR | 127 | 1 | 55 | |
| 65 | CEDCAA001 | SSR | 199 | 6/1 | 56 | |
| 66 | CEDG153 | SSR | 70 | 2 | 53 | |
| 67 | CEDG166 | SSR | 185 | 9 | 54 | |
| 68 | CEDG244 | SSR | 152 | 2 | 58 | |
| 69 | CEDG117 | SSR | 166 | 3 | 58 | |
| 70 | CEDG132 | SSR | 150 | 5 | 57 | |
| 71 | CEDG171 | SSR | 190 | 5 | 56 | |
| 72 | CEDG184 | SSR | 257 | 5 | 54 | |
| 73 | CEDG245 | SSR | 116 | 6 | 53 | |
| 74 | CEDG218 | SSR | 177 | 7 | 57 | |
| 75 | CEDG130 | SSR | 233 | 8 | 54 | |
| 76 | CEDG147 | SSR | 308 | 9/10 | 54 | |
| 77 | CEDG267 | SSR | 209 | 9 | 55 | |
| 78 | CEDG198 | SSR | 227 | 10 | 52 | |
| 79 | CEDG116 | SSR | 120 | 10 | 53 | |
| 80 | CEDG113 | SSR | 97 | 10 | 58 | |

| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
|---|---|---|---|---|---|---|
| 81 | CEDG097 | SSR | 100 | 10 | 54 | |
| 82 | MBSSR140 | SSR | 209 | x | 51 | Somta et al. (2007) |
| 83 | MBSSR14 | SSR | 184 | x | 55 | |
| 84 | MBSSR238 | SSR | 115 | x | 50 | |
| 85 | MBSSR175 | SSR | 157 | x | 57 | |
| 86 | MBSSR42 | SSR | 110 | x | 55 | |
| 87 | MBSSR169 | SSR | 168 | x | 55 | |
| 88 | MBSSR87 | SSR | 148 | x | 55 | |
| 89 | MBSSR114 | SSR | 127 | x | 55 | |
| 90 | MBSSR163 | SSR | 183 | x | 55 | |
| 91 | MBSSR121 | SSR | 147 | x | 55 | |
| 92 | DMBSSR137 | SSR | 167 | x | 55 | Somta et al. (2009) |
| 93 | DMBSSR100 | SSR | 198 | x | 55 | |
| 94 | DMBSSR084 | SSR | 187 | x | 55 | |
| 95 | DMB080 | SSR | 167 | x | 55 | |
| 96 | DMBSSR105 | SSR | 125 | x | 55 | |
| 97 | DMBSSR119 | SSR | 132 | x | 55 | |
| 98 | DMBSSR125 | SSR | 134 | x | 54 | |
| 99 | DMBSSR130 | SSR | 132 | x | 53 | |
| 100 | DMBSSR136 | SSR | 133 | x | 54 | |
| 101 | DMBSSR135 | SSR | 187 | x | 54 | |
| 102 | DMBSSR101 | SSR | 127 | x | 54 | |
| 103 | DMBSSR098 | SSR | 198 | x | 53 | |
| 104 | cp02661 | SSR | 199 | 1 | 55 | |
| 105 | cp06039 | SSR | 208 | 1 | 56 | |
| 106 | cp04220 | SSR | 282 | 1 | 58 | |
| 107 | cp06173 | SSR | 228 | 1 | 57 | |
| 108 | cp03715 | SSR | 156 | 2 | 58 | Isemura et al. (2012) |
| 109 | cp03853 | SSR | 142 | 2 | 58 | |
| 110 | cp10211 | SSR | 270 | 3 | 56 | |
| 111 | cp01713 | SSR | 321 | 1 | 56 | |
| 112 | cp07770 | SSR | 290 | 1 | 58 | |
| 113 | cp00674 | SSR | 343 | 4 | 58 | |
| 114 | cp09781 | SSR | 422 | 6 | 58 | |
| 115 | cp06427 | SSR | 412 | 7 | 58 | |
| 116 | cp05325 | SSR | 195 | 10 | 55 | |
| 117 | cp05914 | SSR | 181 | 10 | 55 | |
| 118 | cp08695 | SSR | 265 | 11 | 55 | |
| 119 | DMBSSR26 | SSR | 117 | x | 55 | Hisano et al. (2007) |
| 120 | DMBSSR24 | SSR | 214 | x | 55 | |
| 121 | DMBSSR34 | SSR | 261 | x | 55 | |
| 122 | DMBSSR139 | SSR | 218 | x | 55 | |
| 123 | DMBSSR30 | SSR | 178 | x | 55 | |
| 124 | DMBSSR14 | SSR | 112 | x | 55 | |
| 125 | DMBSSR20 | SSR | 178 | x | 55 | |
| 126 | DMBSSR1 | SSR | 115 | x | 55 | |
| 127 | DMBSSR18 | SSR | 117 | x | 55 | |
| 128 | DMBSSR16 | SSR | 214 | x | 55 | |
| 129 | DMBSSR38 | SSR | 178 | x | 55 | |
| 130 | GMES4400 | SSR | 275 | 1 | 58 | |
| 131 | GMES3004 | SSR | 266 | 1 | 58 | |
| 132 | GMES0504 | SSR | 225 | 1 | 58 | |
| 133 | GMES2225 | SSR | 272 | 1 | 58 | |
| 134 | GMES0477 | SSR | 318 | 2 | 58 | |
| 135 | GMES1303 | SSR | 434 | 2 | 58 | |
| 136 | GMES5823 | SSR | 149 | 2 | 58 | |
| 137 | GMES0214 | SSR | 490 | 2/3 | 58 | |
| 138 | GMES5091 | SSR | 251 | 3 | 58 | |
| 139 | GMES2040 | SSR | 463 | 3 | 58 | |
| 140 | GMES1820 | SSR | 306 | 4 | 58 | |
| 141 | GMES6901 | SSR | 430 | 4 | 58 | |
| 142 | GMES2063 | SSR | 363 | 5/3 | 58 | |
| 143 | GMES3515 | SSR | 280 | 5 | 58 | |
| 144 | GMES1028 | SSR | 372 | 6 | 58 | |
| 145 | GMES1823 | SSR | 282 | 6 | 58 | |
| 146 | GMES0969 | SSR | 185 | 7 | 58 | |
| 147 | GMES5773 | SSR | 334 | 7 | 58 | |
| 148 | GMES4431 | SSR | 227 | 10 | 58 | |
| 149 | GMES5010 | SSR | 229 | 1 | 57 | |
| 150 | PV-ggc001 | SSR | 240 | 10 | 58 | Yu et al. (2000) |
| 151 | GBssr-MB7 | SSR | 300 | 6 | 53 | Gwag et al. (2000) |
| 152 | GBssr-MB87 | SSR | 250 | x | 54 | |
| 153 | GBssr-MB13 | SSR | 200 | x | 53 | |
| 154 | GBssr-MB17 | SSR | 160 | x | 55 | |
| 155 | GBssr-MB91 | SSR | 180 | x | 54 | |
| 156 | BM212 | SSR | 216 | 9 | 53 | Gaitan-Solis et al. (2002) |
| 157 | BGA9 | SSR | 158 | x | 53 | |
| 158 | BAT49 | SSR | 190 | x | 55 | 159 |
| BAT50 | | SSR | 187 | x | 55 | |
| 160 | BAT82 | SSR | 160 | x | 54 | |
| 161 | BAT76 | SSR | 271 | x | 57 | |
| 162 | BAT68 | SSR | 176 | x | 58 | Gaitan-Solis et al. (2002) |
| 163 | BAT44 | SSR | 187 | x | 58 | |
| 164 | VrD1 | SSR | 252 | 1 | 58 | Chen et al. (2004) |

| (1) | (2) | (3) | (4) | (5) | (6) | (7) |
|---|---|---|---|---|---|---|
| 165 | STSBr1 | STS | 225 | x | 54 | Sarkar et al. (2011) |
| 166 | STSBr2 | STS | 345 | x | 50 | |
| 167 | VR01 | SSR | 145 | x | 55 | Tangpha-tsornruang et al. (2009) |
| 168 | VR03 | SSR | 138 | x | 55 | |
| 169 | VR04 | SSR | 324 | x | 55 | |
| 170 | VR05 | SSR | 132 | x | 55 | |
| 171 | VR07 | SSR | 325 | x | 55 | |
| 172 | VR08 | SSR | 127 | x | 55 | |
| 173 | VR09 | SSR | 291 | x | 55 | |
| 174 | VR010 | SSR | 237 | x | 55 | |
| 175 | VR011 | SSR | 139 | x | 55 | |
| 176 | VR012 | SSR | 100 | x | 55 | |
| 177 | ‘VR016 | SSR | 355 | x | 55 | |
| 178 | VR021 | SSR | 282 | x | 55 | |
| 179 | VR024 | SSR | 223 | x | 55 | |
| 180 | VR025 | SSR | 195 | x | 55 | |
| 181 | VR031 | SSR | 306 | x | 55 | |
| 182 | VR032 | SSR | 306 | x | 55 | |
| 183 | VR037 | SSR | 101 | x | 55 | |
| 184 | VR038 | SSR | 218 | x | 55 | |
| 185 | VR049 | SSR | 147 | x | 55 | |
| 186 | VR056 | SSR | 210 | x | 55 | |
| 187 | VR059 | SSR | 263 | x | 55 | |
| 188 | VR060 | SSR | 309 | x | 55 | |
| 189 | VR066 | SSR | 122 | x | 55 | |
| 190 | VR068 | SSR | 108 | x | 55 | |
| 191 | VR072 | SSR | 106 | x | 55 | |
| 192 | VR074 | SSR | 157 | x | 55 | |
| 193 | VR075 | SSR | 174 | x | 55 | |
| 194 | VR077 | SSR | 110 | x | 55 | |
| 195 | VR081 | SSR | 192 | x | 55 | |
| 196 | VR082 | SSR | 182 | x | 55 | |
| 197 | VR083 | SSR | 103 | x | 55 | |
| 198 | VR085 | SSR | 366 | x | 55 | |
| 199 | VR087 | SSR | 284 | x | 55 | |
| 200 | VR088 | SSR | 110 | x | 55 | |
| 201 | VR089 | SSR | 207 | x | 55 | |
| 202 | VR091 | SSR | 117 | x | 55 | |
| 203 | VR092 | SSR | 152 | x | 55 | |
| 204 | VR096 | SSR | 185 | x | 55 | |
| 205 | VR097 | SSR | 369 | x | 55 | |
| 206 | VR0100 | SSR | 233 | x | 55 | |
| 207 | VR0103 | SSR | 154 | x | 55 | |
| 208 | VR0107 | SSR | 227 | x | 55 | |
| 209 | VR0112 | SSR | 125 | x | 55 | |
| 210 | VR0117 | SSR | 107 | x | 55 | |
| 211 | VR0118 | SSR | 130 | x | 55 | |
| 212 | VR0120 | SSR | 146 | x | 55 | |
| 213 | VR0125 | SSR | 188 | x | 55 | |
| 214 | CEDG256 | SSR | 238 | 1 | 58 | Wang et al. (2004) |
| 215 | CEDG044 | SSR | 150 | 11 | 58 | |
| 216 | MYMVR-583 | SCAR | 545 | x | 58 | Dhole and Reddy ( 2012) |
| 217 | RGASF1/SR1 | RGA | 456 | x | 58 | Maiti et al. (2011) |
| 218 | RGA22F2/24R2 | RGA | 1236 | x | 58 | |
| 219 | RGA-1-F-CGb/R | RGA GA-1-R | 455 | x | 50 | |
| 220 | RGA-1-F-TG/RGA-1-R | RGA | 450 | x | 50 | |
| 221 | RGA-4 | RGA | 423 | x | 50 | |
| 222 | RGA-5 | RGA | 433 | x | 50 | |
| 223 | RGA-6 | RGA | 435 | x | 50 | |
| 224 | RGA-8 | RGA | 420 | x | 50 | |

x= Linkage group not assigned