Published online July 11, 2018

RESEARCH

Mapping of Duplicate Dominant Genes for *Mungbean yellow mosaic India virus* Resistance in *Glycine soja*

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ABSTRACT

Glycine soja Siebold & Zucc. PI 393551 carries gene(s) for resistance to yellow mosaic disease, which is caused by *Mungbean yellow mosaic India virus* (MYMIV). Yellow mosaic disease is a serious constraint to soybean [*Glycine max* (L.) Merr.] production in India. However, the gene(s) imparting resistance to MYMIV in *G. soja* have not yet been mapped. In the present study, three F_2 populations derived from crosses of the three *G. max* cultivars 'Ankur', 'Sawarn Vasundhra', and 'JS335' with PI 393551, the donor for MYMIV resistance, were phenotyped for reaction to MYMIV at the geographic hot spot for yellow mosaic disease to determine inheritance of MYMIV resistance in PI 393551. All three F_2 populations exhibited a 15:1 ratio of individuals resistant and susceptible to MYMIV, indicating duplicate dominant inheritance of MYMIV resistance genes. Further, a large F_2 population (1520 plants) reconstructed from JS335 × PI 393551 was used for mapping MYMIV resistance genes. Bulked segregant analysis identified two genomic regions associated with MYMIV resistance, one on chromosome 8, and another on chromosome 14. A total of 78 plants with 100% MYMIV infection, which were expected to be homozygous for recessive genes, were genotyped using polymorphic simple sequence repeat (SSR) markers near linked markers on chromosome 8 and 14. Genetic analyses revealed tight linkages of MYMIV resistance with SSR marker BARCSOYSSR_08_0867 (15,434,295 bp) on chromosome 8, and with BARCSOYSSR_14_1416 (47,686,933 bp) and BARCSOYSSR_14_1417 (47,738,940 bp) on chromosome 14. The identified SSR markers that are tightly linked to MYMIV resistance genes will be useful for introgression of MYMIV resistance from *G. soja* into *G. max*.

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Abbreviations: InDel, insertion-deletion; miRNA, microRNA; MYMIV, *Mungbean yellow mosaic India virus*; MYMV, *Mungbean yellow mosaic virus*; PCR, polymerase chain reaction; SSR, simple sequence repeat; YMD, yellow mosaic disease.

Soybean [*Glycine max* (L.) Merr.] is the leading oilseed crop in India. In 2016, 11.27 million ha of soybean were grown, which contributed 14.22 Tg to total oilseed production in India (23.66 Tg, http://www.agricoop.nic.in). Further, the soybean crop brings substantial foreign exchange through the export of soy meal from India and has uplifted the rural economy of central India. Domestic consumption of soy meal has increased from 5000 metric tons in 1964 to 4.97 Tg in 2016. However, soybean productivity in India hovers around 1000 kg ha^{-1}, which is much lower than the world average of 2500 kg ha^{-1}. Among several biotic and abiotic factors that appear to be responsible for the low productivity of soybean in India, yellow mosaic disease (YMD) is a major biotic factor affecting soybean yield (Bhatia and Sharma, 2016).

Yellow mosaic virus is one of the most destructive and widely distributed plant pathogenic viruses in the family *Geminiviridae*. This virus causes YMD in legumes and induces typical yellow and golden mosaic symptoms in several legume crops, including soybean (*G. max*), blackgram [*Vigna mungo* (L.) Hepper], mungbean [*Vigna radiata* (L.) R. Wilczek], and cowpea [*Vigna unguicula* (L.) Walp.] (Varma et al., 1992). The virus is transmitted

Published in Crop Sci. 58:1566–1574 (2018).
doi: 10.2135/cropsci2017.11.0652

by the white fly (*Bemisia tabaci* Genn.) (Nariani, 1960; Nene, 1972, 1973), and *Yellow mosaic virus* particles contain bipartite, single-stranded, circular DNA genome known as DNA A and DNA B (Lazarowitz, 1992). Both of these genomes encode necessary components for replication, movement, and symptom development and are 2.5 to 2.7 kb in size (Lazarowitz, 1992; Gutierrez, 1999; Hanley-Bowdoin et al., 1999). Based on nucleotide sequence data of the genomic components of yellow mosaic viruses, two distinct begomoviruses, *Mungbean yellow mosaic India virus* (MYMIV; Mandal et al., 1997) and *Mungbean yellow mosaic virus* (MYMV; Morinaga et al., 1990) were suggested to be associated in the etiology of YMD in legumes in India and South Asia. Nucleotide sequences of a virus isolated from soybean plants affected by YMD in India showed 89% similarity with MYMIV; thus, the virus was designated as a soybean isolate of MYMIV (MYMIV-[Sb]) by Usharani et al. (2004). *Mungbean yellow mosaic India virus* has been reported to infect soybean in Vietnam and Indonesia (Tsai et al., 2013) and, more recently, kidney beans (*Phaseolus vulgaris* L.) in Oman (Shahid et al., 2016). A large survey of different soybean-growing locations in India using a polymerase chain reaction (PCR)-based assay showed that MYMIV is the prevalent virus infecting soybean in northern and central India (Ramesh et al., 2016a).

Glycine soja accession PI 393551, introduced in India courtesy of K.L. Chan of the Taiwan Agricultural Research Institute, Taipei, possesses resistance to yellow mosaic disease (Singh et al., 1974). This accession from Taiwan is available from the USDA Soybean Germplasm Collection and the National Agriculture and Food Research Organization (NARO) Genebank of Japan. PI 393551 is a typical wild-type soybean with very narrow leaves and indeterminate growth habit. It flowers in 85 d and reaches maturity in ∼130 d at Indore, India (22°43′10.4448″ N, 75°51′27.8172″ E). *Glycine soja* can be easily crossed with cultivated soybeans (Ram et al., 1984), as both contain same number of chromosomes ($2n = 40$) and produce vigorous fertile intermediate F_1 hybrids (Singh and Hymowitz, 1999). Moreover, new and unique genes for high yield (Wang et al., 2004), high protein (Nichols et al., 2006), high linolenic acid (Pantalone et al., 1997), resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe; Kabelka et al., 2005), and tolerance to salt (Lee et al., 2009) have been reported in *G. soja* accessions. Therefore, *G. soja*, the progenitor of *G. max*, is an excellent source of genetic variability, although it does possess several undesirable genetic traits such as vining, lodging, susceptibility to pod shattering, lack of complete leaf abscission, and small seed size with black seed coat (Carpenter and Fehr, 1986).

Various efforts have been made to understand the mechanism of natural resistance to YMD in *G. soja* and *G. max*. Ramesh et al. (2016b) conducted in silico analysis of the DNA genomes of MYMIV and MYMV using a microRNA (miRNA) target prediction algorithm at the plant small RNA target analysis server psRNATarget (Dai and Zhao, 2011; http://plantgrn.noble.org/psRNATarget/). The MYMIV genome was vulnerable to 70 plant miRNAs that could target its genome. There were 18 potential target sites for *G. soja*-derived miRNAs and 63 potential target sites for *G. max*-derived miRNAs in the begomovirus genomes. Yadav et al. (2009) reported accumulation of late viral transcripts and DNA replication in a susceptible cultivar and rapid degradation of early viral RNAs in resistant cultivars. This rapid degradation of the early viral transcripts, possibly through a small interfering RNA mechanism, could be a mechanism of natural resistance against geminivirus.

Several studies have examined the inheritance of MYMIV resistance in *G. max*. Rani et al. (2017) reported MYMIV resistance controlled by a single recessive gene in PI 171443, Singh and Mallick (1978) reported MYMIV resistance controlled by double recessive genes in PI 171443, and Talukdar et al. (2013) reported MYMIV resistance controlled by a single dominant gene in YMD-resistant cultivars 'DS97-12' and 'DS98-14'. One report by Bhattacharyya et al. (1999) indicated a single dominant gene controlling YMD resistance gene in *G. soja* accession PI 393551; however, this result has not been further validated. Therefore, the inheritance of MYMIV resistance in *G. soja* was verified in the present study before introgressing the resistance genes into cultivated varieties of soybean. *Mungbean yellow mosaic India virus* resistance from the PI 171443 accession of *G. max* has been used in several soybean cultivars developed for the northern plains of India. There have been no reports of MYMIV resistance from *G. soja* in cultivar development, mainly because many backcrosses are required to introgress these genes into *G. max* cultivars, as most of the *G. soja* genome is not useful in cultivated soybean. Backcrossing without a marker linked closely to a desirable trait is very difficult because segregating material that is generated for introgressing an MYMIV resistance gene into high-yielding adapted cultivars must be screened at hot spots or under artificial conditions. Several reports of molecular markers linked to MYMIV resistance in *G. max* are available (Yadav et al., 2015; Kumar et al., 2015; Rani et al., 2017), and the linked markers identified by Rani et al. (2017) are being actively deployed for introgression of an MYMIV resistance gene into the predominant cultivars used in central India. However, no attempt has been made to map MYMIV resistance gene(s) in *G. soja*. Identification of DNA markers closely linked to MYMIV resistance genes in *G. soja* would help to accelerate introgression of MYMIV resistance genes from *G. soja* into cultivated varieties of soybean. The purpose of the present study was to determine the inheritance of MYMIV resistance gene(s)

in *G. soja* PI 393551 and to identify molecular markers closely linked to the gene(s) for accelerated introgression of the trait into cultivated varieties of soybean.

MATERIALS AND METHODS

Development of Mapping Populations

Three YMD-susceptible soybean genotypes—'JS335', 'Ankur', and 'Sawarn Vasundhra'—were crossed with *G. soja* accession PI 393551, the donor of YMD resistance. JS335, released in 1994, is commonly grown in central India and is resistant to bacterial pustule [*Xanthomonas axonopodis* pv. *glycines* (Nakano) Vauterin et al.] and bacterial blight [*Pseudomonas savastanoi* pv. *glycinea* (Coerper) Gardan et al]. Ankur was released in 1976 for northern plains of India and is resistant to bacterial pustule and tolerant to soybean rust [*Phakopsora meibomiae* (Arthur) Arthur]. Sawarn Vasundhra was released in 2008 for use as a vegetable and is an elite germplasm line (EC 384907) introduced by the Asian Vegetable Research and Development Center (AVRDC) in Taiwan. Concurrently, the F_1 derived from a cross between JS335 and PI 393551 was backcrossed to JS335, and resistant BC_1F_1 plants were again backcrossed to JS335 at Ludhiana, Punjab, India, a hotspot for YMD. A YMD-resistant BC_2F_1 plant that was morphologically similar to JS335 was chosen and was again backcrossed to JS335. One YMD-resistant BC_3F_1 plant similar to JS335 was identified and was allowed to self, and then BC_3F_2 plants were phenotyped and genotyped. A large F_2 population consisting of 1520 plants was reconstructed by crossing JS335 with PI 393551 for mapping.

Phenotyping for Reaction to MYMIV

F_2 populations derived from JS335 × PI 393551, Ankur × PI 393551 and Sawarn Vasundhra ×× PI 393551, BC_3F_2 populations, and reconstructed F_2 populations derived from JS335 × PI 393551 were raised at Ludhiana, a hotspot for YMD in soybean, with a row of susceptible genotypes planted as an infector every third row. Rows were 3 m in length, with row-to-row and plant-to-plant spacing of 45 and 5 cm, respectively. Phenotyping was performed at the R5 stage when YMD symptoms were severe. Plants with no infection or small yellow specks on only one or two leaves were classified as resistant, whereas plants with the majority of leaf area and leaves affected were classified as susceptible (Fig. 1). For the purpose of mapping, only those F_2 plants that showed YMD symptoms on 100% of leaves were considered susceptible. To confirm the species of virus affecting these plants, DNA was extracted and amplified with MYMIV- and MYMV-specific primers designed by Ramesh et al. (2016a).

Molecular Marker Analysis

DNA was extracted from the parental genotypes JS335 and PI 393551 and from F_2 and BC_3F_2 plants of their cross using the method of Doyle and Doyle (1990). A parental polymorphism survey was performed using 15 to 25 primer pairs from each linkage group so as to obtain at least seven regularly spaced polymorphic markers. A total of 156 polymorphic simple sequence repeat (SSR) markers regularly spaced on the 20 soybean genome linkage groups were used for the analysis. The SSR marker sequences were taken from integrated linkage maps published by Hyten et al. (2010) and Song et al. (2010).

Quantified DNA was subjected to PCR amplification in 10-μL reactions containing 2 μL DNA (25 ng μL^{-1}), 1 μL PCR 10× buffer, 1.1 μL $MgCl_2$ (25 mM), 0.1 μL deoxynucleotides (25 mM), 0.4 μL of each forward and reverse SSR primer (30 ng μL^{-1}), 0.068 μL *Taq* DNA polymerase (3 units μL^{-1}), and 4.932 μL distilled water. DNA was denatured at 94°C for 2 min, followed by 30 cycles each of denaturation at 94°C for 1 min, primer annealing at 50°C for 2 min, extension at 72°C for 3 min, and final extension at 72°C for 10 min in the thermocycler (ProFlex PCR System, Model 4484073, ThermoFisher Scientific). Amplified products were resolved on 3% MetaPhor agarose gels (Lonza Groups) along with a 50-bp ladder (BR Biochem LifeSciences) for allele sizing, stained with ethidium bromide to visualize amplification products, and analyzed using a gel documentation system (Syngene, Gene Genius, Bioimaging System). The SSR bands scored manually on gels were denoted as S, R, or H, where S represents a band from the susceptible parent only, R represents a band from the resistant parent only, and H represents bands from both parents (heterozygote).

Bulked Segregant Analysis

Bulked segregant analysis of the JS335 × PI 393551 F_2 was performed following the protocol prescribed by Michelmore et al. (1991). The resistant bulk was created by mixing equal amounts of DNA from 30 resistant plants, whereas the susceptible bulk was created in the same manner from 30 susceptible plants. The bulked DNAs from resistant and susceptible plants, along with DNA from their parents, were subjected to PCR using seven to eight regularly spaced polymorphic SSR primers from each linkage group to identify SSR markers linked to MYMIV resistance.

Data Analysis and Genetic Mapping

A total of 78 highly susceptible F_2 plants of JS335 × PI 393551 were assayed individually following the method of Yao et al. (1997) with linked markers identified in bulked segregant analysis and additional molecular markers present in its proximity. Nine polymorphic SSR markers including one expressed sequence tag-derived SSR marker (Hisano et al., 2007) and one insertion-deletion (InDel) marker (Song et al., 2015) near a linked marker were assayed on chromosome 14, and 12 polymorphic SSR markers near a linked marker were assayed on chromosome 8. The frequency of recombination between a marker locus and the resistance loci was calculated using the maximum likelihood estimator (Allard, 1956), assuming that all of the highly susceptible individuals were homozygous recessive for the targeted resistance loci. All BC_3F_2 plants were also genotyped with linked markers for confirmation.

RESULTS

Phenotyping Mapping Populations for Yellow Mosaic Disease Reaction to Study Inheritance of Resistance

The parental lines, F_1, and F_2 plants derived from the crosses described above were screened for YMD reaction

Fig. 1. Representative photographs of three categories of reaction to *Mungbean yellow mosaic India virus* on F_2 population of JS335 × PI 393551: (A) plants with most leaves affected, (B) small yellow specks on only one or two leaves, and (C) plants with no infection.

in a field under epiphytotic conditions with abundant white fly populations and infector rows of a susceptible cultivar. The susceptible parental genotypes—JS335, Ankur, and Sawarn Vasundhra—showed susceptible reactions to YMD, whereas *G. soja* accession PI 393551 showed a resistant reaction. Amplification of a 391-bp fragment only from susceptible plants with a MYMIV-specific primer confirms the presence of MYMIV infecting these plants. The F_1 plants exhibited resistance, indicating the dominant nature of YMD resistance. A total of 380, 406, and 310 F_2 plants from the crosses JS335 × PI 393551, Ankur × PI 393551, and Sawarn Vasundhra × PI 393551, respectively, were tested for YMD reaction, as shown in Table 1. The observed segregation patterns fit a ratio of 15 resistant to one susceptible, which indicates likely duplicate dominant genes controlling MYMIV resistance in PI 393551. However, Bhattacharyya et al. (1999) reported monogenic inheritance of the trait in the same *G. soja* accession. The discrepancy between these results could be due to differences in the manner of classifying resistant and susceptible phenotypes. In the present investigation, the plants showing small yellow specks on one or two leaves were classified as resistant (Fig. 1), similar to the reaction observed in the resistant parent. In contrast, Bhattacharyya et al. (1999) classified F_2 plants as resistant only if they showed a completely immune response. Phenotyping of reconstructed F_2 population of JS335 × PI 393551 consisting of 1520 plants categorized 90 plants as susceptible and 1408 as resistant. Twenty-two plants did not fit into any category. Data from such plants were not considered in the study to avoid confusion. The BC_3F_2 population developed from the cross JS335 × PI 393551 by repeated backcrossing to JS335 segregated in a ratio of three resistant to one susceptible, indicating segregation of only one MYMIV resistance gene from PI 393551 in this population. It appears that one of the genes responsible for MYMIV resistance was lost on repeated backcrossing to recurrent parent JS335.

Identifying Potential Chromosomal Regions Containing MYMIV Resistance Genes

A parental polymorphism survey performed for the resistant parent PI 393551 and susceptible parent JS335 using 410 SSR markers spanning the 20 chromosomes of the soybean genome revealed 180 polymorphic SSR markers. A total of 156 regularly spaced polymorphic SSR markers across these 20 soybean linkage groups were chosen for genotyping the resistant bulk, the susceptible bulk, and the parents JS335 and PI 393551. Bulked segregant analysis of the F_2 from JS335 × PI 393551 led to the identification of an SSR marker, BARCSOYSSR_08_0867 (15,434,295 bp), on chromosome 8 (LG A2) and another SSR marker, BARCSOYSSR_14_1416 (47,686,933 bp), on chromosome 14 (LG B2) that were linked to MYMIV resistance. A total of 78 highly susceptible F_2 plants from JS335 × PI 393551, assumed to be homozygous recessive

Table 1. Chi-squared test for segregation of *Mungbean yellow mosaic India virus* (MYMIV) resistance gene(s).

Type of population	Cross combination	No. of plants	MYMIV reaction		Expected ratio	χ^2	*P*-value
			Susceptible	Resistant			
F_2	JS335 × PI 393551	380	28	352	15:1	1.103	0.20
F_2	Ankur × PI 393551	406	30	376	15:1	0.899	0.30
F_2	Sawarn Vasundhra × PI 393551	310	23	287	15:1	0.724	0.30
F_2	JS335 × PI 393551 (reconstructed)	1520	90	1408	15:1	0.148	0.70
BC_3F_2	JS335 × PI 393551	98	25	73	3:1	0.014	0.90

for duplicate dominant genes, were then genotyped using these two markers (Fig. 2). The parental polymorphism survey was then analyzed for all of the markers within 30 cM flanking the markers linked to MYMIV resistance. A total of nine SSR markers and one InDel marker on chromosome 14, in addition to the linked marker BARCSOYSSR_14_1416 identified in our study, were found to be polymorphic (Table 2). Further, 12 SSR markers on chromosome 8 in addition to the linked marker BARCSOYSSR_08_0867 were found to be polymorphic (Table 3). All of these markers were tested for segregation distortion by genotyping 96 random samples from the F_2 of JS335 × PI 393551. All of these markers except for BASRCSOYSSR_08_0926 segregated in the expected ratio of 1:2:1. The mapping population of 78 F_2 homozygous recessive plants was then genotyped using all of these polymorphic markers on chromosomes 8 and 14, except BARCSOYSSR_08_0926, near the markers linked to MYMIV resistance.

Determining the Map Locations of the Two Resistance Loci

The recombination frequencies between SSR markers and the MYMIV resistance loci were calculated using the maximum likelihood estimator (Tables 2 and 3). Map distances were obtained by converting recombination frequencies to centimorgans using the Kosambi (1944) mapping function. The order of DNA markers based on their genetic map positions in the present study was same as on the physical map of Song et al. (2010). The data clearly showed that one of the resistance loci (R_1) present on chromosome 8 is closely linked to the BARCSOYSSR_08_0867 SSR marker (Fig. 3A). Another resistance locus (R_2) is located between Satt063 and BARCSOYSSR_14_1422 on chromosome 14 and is tightly linked to BARCSOYSSR_14_1416 and BARCSOYSSR_14_1417 (Fig. 3B). The BC_3F_2 population derived from JS335 × PI 393551 was genotyped with linked markers for both MYMIV resistance loci R_1 and R_2. Genotyping of BC_3F_2 plants with linked markers showed

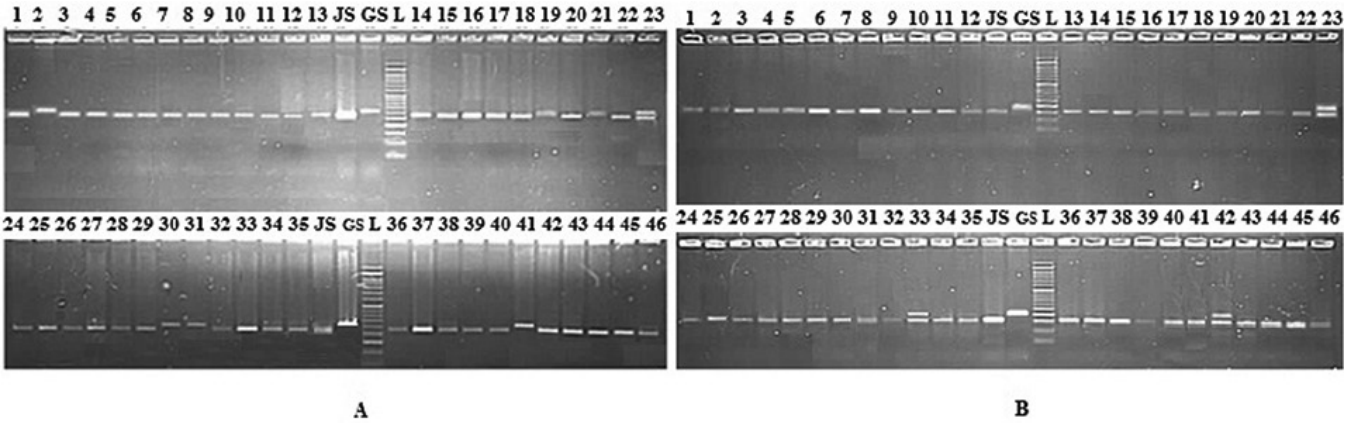

Fig. 2. (A) Polymerase chain reaction (PCR) amplification of BARCSOYSSR_08_0867 on chromosome 8 from DNA of F_2 susceptible plants derived from JS335 × PI 393551, and (B) PCR amplification of BARCSOYSSR_14_1416 on chromosome 14 from DNA of F_2 susceptible plants derived from JS335 × PI 393551. Lanes marked JS represent JS335, lanes marked GS represent *G. soja*, Lanes 1 to 46 represent susceptible plants, and lanes marked L represent the 50-bp ladder DNA size standard.

Table 2. Maximum likelihood estimates of recombination frequencies and genetic distance between markers and the *Mungbean yellow mosaic India virus* resistance gene calculated using the highly susceptible plants, assuming that these plants are homozygous for the recessive allele at the R_2 locus on chromosome 14.

Locus	Physical position (Glyma.Wm82.a1.v1)	Physical position (Glyma.Wm82.a2.v1)	Recombination frequency ± SE	Map distance
			%	cM
Satt687	49,404,136–49,404,162	48,365,147–48,365,173	10.24 ± 2.4	10.38
CSSR377	48,621,990–48,622,013	47,940,909–47,940,947	7.68 ± 2.1	7.74
SSR0169	47,886,063–47,886,090	47,206,947–47,206,974	0.64 ± 0.6	0.64
BARCSOYSSR_14_1422	47,856,087–47,856,122	47,176,971–47,177,006	0.64 ± 0.6	0.64
BARCSOYSSR_14_1417	47,738,940–47,738,961	47,059,638–47,059,659	0.00	0.00
BARCSOYSSR_14_1416	47,686,933–47,686,956	47,007,588–47,007,611	0.00	0.00
Satt063	46,705,840–46,705,899	45,993,741–45,993,800	10.24 ± 2.4	10.38
GMES 6175	46,264,020 (Kazusa soymarker)	46,264,020 (Kazusa soymarker)	21.76 ± 3.3	23.31
Sct_064	45,520,897–45,520,928	44,799,009–44,799,040	21.76 ± 3.3	23.31
MOL0781	41,875,710	NA†	25.6 ± 3.5	27.76
Satt556	39,579,320–39,579,361	38,859,494–38,859,535	32.0 ± 3.7	37.9

† NA, not available.

Table 3. Maximum likelihood estimates of recombination frequencies and genetic distance between markers and the *Mungbean yellow mosaic India virus* resistance gene calculated using the highly susceptible plants, assuming that these plants are homozygous for the recessive allele at the R_1 locus on chromosome 8.

Locus	Physical position (Glyma.Wm82.a1.v1)	Physical position (Glyma.Wm82.a2.v1)	Recombination frequency ± SE	Map distance
	bp		%	cM
Sat_250	18,674,587–18,674,624	18,611,862–18,611,899	30.72 ± 3.6	35.37
CSSR216	16,748,191–16,748,210	16,682,609–16,682,654	12.8 ± 2.5	12.8
BARCSOYSSR_08_0867	15,434,295–15,434,316	15,365,593–15,365,614	0.64 ± 0.6	0.64
BARCSOYSSR_08_0862	15,354,561–15,354,582	15,285,858–15,285,879	1.28 ± 0.9	1.28
BARCSOYSSR_08_0855	15,225,415–15,225,436	15,156,824–15,156,845	1.28 ± 0.9	1.28
BARCSOYSSR_08_0851	15,150,268–15,150,327	15,081,632–15,081,777	1.92 ± 1.1	1.92
BARCSOYSSR_08_0848	15,108,761–15,108,780	15,040,125–15,040,144	1.92 ± 1.1	1.92
BARCSOYSSR_08_0801	14,574,617–14,574,644	NA†	3.84 ± 1.5	3.84
BARCSOYSSR_08_0764	13,971,043–13,971,062	13,901,752–13,901,771	7.68 ± 2.1	7.74
CSSR571	11,868,973–11,869,016	11,786,820–11,786,863	16 ± 2.9	16.58
Satt424	10,721,723–10,721,881	10,633,708–10,633,866	24.32 ± 3.4	26.54
Satt187	9,199,863–9,199,916	9,192,592–9,192,645	30.72 ± 3.6	35.79

† NA, not available.

that the population inherited only the resistance locus present on chromosome 14. The monogenic inheritance observed in this genotyping study corroborates phenotyping data showing segregation for a single gene. The molecular markers BARCSOYSSR_14_1416 and BARCSOYSSR_14_1417 that were linked to MYMIV resistance in the F_2 mapping population also showed complete linkage to the resistance locus in the BC_3F_2 population.

DISCUSSION

Yellow mosaic disease is a serious and widespread disease of soybean in northern India, parts of southern India,

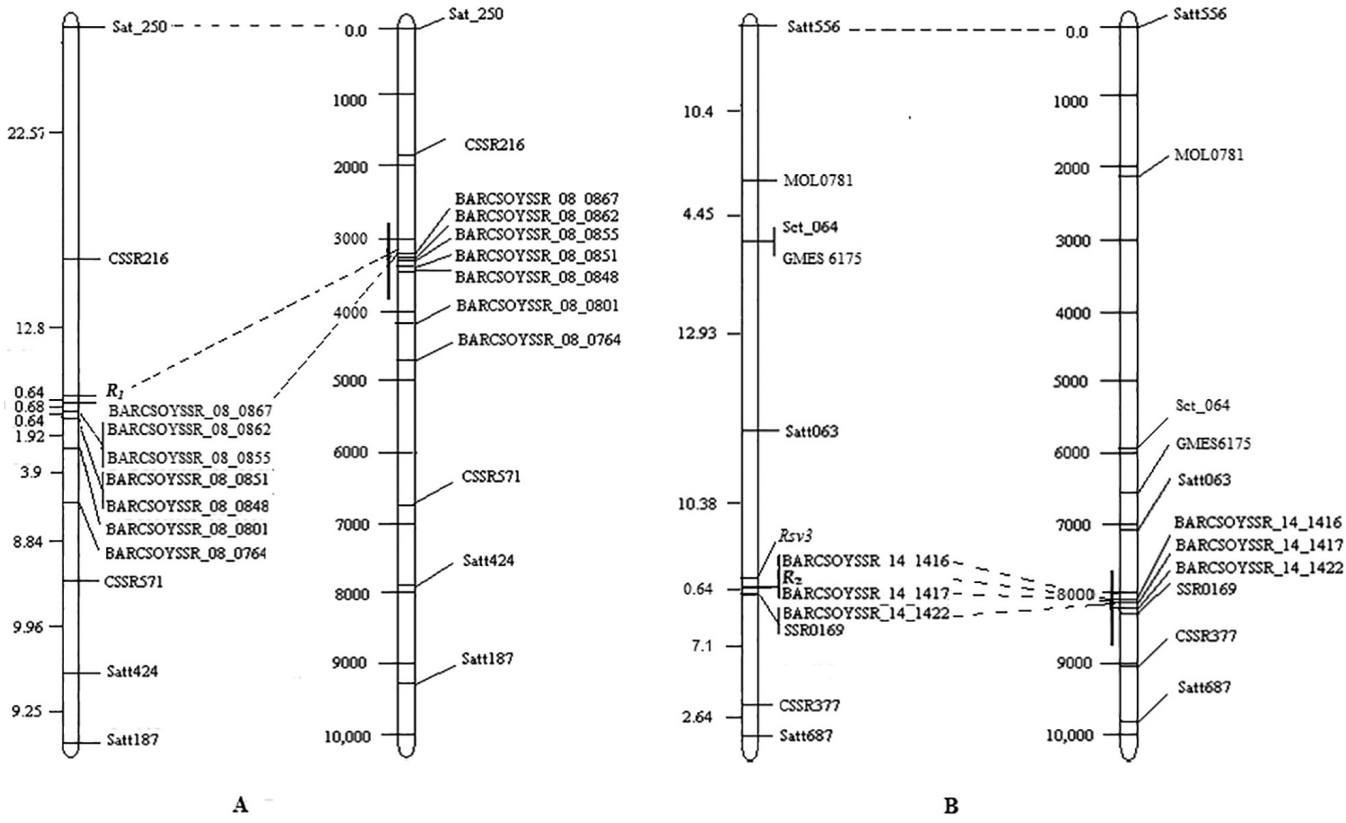

Fig. 3. Genetic and physical maps in the vicinity of the *Mungbean yellow mosaic India virus* resistance genes, (A) R_1 and (B) R_2, on soybean chromosomes 8 and 14, respectively. Values on the left side of the genetic maps are map distances in centimorgans. Values on the left side of the physical map are the physical distance from Sat_250 in kilobase pairs. The bar on the left side of the physical map indicates the genetic region containing the R_1 locus. R_1 and BARCSOYSSR_08_0867 are connected by dotted lines to show the R_1–containing chromosomal region. Values on the left side of the physical map are the physical distance from Satt556 in kilobase pairs. The bar on the left side of the sequence map indicates the genetic region containing the R_2 locus, BARCSOYSSR_14_1416. R_2 and BARCSOYSSR_14_1417 are connected by dotted lines to show the R_2–containing chromosomal region.

and in Sri Lanka, Bangladesh, Pakistan, and Thailand. The magnitude of yield loss due to this disease has been reported to be as high as 80% (Nene, 1972). More recently, the virus has spread to the hub of soybean cultivation in central India and was the main reason for low productivity of soybean in 2015 (Bhatia and Sharma, 2016). To date, *G. max* accession PI 171443 has been the donor of MYMIV resistance for all MYMIV-resistant soybean cultivars developed so far through traditional breeding. Although *G. soja* PI 393551 has been recognized as a source of YMD resistance since 1974 (Singh et al., 1974), its YMD resistance has not been introgressed into cultivated varieties due to the undesirable traits from its background of unimproved *G. soja*. However, these undesirable traits can be eliminated by selection during successive backcross generations (Singh and Hymowitz, 1999). The introgression of MYMIV resistance gene(s) into cultivated varieties will require a number of backcrosses to recover the genome of the cultivated variety. To retain the desired resistance gene(s) during this process in the absence of a suitable molecular marker, backcrossing should be performed in a geographic hot spot for YMD during the soybean growing season or under artificial inoculation. Thus, understanding the nature of inheritance of MYMIV resistance and identifying molecular markers linked to the resistance gene(s) would accelerate introgression of the trait. A previous study of the inheritance of MYMIV resistance demonstrated that it is controlled by a single dominant gene (Bhattacharyya et al., 1999). Our results also validate the dominant nature of the trait, but we also observed duplicate gene action in this study. This discrepancy could be due to differences between these two studies in the classification of resistant and susceptible plants. Another possible reason for the differences between these results is that MYMIV strains in each study could have responded differently to the resistance genes. The strain in the Bhattacharya study might have overcome one of the resistance genes, leading to a 3:1 segregation ratio. Various efforts have been made to map MYMIV resistance genes in *G. max*. Yadav et al. (2015) performed whole-genome sequencing of the MYMIV-susceptible cultivar JS335 and the resistant genotype UPSM534 (PI 171443) to identify genomic regions associated with MYMIV resistance. In that study, a single-nucleotide polymorphism on chromosome 18 showed a possible association with a MYMIV resistance gene. Kumar et al. (2015) found a region on chromosome 17 in significant linkage disequilibrium with MYMIV resistance in an association mapping study. Recently Rani et al. (2017) mapped a MYMIV resistance gene on chromosome 6 very close to the two SSR markers Satt322 and GMAC7L. As viruses continuously mutate to evade resistance mechanisms of host plants, it is essential to continue to map MYMIV resistance genes from new sources to meet future challenges.

In the present study, we identified one MYMIV resistance gene on chromosome 14 and another on chromosome

Table 4. Gene annotations of soybean chromosome 8 between 9,494,946 and 15,515,548 bp.

Gene name	Physical position	Gene annotation†
	bp	
Glyma08g13040	9,494,946–9,505,931	NB-ARC domain (NB-ARC)/protein tyrosine kinase (Pkinase_Tyr)/leucine rich repeat (LRR_8)
Glyma08g16380	11,895,122–11,901,647	Leucine-rich repeat-containing protein
Glyma08g20350	15,331,217–15,335,639	Leucine-rich repeat-containing protein
Glyma08g20581	15,509,858–15,515,548	Leucine-rich repeat-containing protein

† Gene annotation information was retrieved from the soybean gene annotation database accessible at Phytozome version 12.1 (http://www.phytozome.net). Only leucine-rich repeat (LRR), leucine-rich repeat receptor-like kinase (LRR-RLK), and NB-ARC domain (NB-ARC) genes are presented for the chromosomal regions between 9,494,946 and 15,515,548 bp.

Table 5. Gene and marker annotations of soybean chromosome 14 between 45,268,392 and 47,199,983 bp.

Gene name	Physical position	Gene annotation†
Glyma14g36630	45,268,392–45,271,692	Protein kinase domain (Pkinase)/leucine-rich repeat (LRR_1)/leucine-rich repeat N-terminal domain (LRRNT_2)/leucine rich repeat (LRR_8)
Glyma14g37860	46,435,759–46,438,392	Disease resistance protein RPP13-related
Glyma14g38500	46,946,496–46,957,734	Disease resistance protein rpp13-related
Glyma14g38516	46,968,705–46,974,585	Leucine-rich repeat-containing protein
Glyma14g38533	46,981,104–46,996,696	Disease resistance protein RPP13-related
Glyma14g38561	47,005,574–47,019,661	Disease resistance protein RPP13-related
Glyma14g38586	47,046,209–47,053,652	Disease resistance protein RPP13-related
Glyma14g38630	47,096,077–47,101,943	Protein kinase domain (Pkinase)/leucine rich repeat N-terminal domain (LRRNT_2)/leucine rich repeat (LRR_8)
Glyma14g38650	47,136,855–47,148,766	Leucine-rich repeat protein kinase-like protein
Glyma14g38670	47,152,323– 47,167,818	Leucine-rich repeat protein kinase-like protein
Glyma14g38700	47,181,077–47,199,983	Leucine-rich repeat-containing protein

† Gene annotation information was retrieved from the soybean gene annotation database accessible at Phytozome version 12.1 (http://www.phytozome.net). Only leucine-rich repeat (LRR), leucine-rich repeat receptor-like kinase (LRR-RLK), and disease resistance proteins are presented for the chromosomal region between 45,268,392 and 47,199,983 bp.

8. The gene on chromosome 14 is close to the SSR markers BARCSOYSSR_14_1416 and BARCSOYSSR_14_1417 in a euchromatin region 43.7 to 49 Mb in length. The average crossover frequency (cM Mb^{-1}) on chromosome 14 is 6.4 with a crossover frequency of 7.9 in the distal 25% region and of 0.15 in the proximal 25% region, with four recombination hotspots of crossover frequency > 14 (Ott et al., 2011). This genomic region also contains the *Rsv3* gene responsible for *Soybean mosaic virus* resistance (Jeong et al., 2002; Shi et al., 2008). The soybean genomic regions containing mapped genes are rich in nucleotide-binding site leucine-rich repeat-type resistance genes, the most characterized family of plant disease resistance genes (Tables 4 and 5). NBS_C, NBS_D, and NBS_E in this genomic region are the likely functional alleles of the *Rsv3* locus that confer resistance to *Soybean mosaic virus* (Suh et al., 2011; Redekar et al., 2016; Ma et al., 2017). Further studies are required to investigate whether the MYMIV resistance genes mapped in this study are related to the same gene family.

The SSR markers Satt322 and GMAC7L, previously reported as linked to MYMIV resistance in *G. max* (Rani et al., 2017), are being successfully deployed to introgress a MYMIV resistance gene from PI 171443 into the predominant cultivated varieties of central India. However, breakdown of resistance to plant disease can frequently occur, particularly when the resistance to a specific disease is conditioned by a single gene. Pyramiding of multiple resistance genes has been successfully applied for resistance to diseases such as bacterial blight *[Xanthomonas oryzae* (ex Ishiyama) Swings et al. pv. *oryzae* (ex Ishiyama) Swings et al.] (Huang et al., 2004) and blast (*Pyricularia oryzae* Cavara) (Hittalmani et al., 2000) in rice (*Oryza sativa* L.), and powdery mildew [*Blumeria graminis* (DC) Speer f. sp. *tritici* emend. É. J. Marchal] in wheat (*Triticum aestivum* L.) (Liu et al., 2000). Pyramiding several genes conferring resistance to a particular disease would be impossible without molecular markers in the absence of well-characterized strains of causal organisms. The molecular markers linked to MYMIV resistance in *G. soja* identified in the present study will be useful for pyramiding resistance genes from both *G. soja* and *G. max*. Several breeders have been successful in pyramiding disease resistance genes using marker-assisted selection, including genes for *Soybean mosaic virus* resistance by Saghai Maroof et al. (2008) and Shi et al. (2009). The tightly linked markers identified in the present study would also be useful in marker-assisted selection in forward breeding, as well as for marker-assisted pyramiding of MYMIV resistance genes from *G. max* and *G. soja* for marker-assisted backcross breeding in relatively shorter timespans.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

We are thankful to the Director of the ICAR-Indian Institute of Soybean Research at Indore for providing the infrastructure and support for this research.

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