Introduction

Grain legumes and pulses in countries such as India, Thailand, Bangladesh, Sri Lanka, and Pakistan are characteristically affected by yellow mosaic disease (YMD) caused by Mungbean yellow mosaic virus (MYMV), Mungbean yellow mosaic India virus (MYMIV), Dolichos yellow mosaic virus and Horsegram yellow mosaic virus. These viruses are closely related and have distinct but overlapping host ranges. Mungbean yellow mosaic virus (MYMV) and Mungbean yellow mosaic India virus (MYMIV) occur across the Indian subcontinent affecting the majority of legume crops including blackgram (Vigna mungo), cowpea (Vigna unguiculata), dolichos (Lablab purpureus), horsegram (Macrotyloma uniflorum), lima bean (Phaseolus lunatus), mungbean (Vigna radiata), pigeon pea (Cajanus cajan), mothbean (Vigna aconitifolia), common bean (Phaseolus vulgaris) and soybean (Glycine max) (Qazi et al. 2007, Varma et al. 1992). YMD of the leguminous crops causes an estimated annual loss of US$300 million (Varma et al. 1992). Among legumes, soybean is an economically important crop in which YMD causes 15–75% yield loss (Sharma et al. 2014). Nucleotide sequence of the virus isolated from soybean plants in northern and central India affected by YMD showed 89% similarity with Mungbean Yellow Mosaic India Virus (MYMIV) and was designated as soybean isolate of MYMIV (MYMIV-[Sb]) by Usharani et al. (2004). This virus is transmitted by the white fly, Bemisia tabaci Genn. (Nairani 1960, Nene 1972, 1973) and possesses bipartite, single stranded, circular DNA genome referred as DNA A and DNA B (Lazarowitz and Shepherd 1992). MYMIV produces typical yellow and golden mosaic patterns on the leaves of affected plants. Initially symptoms appear as small yellow specks along the veins and then spread over the leaf. In severe infections the entire leaf may become chlorotic. Since 1970s, MYMIV is posing a major threat to Indian soybean cultivation and it is reported to spread throughout India in alarming proportions (Varma and Malathi 2003).

Soybean in India has become a leading oilseed crop with...
41.5% and 28.6% contribution towards total oilseeds and edible oil production in the country during triennium average ending 2013–14. Beside contribution to edible oil pool, the crop is earning huge foreign exchange through export of soy meal, which has uplifted the rural economy of central India. However, the productivity of the crop which hovers around 1.2 tonne per ha in India is the major concern compared to the world average of 2.5 tonne per ha. YMD caused by MYMIV is one of the major constraints in enhancing the yield of soybean crop in India. None of the dominant varieties of central India, hub of soybean cultivation, is resistant to this virus. Therefore, it is imperative to introgress MYMIV resistance gene in elite soybean varieties with durable tolerance to MYMIV.

Various efforts have been made to understand the mechanism of natural resistance and nature of resistant gene in resistant soybean varieties. Yadav et al. (2009) compared the abundance of the viral RNAs in a resistant and a susceptible variety at the early time points after agro infection. Whilst the resistant variety displayed synthesis but rapid degradation of the early viral RNAs; the degradation in the susceptible variety was delayed resulting in accumulation of those transcripts later in infection. Accumulation of the late viral transcripts and DNA replication were detectable only in the susceptible variety that indicates rapid degradation of the early viral transcripts possibly through siRNA mechanism, is one of the probable mechanisms of natural resistance against geminivirus.

There are contradictory reports on the genetic nature of Yellow mosaic virus resistance. It is reported to be controlled by double recessive genes in PI171443 by Singh and Malick (1978) and a single dominant gene by Talukdar et al. (2013). It has become imperative to find out the true nature of MYMIV resistance in soybean. Moreover, segregating material, generated for the introgression of MYMIV resistance gene in high yielding and adapted varieties, has to be screened at hot spots or under artificial conditions for development of MYMIV resistant varieties. Identification of DNA markers linked to the trait will obviate the need of screening the segregating material at hot spot or under artificial conditions. If the trait is governed by recessive gene, selection for the trait in traditional breeding methods has to be deferred because of absence of expression of the trait in heterozygous condition. Marker Assisted Selection using linked marker will help in accelerated introgression of MYMIV resistance gene in dominating but susceptible varieties of central India. Attempts have been made to identify DNA markers linked to this trait. Yadav et al. (2015) did whole genome sequencing of MYMIV susceptible variety JS335 and resistant genotype UPSM534 (PI171443) to find out the genomic regions associated with resistance gene. They indicated a Single Nucleotide Polymorphism (SNP) on chr 18 (LG G) with a possible association with MYMIV resistance gene. Kumar et al. (2015) reported a region on chr 17 (LG D2) in significant linkage disequilibrium with resistance gene in association mapping study. But none of the reported linkage has been validated in mapping population. Efforts were made in this study to investigate the true nature of MYMIV resistance and to map the resistance gene to find the molecular markers linked to the trait.

Materials and Methods

Development of mapping populations

Two susceptible soybean genotypes viz; JS335, a dominant variety of India and NRC101, a newly developed Kunitz trypsin inhibitor free soybean genotype and two resistant genotypes viz; PI171443, donor of MYMIV resistance gene in most of the MYMIV resistant varieties released in India and SL525, a variety released for northern India, which has MYMIV resistance gene from PI171443 were used to develop mapping populations. JS335 was crossed with PI171443 to develop Recombinant Inbred Lines (RILs). F2 mapping population was developed from crossing of SL525 and NRC101. F1s & F2s of SL525 × NRC101 and advancement of RILs of JS335 × PI171443 were raised at Indore in disease free condition for development of mapping populations. RILs and F3 progeny rows were used for phenotyping. Fresh crosses were made for production of F1 seeds for phenotyping.

Phenotyping for reaction to MYMIV

RILs derived from JS335 × PI171443 and F3 progeny rows of SL525 × NRC101 along with 50 F1 plants of each population were raised at Ludhiana, hot spot for YMD, along with infector rows of susceptible genotype after every two rows. A vast survey made by Indian Council of Agriculture Research-Indian Institute of Soybean Research from different locations of India has proved that MYMIV is the prevalent virus infecting soybean at Ludhiana based on PCR based assay (Ramesh et al. 2016). Mapping populations were sown in June 2015. Fifty to sixty plants were raised per progeny row with 45 cm & 5 cm row to row and plant to plant distance respectively. A total of 98 F3 progeny rows, derived from the cross SL525 × NRC101 and 89 progeny rows of RILs derived from JS335 × PI171443 were tested for their reaction to MYMIV. Phenotyping was done at R5 stage when YMD was most severe. Severity of infection was scored from 0 to 9 scales. Progeny rows, which did not show any symptom in any of the plant were given a score of 0, progeny rows with any plant showing infection symptoms in 10% of the leaves were given a score of 1 and progeny rows with any of the plant showing infection symptoms in 20% of the leaves were given a score of 2 and likewise. Progeny rows with all the plants affected with a score more than 3 were classified as susceptible and those showing symptoms up to scale of 2, were classified as resistant. Progeny rows with both types of plants were classified as segregating.

DNA isolation and Molecular marker analysis

Genomic DNA was extracted from individual F2 plants
Mapping of MYMIV resistance gene in soybean

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Quantified DNA was subjected to PCR amplification in 10 µl reaction mixture containing 2 µl DNA (25 ng/µl), 1 µl PCR 10x buffer, 1.1 µl MgCl₂ (25 mM), 0.1 µl dNTPs (25 mM), 0.4 µl each forward and reverse SSR primers (30 ng/µl), 0.068 µl Taq DNA polymerase (3 units/µl) and 4.932 µl distilled water. DNA was denatured at 94°C for 2 min followed by 30 cycles each consisting of denaturation at 94°C for 1 min, primer annealing at 50°C for 2 min, primer elongation at 72°C for 3 min and final elongation at 72°C for 10 min in the thermocycler (MJ Research, model PTC100). Amplified products so obtained were resolved on 3% metaphor agarose gel with 50 bps ladder for allele sizing and analyzed in gel documentation system (Syngene). The SSR bands were scored manually from gel images. The SSR polymorphic between two parents were denoted S, R or H, where S band from susceptible parent only, R band from resistant parent only and H band from both the parents.

**Bulked segregant analysis**

The bulked segregant analysis (BSA) was performed following the protocol described by Michelmore et al. (1991). Resistant bulk were formed by mixing equal amount of DNA from 10 resistant progeny rows showing 9 score in RILs along with parental genotypes viz; JS335 and NRC101 showed susceptible reaction to MYMIV with a score of >8 while resistant parent genotypes showed resistance reaction with a MYMIV reaction score <2. The F1 plants of both the crosses showed susceptible reaction with a score >8 indicating the recessive nature of MYMIV resistance. A total of 98 F3 progeny rows, derived from the cross SL525 × NRC101 and 89 progeny rows of RILs derived from JS335 × PI171443 were tested for their reaction to MYMIV (Table 1). The observed segregation pattern fits almost perfectly into 1 resistant: 2 segregating: 1 susceptible for F3 progeny rows, which indicates the presence of a single recessive gene in the MYMIV resistant variety SL525. Progeny rows of RILs derived from JS335 × PI171443, the donor of MYMIV resistance gene in most of the released varieties, segregated in the ratio of 1:1 again proving monogenic nature of resistance gene.

**Genetic map construction**

DNA from resistant and susceptible bulks of F2 homozygous plants of SL525 × NRC101, and that of RILs developed from JS335 × PI171443 selected based on scoring of progeny rows along with their parents were screened with 144 regularly spaced polymorphic SSR markers from twenty linkage groups (LG) of soybean genome. The list of 144 polymorphic SSR markers, their chromosomal location, genetic positions within the chromosome and ampiclon size of parental lines are presented in Supplemental Tables 1, 2. BSA identified two SSR markers, namely GMAC7L (position of 12,259,594–12,259,701 bp) and Satt322 (position of 12,336,492–12,336,709 bp), located on chr 6 (LG C2) with

### Table 1. $\chi^2$ test for segregation of MYMIV resistance gene

<table>
<thead>
<tr>
<th>Type of mapping population</th>
<th>Cross combination</th>
<th>No. of plants</th>
<th>MYMIV reaction*</th>
<th>Expected ratio</th>
<th>$\chi^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIL</td>
<td>JS335×PI171443</td>
<td>89</td>
<td>S: 43</td>
<td>1:1</td>
<td>.101</td>
<td>0.75</td>
</tr>
<tr>
<td>F2:3</td>
<td>SL525×NRC101</td>
<td>98</td>
<td>S: 23</td>
<td>1:2:1</td>
<td>.186</td>
<td>0.90</td>
</tr>
</tbody>
</table>

* S denotes susceptible progeny rows, Seg denotes progenies segregating for susceptible and resistant plants and R denotes resistant progeny rows.
the genetic position of 69.68 cM and 73.18 cM respectively linked to MYMIV resistance in RILs as well as F2 mapping population. These two markers were used to genotype both the mapping populations (Fig. 1A, 1B) and all the markers within distance of 35 cM on both sides of the linked markers were also studied for parental polymorphism survey. A total of 5 SSR markers viz; Satt281, Sat_153, Satt305, Satt170 and Sat_213 other than the two linked markers identified were found to be polymorphic for RILs mapping population and 6 SSR markers viz; Satt281, Satt305, Satt170, Sat_246, Satt363 and Satt376 were found to be polymorphic for F2 mapping population. All the F2 plants and RILs were screened with these polymorphic markers. All the markers except Satt281 segregated in expected ratio of 1:1 in RILs and all markers other than Satt363 segregated in expected ratio of 1:2:1 in F2 mapping population. The genetic distance mapped between Sat_153 and Sat_213 in RILs is 49.7 cM as compared to genetic distance of 27.4 cM given in Soybase (Grant et al. 2010). The genetic distance mapped between Satt281 and Satt376 is 52.2 almost same as given in Soybase (51.9 cM) (Fig. 2).

**Discussion**

In India, breeders have developed MYMIV resistant soybean varieties through classical breeding. All the MYMIV resistant varieties till now are adapted for northern India as due to heavy infection pressure of MYMIV, nothing susceptible to the virus survives in this part of India. So, there is natural selection for MYMIV resistance while making selection in breeding populations. Lately the virus has spread to central India, which is hub of soybean cultivation. It has created havoc in the present year and is the main reason behind low productivity of soybean in the present year. Unfortunately none of the dominant variety of central India is resistant to this virus. Introgression of MYMIV resistance gene in dominant varieties is the immediate challenge. For speedy introgression of the trait, understanding the nature of genetic inheritance due to contradictory reports on its inheritance and finding molecular markers linked to the gene was the immediate challenge. First report on inheritance of the trait reported that the trait is controlled by double recessive gene (Singh and Malick 1978). Our results also prove the recessive nature of the trait, but segregation ratio obtained in our study has proven it to be monogenic. The discrepancy may result from the classification of resistant and susceptible classes. We classified the progeny rows with a MYMIV reaction score of 0 to 2 as resistant progeny rows as we observed similar score in resistant parental lines also, while
the earlier group classified only those F2 plants as resistant which showed complete immune response. Talukdar et al. (2013) has reported dominant and monogenic nature of genetic inheritance of the trait in two resistant varieties DS9712 and DS9814. The result obtained in their study may either be due to escapes of susceptible plants due to low disease pressure or a totally different gene as ancestry of these two varieties could not be traced to known source of YMV resistance.

Efforts have been made to locate the gene on a linkage map by two groups. Yadav et al. (2015) did whole genome sequencing of MYMIV susceptible variety JS335 and resistant genotype UPSM534 (PI171443) to find out the genomic regions associated with resistance gene. They indicated a SNP on chr 18 (LG G) with a possible association with MYMIV resistance gene. Kumar et al. (2015) found a region on chr 17 (LG D2) in significant linkage disequilibrium with resistance gene in association mapping study. But none of the reported linkage has been validated in mapping population. We studied all the linkage maps including these two regions. We did not find any association between these genetic regions and the location of resistance gene. Association studies are known to lead to spurious associations (Lander and Schork 1994) and recent attempts to map such traits have resulted in extremely high false positive rates (Aranzana et al. 2005). In our study, we have mapped MYMIV resistance gene on chr 6 (LG C2) very close to two SSR markers Satt322 and GMAC7L. The total map distance of the linkage map constructed in F2 mapping population is 52.2 cM, which is very similar to map distance given in Soybase. However map distance of 49.7 cM studied in RILs mapping population is much larger as compared to 27.4 cM given in Soybase. Map positions and distances between loci on a linkage group may vary because of deletions, insertions, inversions, translocation and other chromosomal arrangement in one or both of the parents that could change distance between loci as well as their relative orders (Stam 1993).

There are many viruses that affect soybean, and resistance genes against several viruses have been extensively studied. A lot of work has been done on mapping of soybean mosaic virus resistance genes in soybean. Rsv1 locus was mapped on chr 13 (LG F) (Yu et al. 1994, 1996), Rsv3 locus on chr 14 (LG B2) (Jeong et al. 2002) and another gene, Rsv4 locus was mapped on chr 2 (LG D1b) (Hayes et al. 2000). Six linked resistance genes, Ra, Rsc-7, Rsc8, Rsc-9, Rn1, and Rn3, were mapped on chr 7 (LG M) (Wang et al. 2004). Rsc-11 was mapped on chr 13 (LG F) by Li et al. (2009). The major Soybean dwarf virus resistance gene Rsdv1 was mapped on chr 5 (LG A1) (Yamashita et al. 2013). The nucleotide-binding site leucien-rich repeat type resistance genes for virus have been mapped on chr 2, 8, 9 & 18 in the Phytozone database (https://phytozone.jgi.doe.gov/pz/portal.html). No virus resistance gene has been mapped on chr 6 (LG C2) till now.

Development of an MYMIV resistant soybean variety for central India, hub of soybean cultivation, was hampered due to difficulty in creating artificial epiphytotics for yellow mosaic disease. The only alternative available with breeder was to screen segregating populations in hotspot in main soybean crop season in the absence of reliable of molecular marker. The tightly linked marker identified in this study, will help breeder in screening MYMIV resistant soybean plants in segregating populations at their own station and selection can be carried out in off season also thus aiding in the accelerated development of MYMIV resistant cultivars in relatively shorter time span.

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Literature Cited


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