Indian J. Genet., 74(4) Suppl., 584-588 (2014)
DOI: 10.5958/0975-6906.2014.00894.3

Inheritance of MYMIV tolerance in two RIL populations of greengram on lower Gangetic alluvial zone during summer and their parental molecular diversity

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(Received : October 2014; Revised : November 2014; Accepted: November 2014)

Abstract

Inheritance of MYMIV tolerance was determined in two sets of recombinant inbred lines (RILs) of mungbean. The two sets comprising 143 and 79 RILs each derived from the cross between PM5 x Sub2 and B1 X Sub2, respectively were considered for the study. Sub2 was a Sublobata derived lines whereas, B1 was a popular small seeded cultivar of west Bengal, susceptible against MYMIV. It was observed that one or two major genes with a few modifiers played a significant role in resistance mechanism against MYMIV in the lower Gangetic alluvial zone. Resistance alleles for Sub2 and PM5 are probably allelic as observed from frequency distribution pattern. A set of 177 SSRs were employed for identification of polymorphism between parents. Only 37 SSRs showed polymorphism between Sublobata and B1 or PM5. But only eight SSRs were polymorphic between two high yielding cultivars. Markers linked with MYMIV tolerance like RGA, SCAR and others from earlier studies were also considered and found that only one RGA derived marker showed polymorphism between resistance and susceptible parents.

Key words: Greengram, MYMIV, polymorphism, tolerance

Introduction

Amongst pulses, mungbean is one of the most important pulse crop in India covering up to 55% of the total world acreage and 45% of total production. It serves as a vital source of vegetable protein (19.1-28.3%), mineral (0.18-0.21%), vitamins and can fix atmospheric nitrogen, thus help in improve the soil fertility. Presently this crop is tormenting a harsh abatement in the production due to astringent onrush of viral diseases. Yellow Mosaic Disease (YMD) transmitted (Gemini virus) by different species of white-fly belongs to genus Begomovirus and family Geminiviridae. Major pathogen of YMD in lower gangetic alluvial zone is MYMIV which causes irregular green and yellow patches in older leaves and complete yellowing of younger leaves. In lower Gangetic alluvial region, higher incidence of disease has been observed during summer due to favorable conditions for multiplication of the vector *Bemisiatabaci*. There are various reports of even 100 % incidence of the disease [1]. Resistance against the disease incidence might be due to lack of replication or cell to cell movement of viral particle. They are prevented due to loss of function of host genes [2, 3]. Thus, recessive nature of resistance mechanism is often observed in mungbean. There are several contrasting published reports on the inheritance of resistance to MYMV in mungbean. The monogenic recessive inheritance for MYMV has been reported earlier by Malik *et al.* [4], single major recessive gene with modifiers [5], two recessive genes [6] and complementary recessive genes [7]. Therefore, resistance to YMD in mungbean in a particular cultivar varies with the change in environment. Introduction of YMV-tolerant mungbean in the farmer's field will circumvent the problems associated with the residual effects of the insecticides/pesticides in the soil. Knowledge of the mode of inheritance to MYMIV in mungbean is useful for incorporation of resistance into the wide adoptable cultivar of West Bengal, like B1.

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Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110012
Online management by indianjournals.com

Molecular markers such as simple sequence repeats (SSRs) are commonly used for identification of polymorphism in crop breeding because they are highly informative and reproducible. Several workers have utilized these markers in different pulse crops including mungbean to study polymorphism, [8-10], construction of linkage maps and to identify quantitative trait loci (QTLs) for different agronomic traits. As no such information with respect to mode of inheritance of MYMIV tolerance is available for eastern India, especially, West Bengal, hence effort was initiated to study inheritance of MYMIV in two RIL populations derived from the cross between PM5 x Sub2 and B1 X Sub2. An effort was also made to identify polymorphic markers between three parents.

Materials and methods

Evaluation for MYMIV reaction

Several accessions of mungbean were sown in the third week of February as well as in third week of September at the experimental farm, Bidhan Chandra Krishi Viswavidyalaya, Kalyani in replicated blocks with 30 x 10 cm spacing between rows and plants. One hundred forty seven RILs from B1 x Sub2 and 79 from of PM5 x Sub2 along with parents B1, PM5 and Sub2 were screened against MYMIV under natural conditions. B1 was used as spreader variety after each five rows of RILs and genotypes. No insecticide was sprayed in order to maintain the natural whitefly population in the field. Disease scoring was done following 1-5 scale as per the methods described earlier [11].

Identification of polymorphic markers

Genomic DNA was isolated from the young seedlings [12]. Purification was done by RNase treatment and quantification in 0.8% agarose gel comparing with the known standard procedure. One hundred Seventy one SSR primer pairs derived from *Vigna angularis* [10], *Vigna radiata* [8], *Phaseolus vulgaris* [13] and *Glycine max* [8] were employed for the identification of polymorphism following the methods described earlier [12]. In addition, a few RGA derived primer pairs [14], SCAR-marker [11] linked with the MYMIV tolerance and bruchid linked marker [15] as shown in earlier studies were also considered. Further, 25µl PCR mixture comprising of 50ng of template DNA, 10ng of forward and reverse primers, 1µl 2.5mMdNTP mixture, 10X Taq buffer and 1.0 unit Taq polymerase (Genie, Banglore) 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) above the UV light.

Results and discussion

Genotypes collected from seven states of India, were screened for tolerance against mungbean yellow mosaic India virus (MYMIV) as per the procedure described earlier [12]. Crops, sown on post-*kharif* season did not show significant viral symptoms, whereas same set of genotype showed severe infestation when grown in the same field at pre-*kharif*. Sublobata and PM5 showed highest degree of resistance in lower Gangetic alluvial zone of West Bengal, whereas cultivar B1 was highly susceptible against the MYMIV.

Inheritance of MYMIV resistance

Sub2, a wild accession of V. *radiate* var. sublobata which is also the progenitor of cultivated greengram showed resistance against MYMIV and Bruchid in our earlier studies [12, 15]. PM5, a high yielding bold seeded cultivar showed resistance against this viral disease which was also reported earlier [16] when evaluated in Northern zone. The screening of RIL populations also confirmed Sub2 and Pm5 are a good source of resistance to MYMIV (unfilled bar, Fig. 1). Almost 80% lines derived from PM5 x Sub2 showed resistance while 12% showed either moderate resistance or susceptible reaction. These results indicated that the resistance gene present in both the parents are probably allelic. However, complete resistance was not observed, which may be due to the presence of modifier gene(s) which have played some role in expression of resistance gene because four lines exhibited complete susceptibility. The

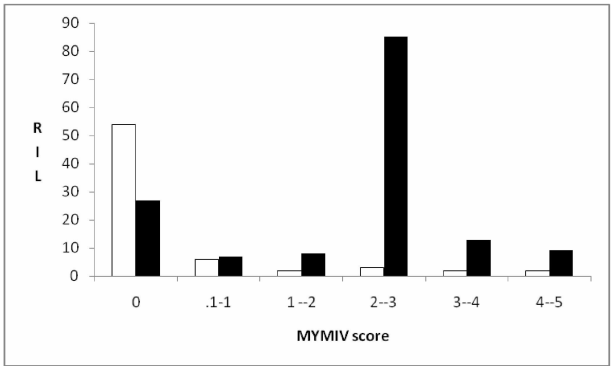

Fig. 1. Frequency distribution for MYMIV score in RIL population derived from PM5 x Sub2 (Unfilled bar) and B1 x Sub2 (filled bar)

Table 1. Description of polymorphic markers among the three parents, used in developing two RIL populations

Marker	Forward primer (5'–3')	Reverse primer (5'–3')	B1	Sub2	PM5	Annealing temp^{o}C
MBSSR238	AGCTATTGGTGCATAGGTTC	GATATGATGAGTATGGTGTAG	115	103	115	55
CEDG228	GTCGTTTCCGGAAACTGTTC	GATCCGAACCTCTTTCTGC	213	225	213	58
CEDG056	TTCCATCTATAGGGGAAGGGAG	GCTATGATGGAAGAGGGCATGG	241	209	241	55
CEDG086	GAGTTTACAACAGATGGGGCTAA	AGGTCTTGATTGACTTTCTGGGT	126	138	130	55
CEDG180	GGTATGGAGCAAAACAATC	GTGCGTGAAGTTGTCTTATC	128	120	122	55
CEDG173	GATAAGAGATGCATCACTC	CTTCTCTTCCATCACATCTG	120	128	120	50
CEDG020	TATCCATACCCAGCTCAAGG	GCCATACCAAGAAAGAGG	206	206	204	58
CEDG143	GATGAACTCGTCTCGCTCATCG	CTGGACGCGTCTACTCAGAC	121	128	124	58
GBSSRMB91	GAGGCCAATCCCATAACTTT	AGCACCACATCAGAGATTCC	171	160	160	55
GBSSRMB7	CTTGCTTGCGAGGATGAC	TCCAGTGCAGCAGATTGA	245	234	234	55
DMBSSR20	TTCTTGTTCTTGCTCCTTCTTG	TTCCCCACCCTTCTTTCTTTAT	250	240	250	55
DMBSSR18	CGGTGTTGTTTGTGTTTTGG	TTTGTGTGGTGAACTGACACT	158	150	158	55
DMBSSR080	CGAGGCAGAGAAACCTTAAGAA	GCTCGATACTCTTGGGTTGAA	105	100	105	55
DMBSSR105	TGATTTAAAGACGGACGGAAA	AGAAGAAAAGCAACCCTTGGAT	98	90	98	55
DMBSSR119	TGGTGTACAACTATTGCCATGAT	GACACTATAGGACCCAAAACATTTC	99	92	99	55
DMBSSR125	AAAATGAGTGACAGAGGTGGAAA	ACATGCACATTCTGAACCACAT	68	60	68	55
CEDG015	CCCGATGAACGCTAATGCTG	CGCCAAAGGAAACGCAGAAC	168	176	174	58
CEDG024	CATCTTCCTCACCTGCATTC	TTTGGTGAAGATGACAGCCC	140	158	140	58
CEDG166	GGTACAACATTCTTCTATTTG	GGCTTATGAGTTTATCTTATC	185	197	185	54
BAT44	TGAGTAAAGCAAGTTTGTATCC	TCAAAATTCGATGAAACTTGAC	142	138	142	55
CEDG041	GCTGCATCTCTATTCTCTGG	GCCAACTAGCCTAATCAG	102	98	102	50
CEDG051	AAACATACCCCTGGCAGTTCC	TTCTGACCTAAGAAAGAGCCTGG	250	240	250	58
CEDG044	TCAGCAACCTTGCATTGCAG	TTTCCCGTCACTCTTCTAGG	133	147	133	58
CEDG067	AGACTAAGTTACTTGGGCAACCAG	TGACGGCCCGGCTCTCC	100	110	110	58
CEDG073	CCCCGAAATTCCCCTACAC	AACACCCGCCTCTTTCTCC	200	170	180	55
CEDG076	GGTGGTTTACTTACTGGCATTT	GGTCTATTCCACCATCTATCAA	176	180	176	55
GMES5010	CACCAAAGCCAACACCTTTT	CAATGCCACCATCTCAACAC	230	225	225	55
VRD1	CAGCTTCTTGTTCTTGCTCC	CGAATGTGCACAGGTGGTGT	245	255	255	55
VRO3	GTCAGAGTGCCTTTGCAGGTA	CTTCCTGGTGTACGGATGAGTT	146	138	140	55
VRO4	GGCTATGGATAAATACTGCTCCC	CAACAGAAACACTTGAGAGACACA	318	325	320	55
VRO9	ATTGTGTTGTGTGTGTTGCAGA	GTAATTTTGGAGGTGCAGGAAG	284	295	295	55
VRO24	CCCTCACCCTAACTGTATAACCA	ACCATGAGCAATCTTTCCAAGT	212	223	220	55
VRO25	ATGGCTGTTGGACATCTTCC	CTTGCTAAAGCTACTGCGATCA	186	193	190	55
VR0120	GTGACCTCAACTAACATAAGCATTC	ACAGGCACAAGGGATTATTACA	180	188	185	55
RGA22F2/24R2	GGGTGGNTTGGGTAAGACCAC	NTCGCGGTGNGTGAAAAGNCT	470	400	400	58
STS br 1	CAGAAAACAAATCACAAGGC	GTAAGCATTGAAAAAGGGTG	-	225	-	58

same population showed differential resistance in multi-environmental trial. The role of modifiers or other minor genes, if present influencing resistance against MYMIV were not detectable. Therefore, population derived from PM5 x Sub2 may be used for mapping minor or modifier genes required for manifestation of resistance gene(s).

Although frequency distribution plotted as bar from the B1 X Sub2 (filled bar, Fig. 1) showed continuous variation but broadly classified into two major classes comprising 28 tolerant (Scale 0) and 89 susceptible (scale 2-3) lines. Therefore, it may be concluded that one or two major loci may be responsible for tolerance against MYMIV in Sub2. In earlier study, Sublobata was shown as susceptible source [17] of MYMIVwhen grown on AVARDC experimental station although manifested tolerance against bruchid. Hence, Sublobata accession, Sub2, used in this study may serve as a source of both MYMIV and bruchid tolerance. So, RIL population derived from B1 X Sub2 is the ideal population for mapping major QTLs for MYMIV tolerance and PM5 X Sub2 for mapping the modifiers.

Parental diversity by SSR

A set of 171 SSR primers comprising of 64 from *Vigna angularis*, 95 from *Vigna radiata*, 11 from *Phaseolus vulgaris* and one from *Glycine max* were employed for identification of polymorphism. Amplification and polymorphic status of these markers among the parental genotypes Sub2, B1, and PM5 is given in Table1. Thirty seven SSRs showed polymorphism among Sub2 and other parents but only eight showed polymorphism between PM5 and B1. So, it also reconfirmed the narrow genetic base among the mungbean varieties of India [12, 18]. Therefore, the Sublobata genome can be utilized for crossing with high yielding cultivars to broaden the genetic base and diversity within Indian cultivars. About 26% of SSRs from *V. angularis* showed polymorphism between Sublobata and other two cultivars, whereas only 18-19% from *Vigna radiata.* Thus, SSR primers derived from *Vigna angularis* may be used more frequently for mapping in mungbean than those of available in public domain from mungbean itself. SCAR marker [11] or most of the RGA markers [14] did not show any polymorphism between resistant and susceptible parents.Only one RGA primer carrying CC-NBS-LRR domain [14] amplified 400bp fragment from the resistant parents, PM5 and Sub2 but 470bp from susceptible parent, B1. Same primer pair amplified a fragment of 1236 bp from the resistant parent as observed earlier [14]; which may be due to the use of different parent(s) by earlier workers. Another STS based primer pair linked with the bruchid tolerance [15] and a QTL for MYMIV tolerance [11] showed polymorphism between Sub2 and other two parents but not between B1 and PM5.All the identified polymorphic markers are in use for mapping of MYMIV tolerance loci and their modifiers.

Acknowledgment

We sincerely acknowledge DBT, Govt. of India for providing financial support.

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